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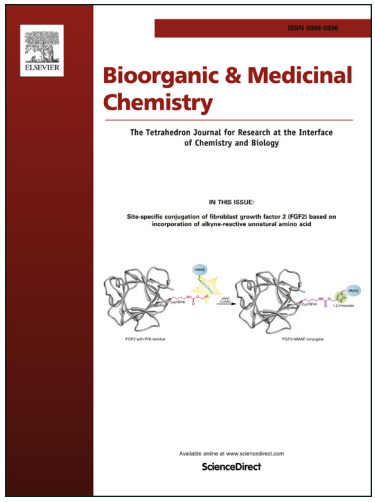

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Conjugates of 18*β*-glycyrrhetinic acid derivatives with 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid as Pin1 inhibitors displaying anti-prostate cancer ability

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Abstract

Twenty-six conjugates of 18*β*-glycyrrhetinic acid derivatives with 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid were designed and synthesized as Pin1 inhibitors. Most of these semi-synthetic compounds showed improved Pin1 inhibitory activity and anti-proliferative effects against prostate cancer cells as compared to 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid and **GA**. Compounds **10a** and **12i** were the most potent to inhibit growth of prostate cancer PC-3 with GI_{50} values of 7.80 μM and 3.52 μM, respectively. The enzyme inhibition ratio of nine compounds at 10 μM was over 90%. Structure-activity relationships indicated that both appropriate structure at ring C of **GA** and suitable length of linker between **GA** skeleton and benzimidazole moiety had significant impact on improving activity. Western blot assay revealed that **10a** decreased the level of cell cycle regulating protein cyclin D1. Thus, these compounds might represent a novel anti-proliferative agent working through Pin1 inhibition.

Keywords: Pin1 inhibitors; 18*β*-glycyrrhetinic acid derivatives; anti-proliferative activity; cyclin D1

1. Introduction

Pin1, which belongs to the peptidyl-prolyl isomerase family, is the only enzyme that catalyzes isomerization of specific phosphorylated motifs in a subset of proteins.[1,2] Pin1 regulates diverse cellular processes, including growth-signal response, cell-cycle regulation, cellular stress response, neuronal function and immune response.[3] High expression level of Pin1 is frequently observed in a variety of cancer cells, especially in prostate cancer. Studies indicated that Pin1 could regulate the level of cancer-related signaling proteins, such as cyclin D1.[4-6] The abnormal increase of cyclin D1 can be attributed to many factors including transcription, translation and protein stability.[7,8] Knockout of Pin1 results in mitotic arrest and apoptosis in tumor cell lines. Therefore, Pin1 has been used as a potential therapeutic target for developing anticancer agent.[9-11]

There has been persistent interest for developing Pin1 inhibitors, and a number of promising entities have been identified by several groups.[12] Structure-guided design was adopted to design small molecule inhibitors, but only a few of them were active in cancer cells due to their poor permeability.[13-17] A couple of natural products have been found to be able to inhibit Pin1 (**Figure 1**); for instance, Juglone and Epigallocatechin-3-gallate (EGCG).[18,19] In our previous studies, lots of triterpenoid derivatives have been reported with effective Pin1 inhibitory ability, including Acetyl-11-keto-β-boswellic acid analogues and Elemonic acid derivatives.[20,21]

(**Figure 1**. should be listed here.)

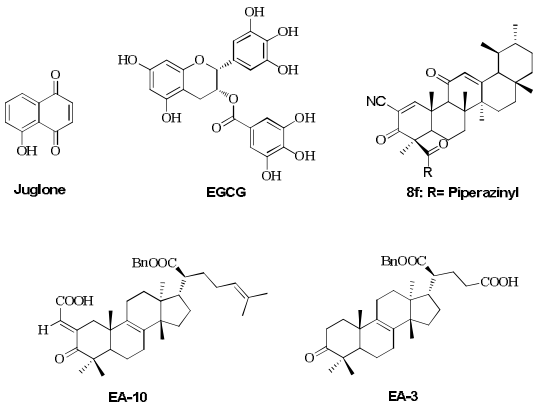


Figure 1. The structures of natural products as Pin1 inhibitors.

The crystal structure of Pin1 protein indicated that PPIase domain consists of two hydrophobic domains and one

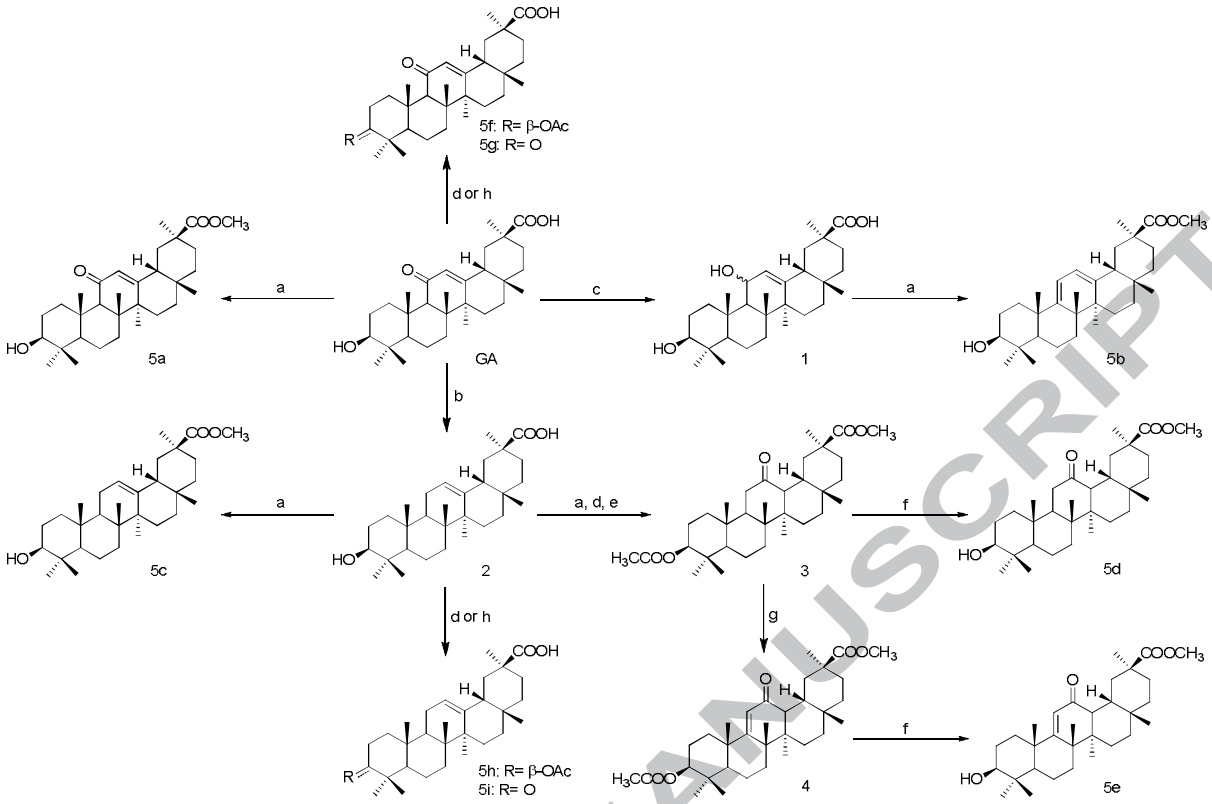
basic cluster. Based on Jonathan D. Moore's research, the 3-(1H-benzo[d]imidazol-2-yl)propanoic acid moiety has been identified to be a suitable core fragment for the prolyl pocket and basic cluster of Pin1 protein, and introducing an extra hydrophobic fragment to access the shallow surface of Pin1 protein could enhance Pin1 inhibitory activity significantly. However, most benzimidazole derivatives were not active in cell-based assays because of the poor permeability.[14,15] As continued research to discover new Pin1 inhibitors with anti-proliferative activity, the 3-(1*H*-benzo[d]imidazol-2-yl) propanoic acid skeleton was adopted for conjugates. Considering the excellent hydrophobicity of triterpenoids, 18*β*-glycyrrhetinic acid derivatives were attached to N1 position of benzimidazole through different flexible linkers.[22] The triterpenoid moiety was supposed to produce a hydrophobic interaction with the shallow surface of Pin1 PPIase domain. It is reasonable to suggest that the construction of triterpenoid–benzimidazole hybrids might develop a series of novel Pin1 inhibitors with anti-proliferative activity.

2. Results and discussion

2.1. Chemistry

The synthetic route adopted for the preparation of key intermediates **5a**–**5i** was outlined in **Scheme 1**, which has been reported previously.[23] Briefly, the esterification of **GA** at C30 was performed by treatment with concentrated sulfuric acid and methanol to give the intermediate **5a**. Reduction of **GA** with sodium borohydride led to **1**, which was then refluxed in methanol and sulfuric acid to obtain **5b**. The intermediate **2** with 12-en moiety at ring C was obtained from **GA** by Clemmensen reduction. Then esterification of **2** was executed to give **5c**, which continued to be acetylated and oxidized resulting **3**. A sequence of bromination/dehydrobromination of **3** provided **4** easily. The deacetylation of **3** and **4** gave **5d** and **5e** in high yield respectively. The acetylation and oxidization of hydroxyl at C3 position of **GA** and **2** provided **5f**–**5i** smoothly.

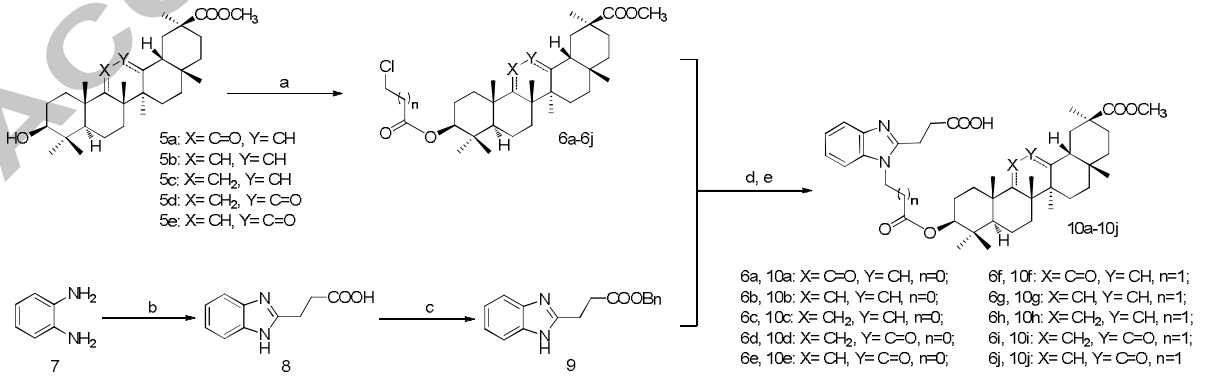
(**Scheme 1**. should be listed here.)



Scheme 1. Synthesis of **5a–5i**. Reagents and Conditions: (a) Concentrated H_2SO_4, methanol, 66 ^oC; (b) Zn-Hg, HCl, 1,4-dioxane, 10 ^oC; (c) $NaBH_4$, $NaOH_{(aq.)}$, THF, 66 ^oC; (d) Ac_2O, pyridine, 115 ^oC; (e) 30% H_2O_2, AcOH, 100 ^oC; (f) KOH, methanol, 66 ^oC; (g) Br_2, HBr, acetic acid, 40 ^oC; (h) Jones' reagent, acetone, r.t..

