SYNTHESIS AND BIOLOGICAL EVALUATION OF OLLEANOLIC ACID DERIVATIVES AS NOVEL INHIBITORS OF PROTEIN TYROSINE PHOSPHATASE 1B

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Abstract – A series of oleanolic acid (OA) derivatives have been synthesized and their inhibitory effects on PTP1B, TCPTP and related PTPs are evaluated. Some compounds with five-membered heterocyclic ring-fused at C-2, C-3 positions showed a dramatic increase in inhibition, the two most potent PTP1B inhibitors 19 (IC_{50} = 0.91 μM) and 21 (IC_{50} = 0.98 μM) showed about 3-fold more potent than lead compound OA. Some C-ring modified OA analogs showed high selectivity for PTP1B over TCPTP, among them, 50 possessed the best selectivity of 6.6-fold.

INTRODUCTION

Protein tyrosine phosphatases (PTPs) are expressed in insulin-sensitive tissues, which can function as negative modulators in insulin signal transduction by dephosphorylation of tyrosyl residues. 1-4 Protein tyrosine phosphatase 1B (PTP1B), a prototypical member of the protein tyrosine phosphate superfamily, is a key negative regulator of both insulin and leptin signaling pathway by dephosphorylating the insulin receptor (IR), 5 insulin receptor substrates (IRS) 6 and Janus kinase 2 (JAK2). 7,8 Previous studies have
reported that PTP1B knockout mice not only display increased insulin sensitivity in liver and muscle tissues but also are resistant to high fat diet-induced obesity.\textsuperscript{7-9,11} PTP1B antisense oligonucleotide treatment can modulate obesity related fat storage and lipogenesis in adipose, and improve insulin sensitivity in animal models of type 2 diabetes.\textsuperscript{12,13} These investigations suggest that inhibition of PTP1B may anti-type 2 diabetes by increasing insulin sensitivity\textsuperscript{9} and resistance in obesity.\textsuperscript{10} In recent years, PTP1B inhibitors are regarded as agents in the treatment of type 2 diabetes and obesity.\textsuperscript{14,15} A series of synthetic inhibitors with submicro-, even nano-molar activity were discovered, however, few was further development to clinical trials, mainly for two reasons. Firstly, the poor bioavailability is an important issue, because of most active site-directed inhibitors reported to date possess a high charge density by mimicking the phosphate group in IRS.\textsuperscript{16} Secondly, the low selectivity between PTP1B and the most homogeneous T-cell protein tyrosine phosphatase (TCPTP) is another important issue, because of all PTPs share a high degree of structural conservation in the active site.\textsuperscript{17}

Natural products play a major role in drug discovery, about 60% of anticancer and 75% of anti-infective drugs approved from 1981-2002 could be traced to natural origins.\textsuperscript{18} In searching for novel PTP1B inhibitors that derived from natural products, researchers have found trodusquemine (is currently conducting a phase I clinical challenge by Genaera\textsuperscript{19}) and pentacyclic triterpenoids,\textsuperscript{20,21} including oleanolic acid (OA) and its derivatives.\textsuperscript{22} It is well-known that pentacyclic triterpenes are too hydrophobic to have acceptable water solubility and related pharmacokinetic properties. Initial SAR studies for analogs of OA with modified various substituents on C-3 and C-28 positions indicated a strong preference for the hydrophobic group and these derivatives had no obvious selectivity for PTP1B over TCPTP (with 77% sequences identity with PTP1B). Our previous study was focused on improving water solubility, increasing inhibitory activity and selectivity between the two homogeneous enzymes by introducing a series of heterocyclic rings at C-2 and C-3 positions of OA.\textsuperscript{23} According to our previous study, the inhibition of the five-membered heterocyclic ring-fused OA derivatives is more potent than the six-membered analogs. Therefore, we herein reported a subsequent work of our previous research. We further introduced five-membered hydrophilic heterocyclic rings at C-2 and C-3 positions and modified the C-ring of OA respectively. All of these OA derivatives were evaluated in the enzyme inhibition assay against PTP1B and TCPTP, and selected compounds of the series were evaluated in related PTPs, such as SHP-1, SHP-2 and LAR.