The synthesis of compounds **10a–10j** was described in **Scheme 2**. Intermediates **5a–5e** were acylated using 2-chloroacetyl chloride or 3-chloropropionyl chloride with the presence of triethylamine or pyridine in dichloromethane to generate **6a–6j**, which were then reacted with **9**. Compounds **10a–10j** could be readily yielded by debenzylation with H_2/Pd-C or $TiCl_4$ at ambient temperature.

(**Scheme 2**. should be listed here.)

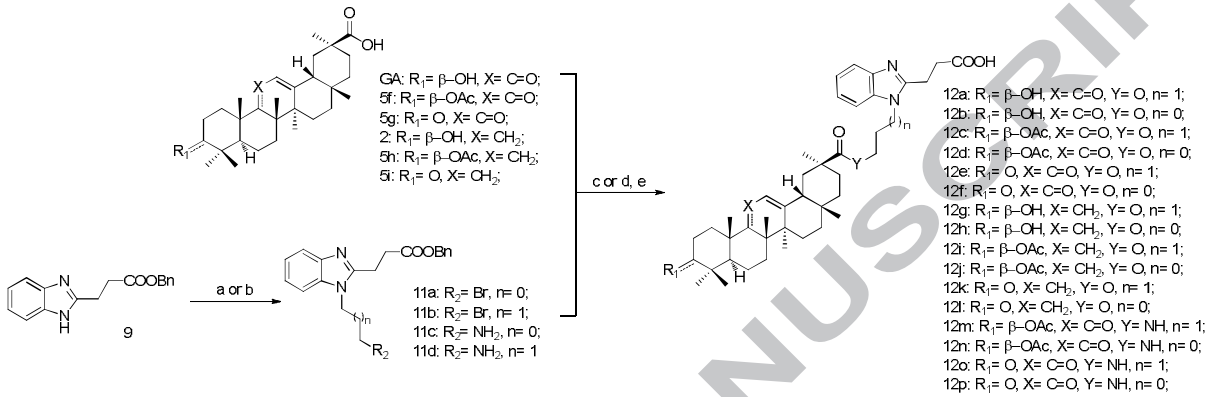


Scheme 2. Synthesis of **10a–10j**. Reagents and Conditions: (a) 2-chloroacetyl chloride or 3-chloropropionyl chloride, triethylamine or pyridine, CH_2Cl_2, r.t.; (b) succinic anhydride, dioxane, reflux; (c) BnBr, K_2CO_3, DMF, r.t.; (d) Cs_2CO_3,

acetonitrile, 80 °C; (e) H_2, 5% Pd/C, methanol, r.t. or $TiCl_4$, CH_2Cl_2, r.t..

Compounds **12a–12p** were prepared as depicted in **Scheme 3**. The coupling of **GA**, **2**, **5f–5i** with **11a–11d** was performed respectively, followed which an efficient debenzylation was carried out in good yield to provide compounds **12a–12p**.

(**Scheme 3**. should be listed here.)



Scheme 3. Synthesis of **12a–12p**. Reagents and Conditions: (a) 1,2-dibromoethane or 1,3-dibromopropane, Cs_2CO_3, acetonitrile, 80 °C; (b) 2-chloroethylamine hydrochloride or 3-bromopropanamin hydrobromide, NaH, DMF, 80 °C; (c) K_2CO_3, DMF, r.t.; (d) $SOCl_2$, CH_2Cl_2, r.t.; (e) H_2, 5% Pd/C, methanol, r.t. or $TiCl_4$, CH_2Cl_2, r.t..

2.2. Biological activity

As Pin1 is often highly expressed in human prostate cancer cells, all target compounds were first evaluated for their anti-proliferative activity against PC-3 and LNCaP cell lines using MTT assay. The results expressed as GI_{50} values were presented in **Table 1**, which were the average of three independent experiments.

As shown in **Table 1**, 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid (**8**) did not show cell growth inhibitory effects might due to its poor permeability and weak activity against Pin1, whereas most of these hybrids exhibited superior anti-proliferative activity to **GA**. This phenomenon indicated that the conjugation is necessary for significant cytotoxicity. Generally, the structural modification of ring A and ring C together with the length of linker between **GA** skeleton and benzimidazole moiety had a notable influence on the anti-proliferative activity. Compounds **10a** and **12i** displayed the most potent inhibitory effects with GI_{50} values of 7.80/13.23 μM and 3.52/7.92 μM, respectively.

As for **10a–10j**, compounds **10a** (GI_{50} = 7.80 μM $_{(PC-3)}$, GI_{50} = 13.23 μM $_{(LNCaP)}$) and **10b** (GI_{50} = 15.31 μM $_{(PC-3)}$, GI_{50} = 21.37 μM $_{(LNCaP)}$) with acetyl as linker showed more potent anti-proliferative activity than compounds **10f** (GI_{50} =

16.05 μM $_{(PC-3)}$, GI_{50} = 26.61 μM $_{(LNCaP)}$) and **10g** (GI_{50} = 47.22 μM $_{(PC-3)}$, GI_{50} > 50 μM $_{(LNCaP)}$) linked by propionyl, indicating that two atoms length is favorable. A tiny tuning of the structure at ring C exerted a tremendous influence on activity. Compounds **10c** (GI_{50} = 29.21 μM $_{(PC-3)}$, GI_{50} = 30.01 μM $_{(LNCaP)}$) and **10h** (GI_{50} > 50 μM $_{(PC-3)}$, GI_{50} > 50 μM $_{(LNCaP)}$) with 12-oxo structure at ring C were inferior to corresponding derivatives with other distinct structures at ring C. When the 11-oxo-12-en (**10a**) at ring C was replaced by 12-en (**10b**) or 9(11),12-dien (**10e** GI_{50} = 14.06/17.39 μM), the inhibitory activities were decreased to about half.

Introducing the benzimidazole moiety to C30 carboxylic acid group of **GA** led to a significant decline of anti-proliferative activity as exemplified by **12a** (GI_{50} > 50 μM $_{(PC-3)}$, GI_{50} > 50 μM $_{(LNCaP)}$) and **12b** (GI_{50} > 50 μM $_{(PC-3)}$, GI_{50} > 50 μM $_{(LNCaP)}$). Replacing the hydroxy group at C3 position of ring A by acetoxyl group or carbonyl caused a remarkable increase of anti-proliferation activity as exemplified by **12c** (GI_{50} = 11.83 μM $_{(PC-3)}$, GI_{50} = 15.51 μM $_{(LNCaP)}$), **12e** (GI_{50} = 35.63 μM $_{(PC-3)}$, GI_{50} = 29.32 μM $_{(LNCaP)}$) vs. **12a**, which could be further confirmed by **12d** (GI_{50} = 27.35 μM $_{(PC-3)}$, GI_{50} = 24.43 μM $_{(LNCaP)}$), **12f** (GI_{50} = 39.93 μM $_{(PC-3)}$, GI_{50} = 48.91 μM $_{(LNCaP)}$) vs. **12b**, suggesting a fine tuning of functional groups at this position was beneficial. However, the anti-proliferative potency of these C30 coupled derivatives was still a little less than **10a**. Subsequently, a small set of C30 coupled analogues **12g**–**12l** with 12-en at ring C were synthesized. Among them, compounds **12i** (GI_{50} = 3.52 μM $_{(PC-3)}$, GI_{50} = 7.92 μM $_{(LNCaP)}$), **12j** (GI_{50} = 10.91 μM $_{(PC-3)}$, GI_{50} = 13.34 μM $_{(LNCaP)}$), **12k** (GI_{50} = 12.98 μM $_{(PC-3)}$, GI_{50} = 19.71 μM $_{(LNCaP)}$) and **12l** (GI_{50} = 18.41 μM $_{(PC-3)}$, GI_{50} = 25.80 μM $_{(LNCaP)}$) were 2~3 fold more potent than their counterparts **12c**, **12d**, **12e** and **12f**, respectively. The activities of compounds **12c** and **12i** were much better than **12d** and **12j**, indicating that for C30 coupled derivatives three atoms length is favorable. In addition, **12i** and **12j** with acetoxyl group at C3 position of **GA** presented superior activity to **12k** and **12l** with carbonyl; this phenomenon was appeared among **12c**, **12d** vs. **12e**, **12f** as well. Nonetheless, once the ester of linker was replaced by amide (**12m**, **12n**, **12o** and **12p**), the anti-proliferative effect decreased sharply.

(**Table 1**. should be listed here.)

Table 1. The anti-proliferative activities of target compounds against PC-3 and LNCaP cell lines.

Compd.	GI_{50} (μM) [a]		Compd.	GI_{50} (μM) [a]	
	PC-3	LNCaP		PC-3	LNCaP
10a	7.80 ± 0.31	13.23 ± 0.29	**12f**	39.93 ± 0.22	48.91 ± 0.41
10b	15.31 ± 0.35	21.37 ± 0.33	**12g**	25.63 ± 0.33	42.66 ± 0.33
10c	29.21 ± 0.59	30.01 ± 0.21	**12h**	> 50	> 50
10d	21.22 ± 0.35	23.45 ± 0.17	**12i**	3.52 ± 0.17	7.92 ± 0.69
10e	14.06 ± 0.25	17.39 ± 0.31	**12j**	10.91 ± 0.28	13.34 ± 0.19
10f	16.05 ± 0.35	26.61 ± 0.44	**12k**	12.98 ± 0.39	19.71 ± 0.52
10g	47.22 ± 0.29	> 50	**12l**	18.41 ± 0.30	25.80 ± 0.77
10h	> 50	> 50	**12m**	> 50	> 50
10i	21.39 ± 0.38	28.95 ± 0.78	**12n**	> 50	> 50
10j	20.41 ± 0.37	25.32 ± 0.39	**12o**	> 50	> 50
12a	> 50	> 50	**12p**	> 50	> 50
12b	> 50	> 50	**8**	> 50	> 50
12c	11.83 ± 0.33	15.51 ± 0.68	**GA**	95.72 ± 0.66	-
12d	27.35 ± 0.19	24.43 ± 0.47	**Juglone**	4.77 ± 0.29	-
12e	35.63 ± 0.27	29.32 ± 0.55			

[a] GI_{50} value is the concentration that inhibits 50% of cell growth compared with the untreated cells. Each value was measured at least three times.

To investigate the Pin1 inhibitory potency of all synthesized compounds, the inhibition ratio at 10 μM was determined using protease-coupled assay, and the results fitted well with the anti-proliferative activity (**Table 2**). The bulk of these triterpenoid–benzimidazole conjugates displayed improved Pin1 inhibitory potency as compared to 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid (**8**) and GA. Among them, the inhibition ratio of nine compounds at 10 μM was over 90%. The IC_{50} values were then determined for those compounds. Compounds **10a** (IC_{50} = 1.07 μM) and **12i** (IC_{50} = 2.10 μM), which displayed the most potent growth inhibition effects, also proved to be outstanding in Pin1 inhibition. On the contrary, compounds **10h**, **12a**, **12m**, **12n**, **12o** and **12p**, which showed terrible anti-proliferative activity, were found to be less potent ones. Therefore, **10a** and **12i** with both good Pin1 inhibitory activity and excellent anti-proliferative effects were regarded as the most promising candidates among those target compounds.

(**Table 2**. should be listed here.)

Table 2. The Pin1 inhibitory activities of target compounds.

Compd.	Pin1 inhibition at 10 μM (%) [a]	IC_{50} (μM) [b]	Compd.	Pin1 inhibition at 10 μM (%) [a]	IC_{50} (μM) [b]
10a	100	1.07	**12f**	63 ± 3	-
10b	96 ± 7	2.31	**12g**	100 ± 2	2.82
10c	67 ± 9	-	**12h**	54 ± 14	-
10d	76 ± 2	-	**12i**	100 ± 3	2.10
10e	74 ± 5	-	**12j**	94 ± 2	4.79
10f	88 ± 3	-	**12k**	98 ± 7	3.80
10g	58 ± 8	-	**12l**	69 ± 5	-
10h	44 ± 1	> 10	**12m**	50 ± 6	-
10i	100	2.35	**12n**	27 ± 4	> 10
10j	70 ± 5	-	**12o**	28 ± 2	> 10
12a	40 ± 4	> 10	**12p**	38 ± 9	> 10
12b	60 ± 9	-	**8**	23 ± 6	> 10
12c	100	1.33	**GA**	34 ± 3	> 10
12d	100 ± 5	1.06	**Juglone**	44 ± 7	10.81
12e	77 ± 7	-			

[a] Values are means of at least three independent experiments.

[b] All results represent the mean of duplicate experiments.

Pin1 directly targeted on the pThr-Pro motifs of cyclin D1 and stabilized cyclin D1 by preventing its nuclear export and ubiquitin-mediated degradation.[3] Thus to further demonstrate the Pin1 inhibitory effect of these compounds, the expression level of cyclin D1 protein was determined by western blot assay in PC-3 cells treated with **10a** at different concentrations for 24 h. The results (**Figure 2**) indicated that **10a** could efficiently decrease the level of cyclin D1 in a concentration-dependent manner, which might be related to the decreased activity of Pin1.

(**Figure 2**. should be listed here.)

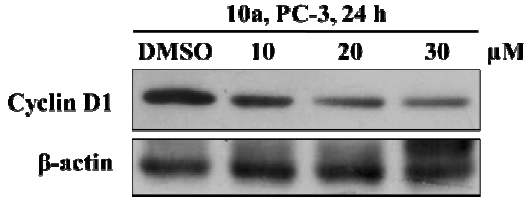


Figure 2. Compound **10a** modulated cyclin D1 level in PC-3.

To demonstrate the binding modes of **10a** and **12i**, molecular docking was carried out using the glide protocol in maestro software package (**Figure 3**). Docking studies revealed that these two compounds interacted with Pin1 in the C-terminal PPIase domain. The catalytic center of PPIase domain composed of two hydrophobic pockets and a basic cluster formed by Lys63, Arg68 and Arg69 residues.[13-15] Analysis of the docked complexes (**Figure 3**) suggested that **10a** and **12i** occupied the active pocket completely and demonstrated interactions with key residues as a result of which they exhibited a high binding affinity of -6.35 kcal/mol and -6.15 kcal/mol, respectively. The benzimidazole moiety of these compounds fit well into the prolyl pocket with a π–π stack between benzene ring and His157. The nitrogen atom on imidazole anchored with residue Ser154 via a crucial hydrogen bond. In addition, the carboxylate of propanoic acid could interacted with the positive charged side chain of Lys63 and Arg69 through salt bridge, and the carbonyl oxygen of carboxyl as a H-bond receptor formed a hydrogen bond with Ser114. Such interactions might have a great contribution to their remarkable Pin1 inhibitory activity. As shown in **Figure 3A**, the pentacyclic triterpenoid scaffold of **10a** showed hydrophobic and van der Waal interactions with the shallow hydrophobic shelf embraces His59, Ala117, Ala118, Leu122 and Ala124 residues, better yet, the carbonyl group at C11 position allowed the H-bond formation with residue Ser114. For compound **12i**, the pentacyclic triterpenoid scaffold connected with benzimidazole moiety at C30 carboxyl. In such circumstance, the acetyl group at C3 position was placed near residue Ala124, which was responsible for the formation of an additional hydrogen bond. By analyzing the events mentioned above, we drew the conclusion that both the benzimidazole moiety and pentacyclic triterpenoid scaffold were greatly helpful to enhance the Pin1 inhibition ability.

(**Figure 3**. should be listed here.)

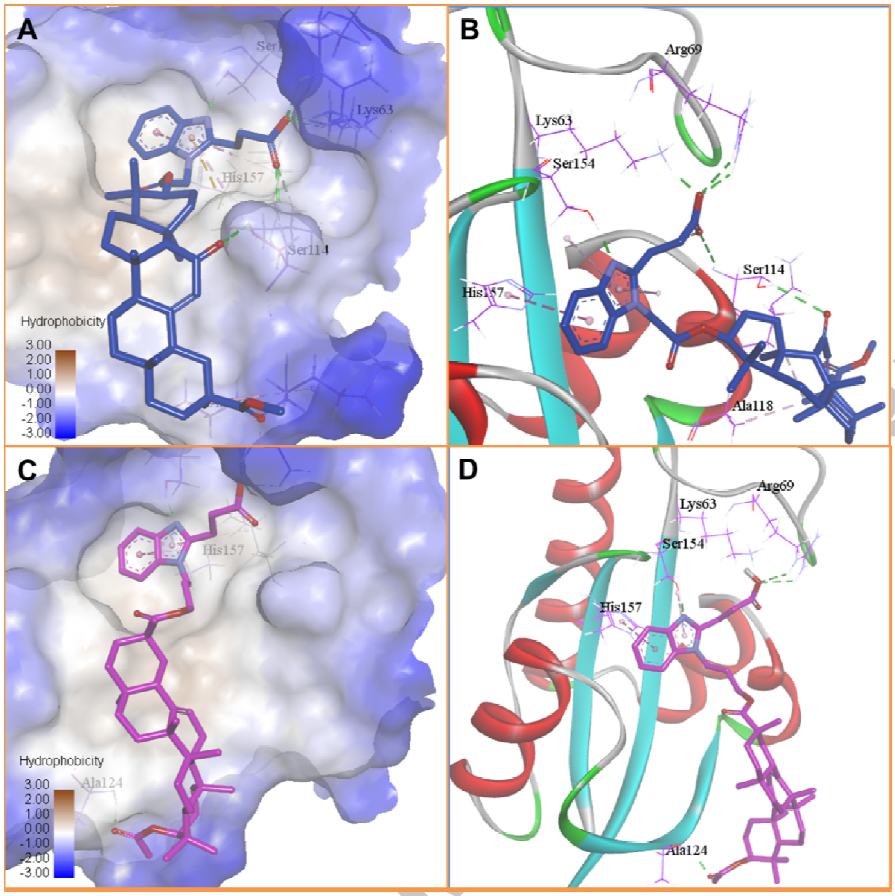


Figure 3. The predicated binding modes of **10a** (A, B) and **12i** (C, D) in the PPIase domain of Pin1 (3KAI in PDB). H-Bonding interactions were presented with green line.

3. Conclusion

We reported herein the discovery and synthesis of novel conjugates of 18β-glycyrrhetinic acid derivatives with 3-(1*H*-benzo[d]imidazol-2-yl)propanoic acid as potential Pin1 inhibitors. Several compounds were identified as promising candidates with both good Pin1 inhibitory activity and excellent anti-proliferative effect against prostate cancer PC-3 and LNCaP cell lines. Compounds **10a** and **12i** were found to be the most potent ones. SAR studies indicated that the structural modification of ring A and ring C together with the length of linker between **GA** skeleton and benzimidazole moiety had a notable influence on the anti-proliferative activity. Western blot assay revealed that **10a** could down-regulate the expression level of cell cycle-related protein cyclin D1 in a concentration-dependent manner, which further demonstrated the Pin1 inhibitory effect of these compounds. Molecular docking studies displayed that both two components of these conjugates were essential for receptor binding. This study provides further possibility to develop novel Pin1 inhibitors with anti-proliferative ability.

4. Experimental

4.1. Chemistry

18β-glycyrrhetinic acid (GA) was purchased from Haokang Chemicals Inc. (Shanghai China) with over 98% purity. The other reagents were obtained commercially and were used with further purification if necessary. The melting points (Mp) were determined on an electrically heated X4 digital visual melting point apparatus which were uncorrected. Mass spectra (MS) were determined on Finnigan MAT/USA spectrometer (LC-MS). Infrared (IR) spectra were recorded on a Bruker IR-27G spectrometer using KBr pellets. High-resolution mass spectra were measured with Bruker microOTOF-Q in ESI mode (HR-MS). Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were measured on Bruker AV-400 or ARX-600 MHz instrument. Chemical shifts were recorded in δ (ppm) with tetramethylsilane (TMS) as the internal standard. All reactions were monitored by thin-layer chromatography (TLC, carried on fluorescent precoated plates GF254, Qingdao Haiyang Chemical, China).

4.1.1. General procedure for the synthesis of 10a–10j

The preparations of key intermediates **5a–5i** were based on our previous research, and the yields of target compounds were quite similar to that reported before.[23]

To a solution of *o*-phenylenediamine **7** (46 mmol) in dioxane (110 mL) was added succinic anhydride (55.5 mmol) at room temperature. Then the mixture was stirred at 80 ^{o}C for 4 h. When the mixture was cooled to room temperature, the precipitate was filtered, washed with dioxane/water/aether, respectively. Finally the precipitate was dried to give **8** as white solid with 57% yield.

To a solution of **8** (5.26 mmol) in DMF (20 mL) was added K_2CO_3 (10 mmol). The mixture was stirred at room temperature for 1 h, then benzyl bromide (6.32 mmol) was added dropwise and stirred for 24 h. The reaction solution was poured into water (50 mL) and filtered. The precipitate was washed with water to PH 7 and dried to give **9** as white solid with 80% yield.

To a solution of **5a-5e** (2.1 mmol) in dry dichloromethane (60 mL), triethylamine or pyridine (4.2 mmol) was

added dropwise under ice-salt bath. Then 2-chloroacetyl chloride or 3-chloropropionyl chloride (3.2 mmol) was slowly added. The mixture was allowed to warm up to room temperature and stirred for 5 h. The reaction was quenched with water, and the water layer was separated, several times extracted with dichloromethane. The combined dichloromethane solutions were dried with Na_2SO_4, filtered and evaporated. The resulting residue was then purified by column chromatography on silica gel to give **6a–6j** as white solid with 70~80% yield.

To a solution of **8** (3 mmol) in dry acetonitrile (50 mL) was added Cs_2CO_3 (6 mmol). The mixture was stirred at room temperature for 1 h, then **6a–6j** (3 mmol) was added slowly. The solution was warmed up to 82 ^{o}C and stirred for additional 4 h. The solvent was evaporated to dryness and added water, extracted with ethyl acetate three times. The organic layers were combined, washed with brine, dried with Na_2SO_4, filtered and evaporated. The resulting residue was then purified by column chromatography on silica gel to give corresponding intermediates as white solid with 60~85% yield.