\textbf{RESULTS AND DISCUSSION}

\textbf{1. Chemistry.} Benzylation of OA, followed by IBX oxidation to give 2. Reaction of 2 with isoamyl nitrite in the presence of t-BuOK in t-BuOH furnished 3.\textsuperscript{24} Compound 3 was treated with hydroxylamine
hydrochloride in refluxing pyridine to give 4. Cyclization of 4 was performed by heating in the presence of KOH to give 5. Compound 6 was produced by debenzylation of 5 over palladium/carbon in MeOH with high yield. Reaction of 3 with zinc powder in AcOH and Ac₂O produced 7. Compound 8 was afforded by reaction of 7 with phosphorus oxychloride in dry pyridine under nitrogen atmosphere. Compound 9 was obtained by debenzylation of 8 with the same procedure as preparation of 6, as shown in scheme 1.

Scheme 1

a) BnBr, K₂CO₃, DMF, rt, 12 h, 92%. b) IBX, DMSO, THF, rt, 6 h, 85%. c) isoamyl nitrite, t-BuOK, t-BuOH, rt, 5 h, 86%. d) hydroxylamine hydrochloride, pyridine, reflux, 5 h, 75%. e) KOH, ethylene glycol, dioxane, reflux, 5 h, 60%. f) 10% Pd/C, H₂, MeOH, rt, 12 h, 92% for 6, 82% for 9. g) Zn dust, AcOH, Ac₂O, rt, 12 h, 86%, h) POCl₃, pyridine, rt, 12 h, 75%.

Compound 10 was produced by Oxidation of OA with IBX. Treatment of 10 with various acylation reagents afforded acylated intermediates 11, 12, 13, 14. Compound 15 was afforded by treatment of 11 with hydroxylamine hydrochloride in refluxing pyridine. Reaction of 12 with hydrazine hydrate in acetic acid produced 16. Compounds 17 and 18 were obtained by treatment of 13 with hydrazine hydrate or hydroxylamine hydrochloride respectively. Compounds 19 and 21 were obtained by cyclization of 14 with the same procedure as preparation of 17 and 18. Compounds 20 and 22 were synthesized by
hydrolysis of 19 and 21 in the presence of NaOH in THF/MeOH, as shown in scheme 2.

Scheme 2

a) IBX, DMSO, THF, rt, 5 h, 90%. b) RCO₂Et (R = CF₃, OEt, CO₂Et), NaH, THF, rt, 12 h or HCO₂Et, MeONa, toluene, rt, 1 h, 70% for 11, 78% for 12, 76% for 13, 78% for 14. c) hydroxylamine hydrochloride, pyridine, reflux, 5 h, 74%. d) 85% hydrazine hydrate, AcOH, reflux, 5 h, 81%. e) 85% hydrazine hydrate, EtOH, reflux, 5 h, 67%. f) hydroxylamine hydrochloride, AcONa, EtOH, reflux, 24 h, 68%. g) hydroxylamine hydrochloride, EtOH, reflux, 5 h. h) 2 M NaOH, THF, MeOH, reflux, 5 h.

Acetylation of 1 followed by oxidation with PDC produced 24. Compound 25 was generated by removal of acetyl group of 24 in the presence of NaOH in MeOH/THF and benzyl group of 24 in the presence of Pd/C respectively. The intermediate 26 was synthesized by reaction of 23 with mCPBA. Compound 27 was obtained by removal of acetyl group and benzyl group of 26 with the same procedure as preparation of 25. Compound 29 was afforded by dehydrogenation of 26 in the presence of Br₂ and HBr in HOAc, then removal of acetyl and benzyl group. Compound 26 reacted with hydroxylamine hydrochloride in pyridine to produce 30. Compound 31 was prepared by deprotection of
acetyl group and benzyl group of 30. Compound 33 was generated by Beckmann rearrangement of 30, then deprotection of acetyl group and benzyl group, as shown in scheme 3.

Scheme 3

a) Ac₂O, DMAP, pyridine, rt, 12 h, 90%. b) pyridinium dichromate, DCM, rt, 3 h, 50%. c) 1. 2 M NaOH, THF, MeOH, rt, 12 h; 2. 10% Pd/C, MeOH, rt, 12 h, 68-80% (over two steps). d) mCPBA, CHCl₃, rt, 24 h, 60%. e) HBr, Br₂, AcOH, 60 °C, 78%. f) hydroxylamine hydrochloride, pyridine, reflux, 5 h. g) SOCl₂, THF, rt, 12 h, 86%.

Compound 1 was protected by TBS group and then reacted with mCPBA to produce 35. Reduction of carbonyl goup of 35 with NaBH₄ yielded 36 and 37; the ratio is 2/3. We also found although 37 could be methylation or acylation, 36 was very difficult to take place these reaction. We speculated that 36 possessed strong steric hindrance. Compounds 40 and 41 were obtained by deprotection of TBS and benzyl group of 36 and 37. The structure and configuration of 39 were proven by single-crystal X-ray
diffraction analysis\textsuperscript{29} (Figure 1). Compound 43 was synthesized by methylation of 37, then removal of TBS and benzyl group of 37. Acylation of 37 with benzoyl chloride or chloroacetyl chloride afforded 44 and 45. Compound 46 was produced by removal of TBS and benzyl group of 44. Deprotection of TBS group of 45 afforded 47. Compounds 50 and 51 were generated by reaction of 47 with piperidine or morpholine, then removal of benzyl group, as shown in scheme 4.

\begin{align*}
\text{Scheme 4} \\
\text{a) TBSCl, imidazole, DMF, 60 °C, 5 h, 83%}. & \quad \text{b) mCPBA, CHCl}_3, \text{rt, 12 h, 67%}. \quad \text{c) NaBH}_4, \text{THF/H}_2\text{O, rt, 5 h, 89%}. \quad \text{(36/37 = 2:3)}. \quad \text{d) BF}_3\text{-Et}_2\text{O, CHCl}_3, \text{0 °C, 5 h, 82%}. \quad \text{e) Pd/C, H}_2, \text{MeOH, rt, 12 h, 78% for 40, 75% for 41, 74% for 43 and 75% for 46 over two steps}. \quad \text{f) MeI, NaH, DMF, rt, 3 h, 85%}. \quad \text{g) benzoyl chloride or chloroacetyl chloride, pyridine, DCM, rt, 5 h, 78% for 44 and 90% for 45}. \quad \text{h) piperidine or morpholine, DCM, reflux, 6 h, 85% for 48 and 49}. 
\end{align*}
In vitro biological evaluation. In this paper, we further synthesized OA derivatives based on our previous work. All these synthetic derivatives were evaluated in the enzyme inhibition assay against PTP1B by the method of p-nitrophenyl phosphate using compound 52 as a reference compound. Homogeneous TCPTP inhibitory activities were investigated simultaneously by the same method for further selectivity studying (Tables 1 and 2). We also tested the inhibitory activity of some synthetic derivatives on several other critical PTPs, which negatively regulate insulin dephosphorylation, such as leukocyte antigen-related phosphatase (LAR), src homology phosphatase-1 (SHP-1) and src homology phosphatase-2 (SHP-2) (Table 3).

The IC\textsubscript{50} of OA derivatives with heterocyclic ring-fused at C-2, C-3 positions (6-22) were tested on PTP1B and TCPTP. The results (Table 1) indicated that we obtained two most potent inhibitors 19 (IC\textsubscript{50} = 0.91 \textmu M) and 21 (IC\textsubscript{50} = 0.98 \textmu M) which showed about 3-fold more potent than lead compound OA on PTP1B. The inhibitory activity was decreased by introducing hydrophilic substituents at the five-membered heterocycles, such as carbonyl group (17 and 18), especially the formyloxy group (20 and 22), and increased by introducing hydrophobic substituents, such as trifluoromethyl group (16), especially the ethyl formyloxy group (19 and 21). The selectivity between PTP1B and TCPTP of these heterocyclic derivatives was not significantly improved, among them 16 possessed the best selectivity of 4.4-fold.
Table 1. Inhibitory activity of OA derivatives with heterocyclic ring-fused at C-2, C-3 positions against PTP1B and TCPTP