The corresponding intermediates (0.25 mmol) were dissolved in methanol (15 mL), and 5% Pd/C (20 mg) was added slowly. The resulting mixture was carefully evacuated, flooded with hydrogen three times, stirred under a hydrogen atmosphere at room temperature for 4 h. The reaction solution was filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to give **10a-10j** as white solid with 90~95% yield.

4.1.1.1. 3-(1-(2-((methyl 11-oxo-18*β*-olean-12-en-30-oate)-3*β*-oxy)-2-oxoethyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10a)

Yield: 91.4%; White solid; Mp: 175-176 ºC; IR (KBr): 3422.3, 2926.5, 1730.5, 1630.3, 1464.7, 1384.4, 1218.1, 1147.4, 1111.9 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H, -COOH), 7.36 (m, 4H, H-4', H-5', H-6', H-7'), 5.26 (m, 2H, H-13'), 5.24 (s, 1H, H-12), 4.45 (m, 1H, H-3), 3.62 (s, 3H, -COOC<u>H</u>$_3$), 3.01 (m, 2H, H-10'), 2.77 (m, 2H, H-11'), 2.38 (s, 1H, H-9), 2.31 (m, 1H), 1.99 (m, 1H), 1.33 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 1.10 (s, 2H), 1.08 (s, 2H), 1.02 (s, 6H), 0.87 (m, 2H), 0.74 (s, 1H), 0.71 (s,3H), 0.64 (s, 1H), 0.58 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 199.3 (C-11),

176.6 (C-30), 173.9 (C-12'), 170.0 (C-14'), 168.3 (C-13), 154.9 (C-2'), 142.4 (C-9'), 136.0 (C-8'), 127.7 (C-12), 122.1 (C-5'), 121.8 (C-6'), 118.8 (C-4'), 110.1 (C-7'), 81.8 (C-3), 61.1, 53.8, 52.1, 52.0, 48.3, 45.2, 45.1, 44.9, 43.9, 43.7, 43.3, 42.4, 40.8, 37.9, 36.8, 35.4, 31.9, 28.6, 28.0, 27.8, 26.4, 26.1, 23.3, 22.2, 20.9, 20.7, 18.6, 16.5, 16.4; LC-MS: 715.7 $[M+H]^+$, 713.6 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{59}N_2O_7$ $[M+H]^+$ 715.4322, found: 715.4324.

4.1.1.2. 3-(1-(2-((methyl 18*β*-olean-12-en-30-oate)-3*β*-oxy)-2-oxoethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (10b)

Yield: 90.0%; White solid; Mp: 125-126 ºC; IR (KBr): 3428.5, 2927.1, 2853.9, 1732.4, 1518.1, 1464.8, 1383.0, 1315.2, 1217.0, 1157.2, 1088.6, 1015.2 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.32 (s, 1H, -COOH), 7.56 (d, 1H, H-4'), 7.47 (d, 1H, H-7'), 7.17 (m, 2H, H-5', H-6'), 5.25 (m, 2H, H-13'), 5.17 (m, 1H, H-12), 4.45 (m, 1H, H-3), 3.61 (s, 3H, -COOC$\underline{H}_3$), 3.01 (m, 2H, H-10'), 2.80 (m, 2H, H-11'), 1.98 (m, 1H), 1.70 (s, 1H), 1.65 (m, 1H), 1.41 (s, 2H), 1.20 (s, 1H), 1.16 (s, 1H), 1.11 (s, 3H), 1.07 (s, 3H), 0.96 (m, 1H), 0.89 (s, 3H), 0.88 (s, 3H), 0.81 (s, 1H), 0.72 (s, 3H), 0.70 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 176.9 (C-30), 173.9 (C-12'), 168.3 (C-14'), 154.8 (C-2'), 144.6 (C-13), 142.4 (C-9'), 136.0 (C-8'), 122.2 (C-5'), 122.2 (C-6'), 121.8 (C-12), 118.8 (C-4'), 110.0 (C-7'), 82.0 (C-3), 54.5, 51.8, 48.2, 47.1, 44.9, 44.0, 42.7, 41.5, 40.4, 38.3, 37.9, 37.6, 36.7, 32.0, 31.1, 28.4, 28.3, 27.9, 26.7, 26.7, 26.1, 25.9, 23.4, 23.3, 22.1, 18.0, 16.8, 16.5, 15.5; LC-MS: 701.4 $[M+H]^+$, 699.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{61}N_2O_6$ $[M+H]^+$ 701.4530, found: 701.4537.

4.1.1.3. 3-(1-(2-((methyl 12-oxo-18*β*-olean-30-oate)-3*β*-oxy)-2-oxoethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (10c)

Yield: 95.2%; White solid; Mp: 122-123 ºC; IR (KBr): 3428.5, 2926.3, 2853.3, 1731.4, 1518.7, 1465.8, 1082.2, 1013.7 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.55 (d, 1H, H-4'), 7.46 (d, 1H, H-7'), 7.16 (m, 2H, H-5', H-6'), 5.23 (m, 2H, H-13'), 4.43 (t, 1H, H-3), 3.58 (s, 3H, -COOC$\underline{H}_3$), 3.00 (m, 2H, H-10'), 2.78 (m, 2H, H-11'), 2.72 (m, 1H), 2.24 (m, 1H), 2.01 (m, 1H), 1.87 (m, 1H), 1.39(s, 3H), 1.23 (s, 8H), 1.09 (s, 3H), 1.05 (s, 3H), 0.85 (s, 3H), 0.79 (s, 6H), 0.69 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 211.1 (C-12), 177.0 (C-30), 173.9 (C-12'), 168.2 (C-14'), 154.9

(C-2'), 142.4 (C-8'), 136.0 (C-9'), 122.1 (C-6'), 121.8 (C-5'), 118.8 (C-4'), 110.0 (C-7'), 81.8 (C-3), 54.1, 51.6, 49.6, 49.2, 44.9, 43.9, 42.0, 41.5, 40.4, 38.5, 37.7, 37.2, 36.6, 33.8, 32.1, 31.5, 31.3, 31.3, 31.0, 29.4, 28.7, 27.7, 27.3, 26.3, 25.9, 23.3, 22.2, 20.8, 18.1, 16.3, 16.0, 15.3; LC-MS: 717.5 $[M+H]^+$, 715.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{61}N_2O_7$ $[M+H]^+$ 717.4479, found: 717.4473.

4.1.1.4. 3-(1-(2-((methyl 12-oxo-18*β*-olean-9(11)-en-30-oate)-3*β*-oxy)-2-oxoethyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10d)

Yield: 94.7%; White solid; Mp: 128-130 ºC; IR (KBr): 3426.7, 2926.4, 1731.9, 1660.3, 1597.7, 1518.8, 1465.3, 1382.6, 1326.3, 1220.4, 1196.1, 1157.5, 1087.1 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.52 (m, 2H, H-4', H-7'), 7.18 (m, 2H, H-5', H-6'), 5.61 (s, 1H, H-11), 5.26 (m, 2H, H-13'), 4.45 (m, 1H, H-3), 3.62 (s, 3H, -COOC$\underline{H_3}$), 3.02 (m, 2H, H-10'), 2.87 (m, 1H), 2.80 (m, 2H, H-11'), 2.09 (m, 1H), 1.69 (m, 2H), 1.58 (m, 4H), 1.24 (s, 6H), 1.14 (s, 3H), 1.05 (s, 3H), 0.91 (s, 3H), 0.87 (s, 5H), 0.75 (s, 3H), 0.60 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 200.0 (C-12), 177.8 (C-30), 176.8 (C-12'), 173.8 (C-14'), 168.2 (C-9), 154.8 (C-2'), 142.1 (C-9'), 135.9 (C-8'), 122.5 (C-11), 122.3 (C-5'), 122.0 (C-6'), 118.7 (C-4'), 110.1 (C-7'), 81.2 (C-3), 51.7, 49.6, 47.6, 45.4, 44.9, 43.9, 41.8, 40.4, 38.5, 38.1, 37.9, 35.8, 33.5, 32.5, 32.0, 31.0, 30.9, 29.4, 28.6, 27.7, 27.4, 26.1, 24.1, 24.0, 23.7, 22.1, 21.9, 17.6, 16.5; LC-MS: 715.5 $[M+H]^+$, 713.3 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{59}N_2O_7$ $[M+H]^+$ 715.4322, found: 715.4317.

4.1.1.5. 3-(1-(2-((methyl 18*β*-olean-9(11),12-dien-30-oate)-3*β*-oxy)-2-oxoethyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10e)

Yield: 91.3%; White solid; Mp: 118-119 ºC; IR (KBr): 3430.9, 2948.1, 2855.8, 1731.9, 1518.8, 1464.9, 1379.6, 1216.2, 1158.2, 1107.2, 1086.8 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.56 (d, 1H, H-4'), 7.48 (d, 1H, H-7'), 7.17 (m, 2H, H-5', H-6'), 5.59 (d, 1H, H-11), 5.51 (d, 1H, H-12), 5.25 (m, 2H, H-13'), 4.46 (m, 1H, H-3), 3.63 (s, 3H, -COOC$\underline{H_3}$), 3.01 (m, 2H, H-10'), 2.79 (m, 2H, H-11'), 1.99 (m, 2H), 1.91 (m, 1H), 1.82 (m, 1H), 1.31 (m, 5H), 1.24 (s, 3H), 1.18 (m, 1H), 1.13 (s, 3H), 1.07 (s, 6H), 0.98 (s, 2H), 0.95 (s, 3H), 0.78 (s, 3H), 0.74 (s, 3H), 0.71 (s, 1H), 0.65 (s, 1H), 0.58 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 176.9 (C-30), 173.8 (C-12'), 168.3 (C-14'), 154.8 (C-9), 154.3 (C-2'), 146.5

(C-13), 142.3 (C-9'), 136.0 (C-8'), 122.2 (C-5'), 121.9 (C-6'), 121.1 (C-4'), 118.8 (C-12), 115.8 (C-11), 110.1 (C-7'), 81.8 (C-3), 51.9, 50.9, 46.4, 44.9, 44.0, 43.7, 42.6, 38.6, 38.2, 37.8, 36.6, 34.6, 31.6, 31.0, 29.4, 28.6, 28.2, 28.0, 27.0, 25.3, 24.0, 22.1, 20.9, 20.3, 19.9, 19.7, 18.0, 16.6; LC-MS: 699.5 $[M+H]^+$, 697.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{59}N_2O_6$ $[M+H]^+$ 699.4373, found: 699.4379.

4.1.1.6. 3-(1-(3-((methyl 11-oxo-18*β*-olean-12-en-30-oate)-3*β*-oxy)-3-oxopropyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10f)

Yield: 93.5%; White solid; Mp: 145-147 ºC; IR (KBr): 3429.3, 2925.6, 2854.1, 1729.1, 1661.8, 1462.8, 1385.3, 1327.2, 1214.7, 1191.1, 1113.3 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 7.52 (m, 2H, H-4', H-7'), 7.16 (m, 2H, H-2', H-5'), 5.37 (d, 1H, H-12), 4.46 (t, 2H, H-13'), 4.39 (m, 1H, H-3), 3.62 (s, 3H, -COOC$\underline{H}_3$), 3.07 (m, 2H, H-14'), 2.85 (m, 2H, H-10'), 2.78 (m, 2H, H-11'), 2.30 (m, 1H), 1.35 (s, 3H), 1.34 (s, 3H), 1.23 (s, 3H), 1.19(s, 2H), 1.10 (s, 2H), 1.07 (s, 2H), 1.02 (s, 6H), 0.83 (m, 2H), 0.74 (s, 1H), 0.71 (s, 3H), 0.66 (s, 3H), 0.64 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 199.3 (C-11), 178.4 (C-30), 176.7 (C-12'), 170.9 (C-15'), 170.0 (C-13), 154.5 (C-2'), 142.6 (C-9'), 135.1 (C-8'), 127.6 (C-12), 123.3 (C-5'), 121.9 (C-6'), 118.7 (C-4'), 110.4 (C-7'), 80.7 (C-3), 61.2, 59.9, 53.9, 52.0, 48.3, 45.2, 43.9, 43.7, 43.3, 42.4, 39.1, 37.8, 36.6, 35.4, 34.2, 31.9, 31.5, 28.6, 28.0, 27.9, 26.1, 23.3, 22.3, 20.9, 20.7, 18.5, 16.8, 16.7, 16.5, 16.0; LC-MS: 729.5 $[M+H]^+$, 727.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{61}N_2O_7$ $[M+H]^+$ 729.4479, found: 729.4421.

4.1.1.7. 3-(1-(3-((methyl 18*β*-olean-12-en-30-oate)-3*β*-oxy)-3-oxopropyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (10g)

Yield: 96.7%; White solid; Mp: 215-216 ºC; IR (KBr): 3431.6, 2949.6, 2924.7, 2853.8, 1728.6, 1631.4, 1512.5, 1462.9, 1384.0, 1216.8, 1187.5, 1155.7, 1088.6 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.51 (m, 2H, H-4', H-7'), 7.14 (m, 2H, H-5', H-6'), 5.32 (t, 1H, H-12), 4.45 (t, 2H, H-13'), 4.37 (m, 1H, H-3), 3.61 (s, 3H, -COOC$\underline{H}_3$), 3.06 (m, 2H, H-14'), 2.83 (m, 2H, H-10'), 2.77 (m, 2H, H-11'), 2.37 (m, 1H), 1.34 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H), 1.17(s, 2H), 1.09 (s, 1H), 1.06 (s, 2H), 1.00 (s, 6H), 0.73 (m, 1H), 0.69 (s, 3H), 0.65 (s, 3H), 0.63 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 177.0 (C-30), 174.0 (C-12'), 170.9 (C-15'), 154.3 (C-2'), 144.6 (C-13), 142.6 (C-9'), 135.2 (C-8'), 122.3 (C-5'), 122.0

(C-6'), 121.6 (C-12), 118.7 (C-4'), 110.4 (C-7'), 80.9 (C-3), 54.7, 51.9, 48.2, 47.1, 44.1, 42.7, 41.5, 39.1, 38.3, 38.0, 37.6, 36.7, 34.2, 32.3, 32.0, 31.1, 30.9, 29.4, 29.1, 28.4, 28.3, 27.9, 26.7, 26.1, 26.0, 23.3, 22.5, 22.2, 18.1, 16.9, 15.5; LC-MS: 715.5 $[M+H]^+$, 713.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{63}N_2O_6$ $[M+H]^+$ 715.4686, found: 715.4639.

4.1.1.8. 3-(1-(3-((methyl 12-oxo-18*β*-olean-30-oate)-3*β*-oxy)-3-oxopropyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (10h)

Yield: 93.3%; White solid; Mp: 136-137 ºC; IR (KBr): 3421.7, 2946.1, 2875.1, 1734.0, 1719.4, 1656.8, 1257.4, 1232.8, 1166.2, 1019.1 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.53 (m, 2H, H-4', H-7'), 7.16 (m, 2H, H-5', H-6'), 4.47 (t, 2H, H-13'), 4.37 (m, 1H, H-3), 3.59 (s, 3H, -COOC$\underline{H}_3$), 3.17 (s, 1H), 3.10 (m, 2H, H-14'), 2.86 (m, 2H, H-10'), 2.80 (m, 2H, H-11'), 2.73 (m, 1H), 2.25 (m, 1H), 2.02 (m, 1H), 1.89 (m, 1H), 1.78 (m, 1H), 1.72 (m, 1H), 1.62 (m, 2H), 1.50 (m, 2H), 1.41 (m, 4H), 1.33 (m, 2H), 1.24 (m, 5H), 1.10 (s, 3H), 1.07 (s, 3H), 0.93 (m, 2H), 0.87 (s, 3H), 0.81 (s, 3H), 0.80 (s, 3H), 0.71 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 211.2 (C-12), 177.0 (C-30), 174.1 (C-12'), 170.9 (C-15'), 154.3 (C-2'), 142.6 (C-8'), 135.1 (C-9'), 122.0 (C-6'), 121.6 (C-5'), 118.7 (C-4'), 110.4 (C-7'), 80.7 (C-3), 54.3, 51.6, 49.6, 49.2, 43.9, 42.1, 41.5, 40.4, 39.1, 38.7, 38.5, 37.6, 37.3, 36.6, 34.1, 33.8, 32.1, 31.6, 31.2, 31.0, 28.7, 27.4, 27.2, 26.3, 25.9, 23.3, 22.2, 20.8, 18.1, 16.6, 16.0, 15.3; LC-MS: 731.5 $[M+H]^+$, 729.3 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{63}N_2O_7$ $[M+H]^+$ 731.4635, found: 731.4634.

4.1.1.9. 3-(1-(3-((methyl 12-oxo-18*β*-olean-9(11)-en-30-oate)-3*β*-oxy)-3-oxopropyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10i)

Yield: 94.0%; White solid; Mp: 110-111 ºC; IR (KBr): 3429.0, 2927.3, 2852.1, 1730.4, 1661.3, 1513.2, 1463.6, 1383.0, 1220.9, 1192.1, 1088.1 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.53 (m, 2H, H-4', H-7'), 7.15 (m, 2H, H-5', H-6'), 5.60 (s, 1H, H-11), 4.47 (t, 2H, H-13'), 4.38 (m, 1H, H-3), 3.61 (s, 3H, -COOC$\underline{H}_3$), 3.10 (t, 2H, H-14'), 2.88 (m, 2H, H-10'), 2.81 (m, 2H, H-11'), 2.09 (m, 1H), 1.88 (m, 2H), 1.79 (m, 2H), 1.58 (m, 4H), 1.39 (s, 9H), 1.33 (s, 3H), 1.23 (m, 3H), 1.13 (s, 3H), 1.06 (s, 3H), 0.92 (s, 3H), 0.86 (s, 3H), 0.75 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 200.0 (C-12), 177.9 (C-30), 176.9 (C-12'), 174.0 (C-15', C-9), 170.9 (C-2'), 154.3 (C-9'), 135.0 (C-8'), 122.5 (C-11),

122.1 (C-5'), 121.8 (C-6'), 118.6 (C-4'), 110.5 (C-7'), 80.0 (C-3), 51.7, 49.7, 47.6, 45.4, 43.9, 41.8, 40.4, 39.2, 38.5, 38.1, 37.9, 35.9, 34.1, 33.5, 32.6, 32.1, 31.2, 30.9, 28.7, 27.8, 27.4, 26.1, 26.0, 24.1, 24.0, 23.7, 22.1, 21.9, 17.7, 16.8; LC-MS: 729.5 $[M+H]^+$, 727.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{61}N_2O_7$ $[M+H]^+$ 729.4479, found: 729.4468.

4.1.1.10. 3-(1-(3-((methyl 18*β*-olean-9(11),12-dien-30-oate)-3*β*-oxy)-3-oxopropyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (10j)

Yield: 92.1%; White solid; Mp: 154-155 ºC; IR (KBr): 3427.4, 2925.7, 2853.7, 1728.2, 1631.7, 1462.5, 1382.6, 1216.6, 1187.1, 1155.6 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.53 (m, 2H, H-4', H-7'), 7.16 (m, 2H, H-5', H-6'), 5.59 (d, 1H, H-11), 5.51 (d, 1H, H-12), 4.47 (t, 2H, H-13'), 4.38 (m, 1H, H-3), 3.63 (s, 3H, -COOC$\underline{H}_3$), 3.11 (t, 2H, H-14'), 2.87 (m, 2H, H-10'), 2.82 (m, 2H, H-11'), 1.12 (m, 2H), 1.13 (s, 3H), 1.08 (s, 3H), 1.07 (s, 3H), 1.04 (s, 1H), 0.99 (s, 1H), 0.96 (s, 3H), 0.82 (s, 1H), 0.78 (s, 3H), 0.73 (s, 3H), 0.70 (s, 3H), 0.67 (s, 1H), 0.65 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 176.9 (C-30), 174.0 (C-12'), 170.9 (C-15'), 154.4 (C-9), 154.3 (C-2'), 146.4 (C-13), 142.5 (C-9'), 135.1 (C-8'), 122.0 (C-5'), 121.7 (C-6'), 121.1 (C-4'), 118.7 (C-12), 115.7 (C-11), 110.4 (C-7'), 80.6 (C-3), 51.9, 51.0, 46.4, 44.0, 42.6, 39.1, 38.6, 38.2, 37.8, 36.8, 36.4, 34.6, 34.2, 31.6, 31.2, 30.8, 28.6, 28.1, 27.0, 25.5, 25.3, 23.9, 22.1, 20.9, 20.3, 19.9, 18.0, 16.9; LC-MS: 713.5 $[M+H]^+$, 711.2 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{61}N_2O_6$ $[M+H]^+$ 713.4530, found: 713.4531.

4.1.2. General procedure for the synthesis of 12a–12p

To a solution of **9** (3 mmol) in dry acetonitrile (30 mL) was added Cs_2CO_3 (4.5 mmol). The mixture was stirred at room temperature for 1 h, then 1,2-dibromoethane or 1,3-dibromopropane (9 mmol) was added slowly. The solution was warmed up to 82 ^oC and stirred for additional 4 h. The solvent was evaporated to dryness and added water, extracted with ethyl acetate three times. The organic layers were combined, washed with brine, dried with Na_2SO_4, filtered and evaporated. The resulting residue was then purified by column chromatography on silica gel to give **11a** and **11b** as colorless transparent oily liquid with 40~55% yield.

To a solution of **9** (3 mmol) in dry DMF (20 mL) was added sodium hydride (60% suspension in mineral oil, 6

mmol) in batches under ice-salt bath. The reaction mixture was allowed to warm up to room temperature and stirred for 0.5 h. Then added sodium hydride (6 mmol) in batches again followed by 2-chloroethylamine hydrochloride or 3-bromopropanamin hydrobromide (4.5 mmol) and the mixture was stirred at 80 ^{o}C for 1 h. Cooled to room temperature, and poured into water, extracted with dichloromethane five times. The organic layers were combined, washed with brine, dried with Na_2SO_4, filtered and evaporated. The resulting residue was then purified by column chromatography on silica gel to give **11c** and **11d** as colorless transparent oily liquid with 30~48% yield.

To a solution of **GA/5f/5g/2/5h/5i** (0.5 mmol) in dry DMF (20 mL) was added K_2CO_3 (1 mmol). The mixture was stirred at room temperature for 1 h, then **11a** or **11b** (0.5 mmol) was added and stirred for 4 h. The reaction solution was poured into water (50 mL), and filtered. The precipitate was washed with water to PH 7 and dried to give corresponding intermediates as white solid with 70~89% yield. The corresponding intermediates were debenzylated at the same reaction condition as making **10a–10j** to give **12a-12l** as white solid with 90~95% yield.