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\textsubscript{50} (μM)</th>
<th>PTP1B</th>
<th>TCPTP</th>
<th>TCPTP/PTP1B\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>2.96 ± 0.26</td>
<td>7.78 ± 1.55</td>
<td>2.6</td>
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<tr>
<td>6</td>
<td>2.71 ± 0.35</td>
<td>9.58 ± 1.64</td>
<td>3.5</td>
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<tr>
<td>9</td>
<td>2.86 ± 0.34</td>
<td>6.59 ± 1.10</td>
<td>2.3</td>
<td></td>
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<tr>
<td>15</td>
<td>3.05 ± 0.23</td>
<td>7.99 ± 1.29</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.39 ± 0.27</td>
<td>6.15 ± 1.40</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2.99 ± 0.44</td>
<td>6.09 ± 0.58</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>3.89 ± 0.63</td>
<td>7.89 ± 0.95</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.91 ± 0.13</td>
<td>2.01 ± 0.34</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>8.87 ± 1.55</td>
<td>23.8 ± 7.12</td>
<td>2.7</td>
<td></td>
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<tr>
<td>21</td>
<td>0.98 ± 0.14</td>
<td>2.46 ± 0.22</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>6.54 ± 0.68</td>
<td>7.93 ± 3.69</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.89 ± 0.08</td>
<td>6.24 ± 0.19</td>
<td>1.6</td>
<td></td>
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</tbody>
</table>

\textsuperscript{a)} TCPTP/PTP1B, the ratio of IC\textsubscript{50} of TCPTP and PTP1B. \textsuperscript{b)} Positive control.

The PTP1B inhibition of the C-ring modified derivatives (25-51) was decreased dramatically, with the exception of 46. The ring expansion compound 33 was obtained by Beckman rearrangement of 31, which PTP1B inhibitory potency was decreased at least 2-fold. The stereo configuration of C-12 hydroxyl is important since the activity of β-hydroxyl compound (41) was about half of α-hydroxyl compound (40). Compared with 41, the C-12 β-hydroxyl substituted with hydrophobic groups, such as methyl, benzoyl, piperidine N-acetyl and morpholine N-acetyl group (43, 46, 50 and 51) could ameliorate the PTP1B inhibitory potency. Although the PTP1B inhibitory potency of these C-ring modified derivatives was decreased dramatically, the selectivity between PTP1B and TCPTP of some derivatives was improved significantly, especially 25, 50 and 51 possessed more than 6-fold selectivity.

Table 2. Inhibitory activity of C-ring modified OA derivatives against PTP1B and TCPTP

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\textsubscript{50} (μM)</th>
<th>PTP1B</th>
<th>TCPTP</th>
<th>TCPTP/PTP1B\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>2.96 ± 0.26</td>
<td>7.78 ± 1.55</td>
<td>2.6</td>
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<tr>
<td>25</td>
<td>9.07 ± 0.44</td>
<td>57.8 ± 9.38</td>
<td>6.4</td>
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<tr>
<td>27</td>
<td>13.4 ± 0.47</td>
<td>66.1 ± 7.50</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>&gt;40</td>
<td>nd\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>9.34 ± 2.95</td>
<td>36.2 ± 6.73</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>21.2 ± 5.64</td>
<td>36.8 ± 3.75</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>16.1 ± 1.30</td>
<td>44.6 ± 6.05</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>31.4 ± 2.32</td>
<td>61.5 ± 5.12</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>7.56 ± 1.17</td>
<td>18.9 ± 3.11</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>2.44 ± 0.21</td>
<td>9.06 ± 1.33</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>6.35 ± 0.24</td>
<td>41.6 ± 10.5</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>5.71 ± 0.84</td>
<td>35.4 ± 3.47</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>52\textsuperscript{c}</td>
<td>3.89 ± 0.08</td>
<td>6.24 ± 0.19</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Not determined. \textsuperscript{b)} TCPTP/PTP1B, the ratio of IC\textsubscript{50} of TCPTP and PTP1B. \textsuperscript{c)} Positive control.
Moreover, some derivatives (16, 19, 21, 25, 50 and 51), which had potent inhibition or high selectivity, were also evaluated on homogenous enzymes LAR, SHP-1 and SHP-2 (Table 3). The results showed that these compounds had no obvious inhibition against LAR, SHP-1 and SHP-2. Compared to OA, these tested derivatives developed by our group clearly have better selectivity between PTP1B and SHP-1.