To a solution of **5f/5g/5h/5i** (0.5 mmol) in dry chloroform (25 mL) was added oxalyl chloride (2 mL). The mixture was stirred at room temperature for 2 h, and evaporated to dryness. The crude product as white solid was directly used in the next step without further purification. The corresponding acid chloride was dissolved in dry chloroform (20 mL), then the solution of **11c** or **11d** in dry chloroform was added dropwise under ice-salt bath. The mixture was warmed up to room temperature and stirred overnight. The solvent was evaporated to dryness, and the resulting residue was then purified by column chromatography on silica gel to give corresponding intermediates as white solid with 50~58% yield. The corresponding intermediates were debenzylated at the same reaction condition as making **10a–10j** to yield **12m–12p** as white solid with 90~95% yield.

4.1.2.1. 3-(1-(3-(3*β*-hydroxy-11-oxo-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12a)

Yield: 91.6%; White solid; Mp: 100-101 ºC; IR (KBr): 3403.3, 2925.9, 2870.9, 1730.0, 1656.9, 1522.0, 1464.5, 1384.9, 1246.1, 1030.3 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H, -COOH), 7.55 (d, 1H, H-4'), 7.48 (d, 1H,

H-7'), 7.16 (m, 2H, H-5', H-6'), 5.43 (s, 1H, H-12), 4.30 (t, 2H, H-13'), 4.12 (t, 2H, H-15'), 3.76 (m, 1H, -OH), 3.59 (m, 1H, H-3), 3.06 (t, 2H, H-10'), 2.84 (t, 2H, H-11'), 2.58 (m, 1H), 2.34 (s, 1H), 2.06 (m, 2H), 1.99 (m, 2H), 1.53 (m, 2H), 1.36 (s, 3H), 1.24 (s, 2H), 1.10 (s, 3H), 1.03 (s, 6H), 0.91 (s, 3H), 0.75 (s, 3H), 0.69 (m, 3H); LC-MS: 701.7 $[M+H]^+$, 699.4 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{61}N_2O_6$ $[M+H]^+$ 701.4530, found: 701.4517.

4.1.2.2. 3-(1-(2-(3*β*-hydroxy-11-oxo-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12b)

Yield: 93.6%; White solid; Mp: 143-144 ℃; IR (KBr): 3424.2, 2926.8, 1730.1, 1657.3, 1643.0, 1384.9, 1115.4 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, -COOH), 7.54 (m, 2H, H-4', H-7'), 7.15 (m, 2H, H-5', H-6'), 5.39 (s, 1H, H-12), 4.54 (m, 2H, H-13'), 4.42 (m, 1H, -OH), 4.30 (t, 2H, H-14'), 3.12 (t, 2H, H-10'), 3.02 (m, 1H, H-3), 2.84 (t, 2H, H-11'), 2.58 (m, 1H), 2.31 (s, 1H), 1.33 (s, 1H), 1.31(s, 3H), 1.24 (m, 1H), 1.12 (m, 1H), 1.03 (s, 3H), 1.01 (s, 3H), 0.91 (s, 3H), 0.85 (s, 3H), 0.69 (s, 3H), 0.62 (m, 3H); ^{13}C NMR (DMSO-d_6): δ 199.4 (C-11), 176.1 (C-30), 173.9 (C-12'), 169.5 (C-13), 154.4 (C-2'), 142.5 (C-8'), 135.6 (C-9'), 127.8 (C-12), 121.9 (C-5'), 121.6 (C-6'), 118.8 (C-4'), 110.4 (C-7'), 70.0 (C-3), 62.9, 61.5, 54.4, 48.0, 45.2, 43.8, 43.2, 41.8, 40.6, 40.4, 39.1, 38.9, 37.5, 37.0, 32.5, 31.8, 31.1, 30.4, 28.5, 28.4, 27.6, 27.3, 26.4, 26.0, 23.3, 22.3, 18.7, 17.5, 16.5, 16.4; LC-MS: 687.7 $[M+H]^+$, 685.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{42}H_{59}N_2O_6$ $[M+H]^+$ 687.4373, found: 687.4388.

4.1.2.3. 3-(1-(3-(3*β*-acetyloxy-11-oxo-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12c)

Yield: 93.1%; White solid; Mp: 125-126 ℃; IR (KBr): 3432.3, 2927.8, 2853.4, 1729.2, 1658.9, 1512.8, 1463.4, 1385.8, 1326.3, 1246.6, 1153.6, 1029.6 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (m, 1H, H-4'), 7.30 (m, 3H, H-5', H-6', H-7'), 5.79 (s, 1H, H-12), 4.50 (m, 1H, H-3), 4.30 (t, 2H, H-13'), 4.18 (t, 2H, H-15'), 3.21 (m, 2H, H-10'), 3.15 (m, 2H, H-11'), 2.75 (m, 1H), 2.37 (s, 1H), 2.05 (s, 3H, $C\underline{H}_3COO$-), 1.64 (m, 5H), 1.35 (s, 3H), 1.16 (s, 3H), 1.14 (s, 3H), 1.11 (s, 3H), 0.87 (s, 6H), 0.79 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 211.1 (C-11), 177.0 (C-30), 174.1 (C-12'), 170.8 ($CH_3\underline{C}OO$-), 154.5 (C-2'), 142.6 (C-13), 135.1 (C-9'), 129.9 (C-8'), 121.9 (C-5'), 121.6 (C-6'), 118.7 (C-12), 110.4

(C-7'), 80.7 (C-3), 54.3, 51.6, 49.6, 49.3, 43.9, 42.1, 41.5, 40.4, 39.1, 38.7, 38.5, 37.6, 37.3, 36.6, 34.2, 33.8, 32.1, 31.6, 31.0, 28.7, 27.7, 27.2, 26.3, 25.9, 23.3, 22.3, 20.8, 18.1, 16.6, 16.0, 15.3; LC-MS: 743.7 $[M+H]^+$, 741.7 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{45}H_{63}N_2O_7$ $[M+H]^+$ 743.4635, found: 743.4618.

4.1.2.4. 3-(1-(2-(3*β*-acetyloxy-11-oxo-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12d)

Yield: 95.7%; White solid; Mp: 114-115 ºC; IR (KBr): 3428.4, 2926.8, 2855.8, 1730.5, 1513.2, 1645.1, 1463.6, 1385.4, 1246.9, 1147.8, 1030.0 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, -COOH), 7.52 (m, 2H, H-4', H-7'), 7.13 (m, 2H, H-5', H-6'), 5.38 (s, 1H, H-12), 4.53 (m, 2H, H-13'), 4.41 (m, 2H, H-14'), 4.31 (m, 1H, H-3), 3.11 (t, 2H, H-10'), 2.82 (m, 2H, H-11'), 2.62 (m, 1H), 2.37 (s, 1H), 2.22 (m, 2H), 1.99 (s, 3H, C<u>H</u>$_3$COO-), 1.62 (m, 6H), 1.31 (s, 3H), 1.22 (m, 2H), 1.11 (m, 2H), 1.05 (s, 3H), 1.00 (s, 3H), 0.84 (s, 3H), 0.81 (s, 6H), 0.60 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 199.3 (C-11), 176.1 (C-30), 174.9 (C-12'), 173.9 (CH$_3$<u>C</u>OO-), 170.6 (C-13), 154.5 (C-2'), 135.5 (C-9'), 127.6 (C-8'), 122.0 (C-12), 121.8 (C-6'), 118.6 (C-5'), 110.6 (C-4'), 100.0 (C-7'), 80.1 (C-3), 67.5, 65.4, 62.9, 61.1, 60.3, 54.0, 51.8, 48.0, 45.2, 43.8, 43.2, 40.4, 37.9, 36.8, 31.8, 31.1, 30.7, 29.8, 28.4, 28.2, 28.0, 27.6, 26.3, 26.0, 23.3, 22.3, 21.3, 18.6, 16.9, 16.5; LC-MS: 729.7 $[M+H]^+$, 727.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{61}N_2O_7$ $[M+H]^+$ 729.4479, found: 729.4482.

4.1.2.5. 3-(1-(3-(3,11-dioxo-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12e)

Yield: 90.4%; White solid; Mp: 109-110 ºC; IR (KBr): 3427.3, 2927.0, 2853.0, 1727.8, 1705.1, 1658.2, 1511.3, 1461.8, 1280.3, 1247.7, 1210.6, 1154.0, 1087.0, 1032.2 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H, -COOH), 7.55 (d, 1H, H-4'), 7.49 (d, 1H, H-7'), 7.16 (m, 2H, H-5', H-6'), 5.48 (s, 1H, H-12), 4.31 (m, 2H, H-13'), 4.13 (m, 2H, H-15'), 3.07 (t, 2H, H-10'), 2.85 (t, 2H, H-11'), 2.70 (m, 1H), 2.42 (m, 1H), 1.48 (m, 4H), 1.38 (s, 3H), 1.24 (s, 2H), 1.15 (s, 3H), 1.11 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 216.2 (C-3), 198.9 (C-11), 176.0 (C-30), 173.9 (C-12'), 170.0 (C-13), 154.2 (C-2'), 142.5 (C-8'), 135.5 (C-9'), 127.7 (C-12), 121.9

(C-5'), 121.6 (C-6'), 118.9 (C-4'), 109.9 (C-7'), 68.6, 61.9, 60.6, 54.2, 48.4, 47.3, 45.1, 43.9, 43.4, 40.8, 39.3, 37.8, 36.5, 34.1, 31.9, 31.7, 31.1, 30.6, 29.0, 28.6, 28.0, 27.8, 26.5, 26.1, 23.3, 22.1, 21.3, 18.7, 18.4, 15.9; LC-MS: 699.8 $[M+H]^+$, 697.7 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{59}N_2O_6$ $[M+H]^+$ 699.4373, found: 699.4376.

4.1.2.6. 3-(1-(2-(3,11-dioxo-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12f)

Yield: 91.1%; White solid; Mp: 114-115 ℃; IR (KBr): 3426.3, 2926.7, 2855.3, 1729.4, 1704.9, 1657.6, 1462.3, 1384.7, 1210.1, 1150.7, 1086.6 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.22 (s, 1H, -COOH), 7.54 (m, 2H, H-4', H-7'), 7.15 (m, 2H, H-5', H-6'), 5.44 (s, 1H, H-12), 4.54 (m, 2H, H-13'), 4.38 (m, 2H, H-14'), 3.13 (t, 2H, H-10'), 2.84 (t, 2H, H-11'), 2.70 (m, 1H), 2.48 (s, 1H), 2.31 (m, 1H), 1.62 (s, 2H), 1.59 (s, 1H), 1.47 (m, 4H), 1.33 (s, 3H), 1.15 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H), 0.91 (m, 1H), 0.86 (s, 3H), 0.64 (s, 3H); LC-MS: 685.4 $[M+H]^+$, 683.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{42}H_{57}N_2O_6$ $[M+H]^+$ 685.4217, found: 685.4223.

4.1.2.7. 3-(1-(3-(3β-hydroxy-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12g)

Yield: 91.2%; White solid; Mp: 119-120 ℃; IR (KBr): 3435.3, 2926.9, 1728.9, 1615.7, 1512.2, 1463.3, 1383.4, 1312.1, 1212.4, 1152.0, 1030.1 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H, -COOH), 7.55 (d, 1H, H-4'), 7.48 (d, 1H, H-7'), 7.16 (m, 2H, H-5', H-6'), 5.19 (t, 1H, H-12), 4.30 (m, 3H, H-13', -OH), 4.11 (m, 2H, H-15'), 3.07 (t, 2H, H-10'), 3.01 (m, 1H, H-3), 2.85 (t, 2H, H-11'), 2.05 (m, 2H), 1.23 (s, 2H), 1.13 (s, 3H), 1.08 (s, 3H), 0.91 (s, 6H), 0.87 (s, 3H), 0.74 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 176.4 (C-30), 173.9 (C-12'), 154.2 (C-2'), 144.6 (C-13), 142.5 (C-9'), 135.4 (C-8'), 122.4 (C-12), 121.9 (C-5'), 121.6 (C-6'), 118.9 (C-4'), 109.9 (C-7'), 77.2 (C-3), 61.7, 55.1, 48.3, 47.4, 44.1, 42.6, 41.5, 40.4, 39.5, 38.8, 38.6, 38.3, 36.8, 32.6, 32.0, 31.0, 30.9, 29.0, 28.6, 28.4, 28.3, 27.4, 26.7, 26.1, 26.0, 23.4, 22.1, 18.3, 16.9, 16.4, 15.6; LC-MS: 687.8 $[M+H]^+$, 685.6 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{63}N_2O_5$ $[M+H]^+$ 687.4737, found: 687.4728.

4.1.2.8. 3-(1-(2-(3*β*-hydroxy-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12h)

Yield: 94.9%; White solid; Mp: 120-121 ℃; IR (KBr): 3428.2, 2926.6, 1731.4, 1664.0, 1383.5, 1331.1, 1311.4,

1211.1, 1149.5, 1085.5, 1030.6 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, -COOH), 7.54 (m, 2H, H-4', H-7'), 7.15 (m, 2H, H-5', H-6'), 4.89 (t, 1H, H-12), 4.53 (t, 2H, H-13'), 4.40 (m, 1H, -OH), 4.29 (m, 2H, H-14'), 3.13 (t, 2H, H-10'), 3.01 (m, 1H, H-3), 2.84 (t, 2H, H-11'), 1.40 (s, 1H), 1.24 (s, 2H), 1.05 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.68 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 176.4 (C-30), 174.0 (C-12'), 154.4 (C-2'), 144.2 (C-13), 142.5 (C-9'), 135.6 (C-8'), 122.4 (C-12), 121.9 (C-5'), 121.6 (C-6'), 118.7 (C-4'), 110.4 (C-7'), 77.2 (C-3), 62.9, 55.1, 48.0, 47.4, 43.9, 42.4, 41.9, 41.3, 38.8, 38.6, 38.1, 36.8, 32.5, 31.8, 31.1, 30.7, 28.6, 28.2, 28.0, 27.4, 26.7, 26.6, 26.0, 25.9, 23.3, 22.3, 18.4, 16.9, 16.4, 15.6; LC-MS: 673.8 $[M+H]^+$, 671.6 $[M-H]^-$; HR-MS: *m/z*, calcdfor $C_{42}H_{61}N_2O_5$ $[M+H]^+$ 673.4580, found: 673.4578.

4.1.2.9. 3-(1-(3-(3*β*-acetyloxy-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12i)

Yield: 90.4%; White solid; Mp: 212-213 ºC; IR (KBr): 3418.6, 2949.0, 2924.0, 1729.9, 1618.6, 1465.0, 1383.0, 1248.7, 1252.2, 1085.4, 1026.8 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 12.10 (s, 1H, -COOH), 7.55 (d, 1H, H-4'), 7.48 (d, 1H, H-7'), 7.16 (m, 2H, H-5', H-6'), 5.18 (t, 1H, H-12), 4.41 (m, 1H, H-3), 4.29 (m, 2H, H-13'), 4.09 (m, 2H, H-15'), 3.07 (t, 2H, H-10'), 2.85 (m, 2H, H-11'), 2.05 (m, 2H), 2.00 (s, 3H, $C\underline{H_3}COO$-), 1.14 (s, 3H), 1.08 (s, 3H), 0.90 (s, 6H), 0.83 (s, 3H), 0.82 (s, 3H), 0.73 (s, 3H); LC-MS: 729.6 $[M+H]^+$, 727.3 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{45}H_{65}N_2O_6$ $[M+H]^+$ 729.4843, found: 729.4829.

4.1.2.10. 3-(1-(2-(3*β*-acetyloxy-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12j)

Yield: 93.7%; White solid; Mp: 168-169 ºC; IR (KBr): 3427.9, 2948.7, 1731.2, 1629.4, 1517.1, 1484.5, 1465.2, 1382.8, 1313.5, 1248.8, 1210.1, 1027.2 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 7.95 (m, 1H, H-4'), 7.75 (m, 1H, H-7'), 7.51 (m, 2H, H-5', H-6'), 4.84 (m, 2H, H-13'), 4.81 (t, 1H, H-12), 4.50 (m, 1H, H-3), 4.37 (m, 2H, H-14'), 3.41 (t, 2H, H-10'), 3.30 (t, 2H, H-11'), 2.00 (s, 3H,$C\underline{H_3}COO$-), 1.04 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.82 (s, 6H), 0.58 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 176.6 (C-30), 172.9 (C-12'), 171.0 ($CH_3\underline{C}OO$-), 152.8 (C-2'), 144.0 (C-13),

131.5 (C-9'), 130.5 (C-8'), 126.5 (C-5'), 126.0 (C-6'), 122.6 (C-4'), 115.2 (C-12), 111.9 (C-7'), 80.9 (C-3), 61.4, 55.2, 48.0, 47.4, 44.1, 43.9, 42.5, 41.4, 39.7, 38.7, 38.2, 37.6, 36.7, 32.5, 31.8, 31.7, 31.0, 28.2, 28.0, 27.9, 26.7, 25.8, 23.5, 23.4, 21.2, 20.9, 18.1, 16.6, 16.5, 15.5, 15.4; LC-MS: 715.7 $[M+H]^+$, 713.6 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{63}N_2O_6$ $[M+H]^+$ 715.4686, found: 715.4687.

4.1.2.11. 3-(1-(3-(3-oxo-18*β*-olean-12-en-30-methanoyl)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12k)

Yield: 94.8%; White solid; Mp: 93-94 ºC; IR (KBr): 3427.4, 2926.2, 2855.3, 1728.3, 1704.9, 1512.2, 1461.3, 1383.2, 1311.6, 1212.9, 1155.3, 1085.9 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, -COOH), 7.55 (d, 1H, H-4'), 7.49 (d, 1H, H-7'), 7.17 (m, 2H, H-5', H-6'), 5.21 (t, 1H, H-12), 4.30 (t, 2H, H-13'), 4.11 (m, 2H, H-15'), 3.08 (t, 2H, H-10'), 2.86 (t, 2H, H-11'), 2.30 (m, 1H), 2.06 (m, 2H), 1.24 (s, 2H), 1.17 (s, 2H), 1.15 (s, 3H), 1.09 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 216.5 (C-3), 176.4 (C-30), 173.9 (C-12'), 154.2 (C-2'), 144.6 (C-13), 142.5 (C-9'), 135.4 (C-8'), 122.3 (C-12), 122.0 (C-5'), 121.6 (C-6'), 118.9 (C-4'), 110.0 (C-7'), 61.7, 54.7, 48.4, 47.0, 46.5, 44.1, 42.6, 41.6, 40.4, 38.9, 38.3, 36.5, 34.1, 32.1, 32.0, 31.0, 30.9, 29.0, 28.4, 28.3, 26.7, 26.6, 26.1, 25.9, 23.5, 22.1, 21.5, 19.5, 19.0, 16.7, 15.2; LC-MS: 685.8 $[M+H]^+$, 683.6 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{61}N_2O_5$ $[M+H]^+$ 685.4580, found: 685.4545.

4.1.2.12. 3-(1-(2-(3-oxo-18*β*-olean-12-en-30-methanoyl)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12l)

Yield: 91.9%; White solid; Mp: 97-98 ºC; IR (KBr): 3428.8, 2925.9, 2854.6, 1730.4, 1704.1, 1616.1, 1514.2, 1462.7, 1383.4, 1210.8, 1085.6, 1009.8 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, -COOH), 7.55 (m, 1H, H-4', H-7'), 7.16 (m, 2H, H-5', H-6'), 4.91 (t, 1H, H-12), 4.54 (t, 2H, H-13'), 4.31 (m, 2H, H-14'), 3.14 (t, 2H, H-10'), 2.84 (t, 2H, H-11'), 2.32 (m, 1H), 1.84 (m, 4H), 1.24 (s, 2H), 1.07 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.60 (s, 3H); LC-MS: 671.7 $[M+H]^+$, 669.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{42}H_{59}N_2O_5$ $[M+H]^+$ 671.4424, found: 671.4435.

4.1.2.13. 3-(1-(3-(3*β*-acetyloxy-11-oxo-18*β*-olean-12-en-30-carboxamido)propyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (12m)

Yield: 95.3%; White solid; Mp: 140-141 ℃; IR (KBr): 3427.8, 2869.5, 1728.4, 1653.1, 1461.7, 1385.2, 1330.7, 1281.5, 1258.4, 1211.6, 1169.5, 1085.5, 1038.0 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.67 (t, 1H, -CONH-), 7.52 (d, 1H, H-4'), 7.47 (d, 1H, H-7'), 7.14 (m, 2H, H-5', H-6'), 5.51 (s, 1H, H-12), 4.41 (m, 1H, H-3), 4.18 (m, 2H, H-13'), 3.18 (m, 2H, H-15'), 3.08 (m, 2H, H-10'), 2.91 (m, 2H, H-11'), 2.63 (m, 1H), 2.38 (s, 1H), 1.99 (s, CH_3COO-), 1.87 (m, 4H), 1.74 (m, 1H), 1.64 (m, 4H), 1.38 (s, 3H), 1.36 (s, 3H), 1.16 (s, 2H), 1.14 (s, 2H),1.12 (s, 2H), 1.05 (s, 3H), 1.03 (s, 3H), 1.02 (s, 3H), 0.81 (s, 3H), 0.71 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 199.4 (C-11), 175.7 (C-30), 173.9 (C-12', CH_3COO-), 170.5 (C-13), 170.2 (C-2'), 154.1 (C-9'), 135.3 (C-8'), 127.8 (C-12), 122.0 (C-5'), 121.7 (C-6'), 118.7 (C-4'), 110.1 (C-7'), 80.1 (C-3), 68.6, 61.3, 54.1, 48.2, 45.2, 43.3, 41.3, 41.0, 40.4, 38.2, 37.9, 37.7, 36.8, 36.7, 32.3, 31.8, 30.9, 30.0, 29.1, 28.0, 27.8, 26.4, 26.3, 23.6, 23.4, 22.1, 21.4, 18.7, 17.3, 16.9, 16.5; LC-MS: 742.6 $[M+H]^+$, 740.4 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{45}H_{64}N_3O_6$ $[M+H]^+$ 742.4795, found: 742.4768.

4.1.2.14. 3-(1-(2-(3*β*-acetyloxy-11-oxo-18*β*-olean-12-en-30-carboxamido)ethyl)-1*H*-benzo[d]imidazol-2-yl) propanoic acid (12n)

Yield: 92.4%; White solid; Mp: 164-165 ℃; IR (KBr): 3418.5, 2926.0, 2855.6, 1731.1, 1658.4, 1516.6, 1464.5, 1384.9, 1246.5, 1116.2, 1029.9 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.22 (s, 1H, -COOH), 7.77 (t, 1H, -CONH-), 7.50 (m, 2H, H-4', H-7'), 7.14 (m, 2H, H-5', H-6'), 5.47 (s, 1H, H-12), 4.41 (m, 1H, H-3), 4.21 (t, 2H, H-13'), 3.44 (m, 2H, H-14'), 3.08 (m, 2H, H-10'), 2.84 (m, 2H, H-11'), 2.63 (m, 1H), 2.37 (s, 1H), 1.99 (s, CH_3COO-), 1.71 (m, 1H), 1.57 (m, 1H), 1.48 (m, 2H), 1.38 (s, 3H), 1.34 (s, 1H), 1.33 (s, 3H), 1.22 (s, 2H), 1.08 (s, 2H), 1.06 (s, 3H), 1.02 (s, 3H), 0.89 (s, 3H), 0.81 (s, 6H), 0.66 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 199.4 (C-11), 176.3 (C-30), 172.4 (C-12'), 170.6 (CH_3COO-), 170.2 (C-13), 154.0 (C-2'), 135.5 (C-9'), 129.4 (C-8'), 127.8 (C-12), 122.3 (C-5'), 122.0 (C-6'), 118.4 (C-4'), 110.5 (C-7'), 80.1 (C-3), 61.2, 60.4, 54.1, 47.9, 45.2, 43.3, 41.0, 40.4, 38.6, 37.9, 36.8, 32.3, 31.7, 31.1, 30.7, 28.9, 28.6, 28.0, 26.4, 26.2, 23.6, 23.4, 22.1, 21.3, 18.6, 17.4, 17.3, 16.9, 16.5, 14.4; LC-MS: 728.8 $[M+H]^+$, 726.7 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{44}H_{62}N_3O_6$ $[M+H]^+$ 728.4639, found: 728.4618.

4.1.2.15. 3-(1-(3-(3,11-dioxo-18*β*-olean-12-en-30-carboxamido)propyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid

(12o)

Yield: 92.7%; White solid; Mp: 136-137 ºC; IR (KBr): 3420.8, 2926.5, 2868.5, 1704.7, 1656.7, 1518.2, 1462.2, 1385.1, 1328.0, 1259.4, 1167.9, 1113.2, 1012.3 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H, -COOH), 7.71 (t, 1H, -CONH-), 7.54 (d, 1H, H-4'), 7.48 (d, 1H, H-7'), 7.16 (m, 2H, H-5', H-6'), 5.56 (s, 1H, H-12), 4.20 (t, 2H, H-13'), 3.19 (m, 2H, H-15'), 3.06 (m, 2H, H-10'), 2.84 (m, 2H, H-11'), 2.72 (m, 1H), 2.30 (s, 1H), 2.11 (m, 2H), 1.86 (m, 4H), 1.63 (m, 2H), 1.40 (m, 4H), 1.38 (s, 3H), 1.24 (s, 2H), 1.15 (s, 3H), 1.07 (s, 3H), 1.05 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H), 0.75 (s, 3H); LC-MS: 698.7 $[M+H]^+$, 696.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{43}H_{60}N_3O_5$ $[M+H]^+$ 698.4533, found: 698.4531.

4.1.2.16. 3-(1-(2-(3,11-dioxo-18*β*-olean-12-en-30-carboxamido)ethyl)-1*H*-benzo[d]imidazol-2-yl)propanoic acid (12p)

Yield: 93.3%; White solid; Mp: 146-147 ºC; IR (KBr): 3419.2, 2926.1, 2868.8, 1705.1, 1657.4, 1516.9, 1463.0, 1385.2, 1329.0, 1248.7, 1115.2 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.97 (t, 1H, -CONH-), 7.48 (m, 2H, H-4', H-7'), 7.11 (m, 2H, H-5', H-6'), 5.52 (s, 1H, H-12), 4.23 (t, 2H, H-13'), 3.38 (m, 2H, H-14'), 2.99 (m, 2H, H-10'), 2.70 (m, 2H, H-11'), 2.28 (m, 2H), 1.33 (s, 3H), 1.13 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.82 (m, 2H), 0.67 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 216.3 (C-3), 198.9 (C-11), 176.2 (C-30), 174.0 (C-12'), 170.5 (C-13), 154.3 (C-2'), 142.5 (C-9'), 135.8 (C-8'), 127.8 (C-12), 121.9 (C-5'), 121.5 (C-6'), 118.8 (C-4'), 110.2 (C-7'), 60.6, 54.2, 47.9, 47.4, 45.0, 43.4, 43.3, 42.4, 41.0, 40.4, 39.3, 38.6, 37.5, 36.5, 34.0, 31.7, 31.2, 30.7, 28.9, 28.7, 26.6, 26.5, 26.3, 23.4, 22.3, 21.3, 18.7, 18.4, 15.9; LC-MS: 684.7 $[M+H]^+$, 682.5 $[M-H]^-$; HR-MS: *m/z*, calcd for $C_{42}H_{58}N_3O_5$ $[M+H]^+$ 684.4376, found: 684.4381.

4.2. Biological assays

4.2.1. Anti-proliferative assays [21]

The human prostatic cancer PC-3 cell lines were cultured in RPMI1640 with 10% (v/v) heat-inactivated fetal bovine serum. The anti-proliferative activities of semi-synthetic compounds were evaluated by the methyl

thiazolyltetrazolium bromide (MTT) assay. Cells (2×10^4 cells/well) were incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h, then various concentrations of each compound mixed in 100 μL medium were added to each well. After incubated for another 96 h, MTT solution (50 μL of 2 mg/mL) was added to each well and incubated for an additional 4 h. The medium was removed by aspiration and the cells were dissolved in 200 μL DMSO. The absorbance at 570 nm was measured in the 96-well plate reader. Growth inhibition was calculated and expressed as the ratio of the cell number in the treated group to that of the untreated group. The GI_{50} values were calculated according to inhibition ratios. All experiments were performed three times independently, and the results were reported presented as mean.

4.2.2. Pin1 PPIase assay [20]

PPIase inhibitory activities were measured at 10 ºC by protease-coupled assay as reported before. Suc-Ala-Glu-cis-Pro-Phe-4-nitroanilide (Bachem) in 0.47 mol/L LiCl/trifluoroethanol was used as the substrate. The assay buffer (860 μL of 35 mM HEPES at pH 7.8), Pin1 (20 μL, Bought from Sino Biological Inc.) and target compounds (10 μL of varying concentrations in DMSO) were pre-equilibrated in the cuvette at 10 °C for 30 min. Then, 150 μL of ice-cooled chymotrypsin (60 mg/mL in 0.001 M HCl) was added and mixed immediately. Additional substrate (40 μL) was added to start the assay and the reaction was monitored by absorbance at 390 nm for 90 s. The data was analyzed by Graphpad Prism 6.0.

4.2.3. Western blot analysis

The human prostatic cancer PC-3 cells were incubated in presence of **10a** at 10, 20, 30 μM or DMSO for 24 h, harvested, and rinsed with ice-cold PBS. Total protein extracts (40 μg) were prepared with RIPA lysis buffer [50 mMTris-HCl, 150 mMNaCl, 0.1% sodium dodecyl sulfate (SDS), 1% NP-40, 0.5% sodium deoxycholate, 1 mMphenylmethylsulfonyl fluoride (PMSF), 100 μMleupeptin, and 2 μg/mL aprotinin (pH 8.0)]. Samples were loaded on 10% SDS PAGE and transferred to nitrocellulose membranes. The membranes were stained with 0.2% Ponceau S red to assure equal protein loading and transfer. After blocking with 5% nonfat milk, the membranes were incubated with a specific antibody of cyclin D1 and β-actin overnight at 4 °C. Then they were incubated with secondary antibodies at 37 °C for 1 h. Finally, detection was performed with an improved ECL reagent.

4.3. Molecular modeling

We performed a molecule docking using glide protocol in maestro software package based on crystal structure of Pin1 (PDB code 3KAI). The crystal structure was retrieved from the Protein Data Bank. All of the water molecules and other subunits were removed and hydrogen atoms were added. The 3D structures of **10a** and **12i** were constructed using the Sketch Molecule module in maestro molecular modeling software package and subjected to energy minimization. After the structures of ligands and protein were prepared, molecular docking was performed with glide protocol. Default search parameters were used. The docking mode was chosen on the basis of docking scores.

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Legends:

Figure 1. The structures of natural products as Pin1 inhibitors.

Figure 2. Compound **10a** modulated cyclin D1 level in PC-3.

Figure 3. The predicated binding modes of **10a** (A, B) and **12i** (C, D) in the PPIase domain of Pin1 (3KAI in PDB). H-Bonding interactions were presented with green line.

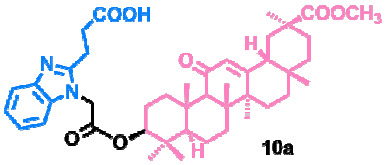
Scheme 1. Synthesis of **5a–5i**. Reagents and Conditions: (a) Concentrated H_2SO_4, methanol, 66 ^oC; (b) Zn-Hg, HCl, 1,4-dioxane, 10 ^oC; (c) $NaBH_4$, $NaOH_{(aq.)}$, THF, 66 ^oC; (d) Ac_2O, pyridine, 115 ^oC; (e) 30% H_2O_2, AcOH, 100 ^oC; (f) KOH, methanol, 66 ^oC; (g) Br_2, HBr, acetic acid, 40 ^oC; (h) Jones' reagent, acetone, r.t..

Scheme 2. Synthesis of **10a–10j**. Reagents and Conditions: (a) 2-chloroacetyl chloride or 3-chloropropionyl chloride, triethylamine or pyridine, CH_2Cl_2, r.t.; (b) succinic anhydride, dioxane, reflux; (c) BnBr, K_2CO_3, DMF, r.t.; (d) Cs_2CO_3, acetonitrile, 80 ^oC; (e) H_2, 5% Pd/C, methanol, r.t. or $TiCl_4$, CH_2Cl_2, r.t..

Scheme 3. Synthesis of **12a–12p**. Reagents and Conditions: (a) 1,2-dibromoethane or 1,3-dibromopropane, Cs_2CO_3, acetonitrile, 80 ^oC; (b) 2-chloroethylamine hydrochloride or 3-bromopropanamin hydrobromide, NaH, DMF, 80 ^oC; (c) K_2CO_3, DMF, r.t.; (d) $SOCl_2$, CH_2Cl_2, r.t.; (e) H_2, 5% Pd/C, methanol, r.t. or $TiCl_4$, CH_2Cl_2, r.t..

Table 1. The anti-proliferative activities of target compounds against PC-3 and LNCaP cell lines.

Table 2. The Pin1 inhibitory activities of target compounds.



10a

PC-3 Cell: GI_{50} = 7.80 μM

Pin1 Inhibition: IC_{50} = 1.07 μM