Table 3. Inhibitory activity of selected OA derivatives against related PTPs

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAR</td>
</tr>
<tr>
<td>OA</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>16</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>19</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>21</td>
<td>&gt; 40</td>
</tr>
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<td>25</td>
<td>&gt; 40</td>
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<tr>
<td>50</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>51</td>
<td>&gt; 40</td>
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</tbody>
</table>

CONCLUSION
In summary, we synthesized a series of novel OA derivatives with five-membered heterocyclic ring-fused at C-2, C-3 positions and the C-ring modified OA analogs efficiently. The five-membered heterocyclic ring-fused compounds had improved inhibitory activity on PTP1B, and the most potent compounds possessed 3-fold potency compare to OA; however, the selectivity between PTP1B and TCPTP was not obviously improved. The C-ring modified compounds had improved selectivity for PTP1B over TCPTP, some compounds possessed up to 6-fold selectivity; however, the inhibitory potency on PTP1B was decreased dramatically. We hope that merge the two parts of works, modification of the C-2, C-3 positions and the C-ring simultaneously, would afford the potent and high selectivity PTP1B inhibitors.

EXPERIMENTAL
General. 1H (400 and 500 MHz) and 13C (100 and 125 MHz) NMR spectra were recorded on a JEOL-400 or Bruker AM-500 Fourier transform spectrometer. The chemical shifts were reported (δ in ppm) using the δ = 7.26, 2.5 signals of CDCl3, DMSO-d6 (1H NMR), and using the δ = 77.23, 39.51 signals of CDCl3, DMSO-d6 (13C NMR) as internal standards. High-resolution mass data were obtained on a Micromass Tof II spectrometer.

General procedure A, oxidation of 3-OH group of OA derivatives. OA derivative (1 mmol) was dissolved in DMSO (30 mL). To this soln., IBX (0.56 g, 2 mmol) was added. After stirring for 12 h at rt,
the reaction mixture was poured into H₂O (30 mL), the precipitate was filtered, and the filtrate was extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (CC).

**General procedure B, acylation of C-2 position of 10.** OA derivative (0.2 mmol) was dissolved in dry THF (20 mL). To this soln., NaH (40 mg, 1 mmol, 60% in oil) was added. After stirring for 1 h at rt, the acylation reagent RCO₂Et (R = CF₃, OEt, CO₂Et) (1 mmol) was added. After stirring for 12 h at rt, the reaction mixture was poured into H₂O (40 mL), the pH was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC.

**General procedure C, deprotection of benzyl group of OA derivatives.** OA derivative (1 mmol) was dissolved in MeOH (50 mL). To this soln., 10% Pd on carbon (30 mg) was added and the reaction was subjected to 1 atm of H₂. After stirring for 12 h at rt, the reaction mixture was filtered, and the filtrate was concentrated to give the target product.

**General procedure D, hydrolysis of OA derivatives.** OA derivative (1 mmol) was dissolved in THF (20 mL) and MeOH (20 mL). To this soln., 2 M NaOH solution (12 mL) was added. After stirring for 12 h at rt, the reaction mixture was poured into H₂O (40 ml), the pH was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC.

**General procedure E, deprotection of TBS group of OA derivatives.** OA derivative (1 mmol) was dissolved in DCM (20 mL). To this soln., boron trifluoride ether etherate (6 mL) was added at 0 °C. After stirring for 5 h at 0 °C, the reaction mixture was poured into H₂O (40 mL), and the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The residue was purified by CC.

**Compound 1.** OA (10 g, 21.9 mmol) was dissolved in dry DMF (100 mL). To this soln., K₂CO₃ (6.04 g, 43.8 mmol) and BnBr (3.33 g, 26.3 mmol) were added. After stirring for 12 h at rt, the reaction mixture was poured into ice H₂O (200 mL). The precipitate was filtered and washed with water, then dried under reduced pressure to give 1 as a white solid powder (11 g, 92%).

**Compound 2.** General procedure A, CC (PE/AcOEt 10:1), afforded 2 (840 mg, 85%) as a white solid. 

**Compound 3.** t-BuOK (515 mg, 4.2 mmol) and 2 (500 mg, 0.92 mmol) were dissolved in t-BuOH (30
mL) and stirred for 1 h at rt. To this soln. isoamyl nitrite (493 mg, 4.2 mmol) was added. After stirring for 5 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give 3 (450 mg, 86%) as a white solid. ¹H NMR (400 MHz, CDCl₃): 7.29-7.32 (m, 5 H); 5.31 (s, 1 H); 5.00-5.09 (m, 2 H); 2.96-3.00 (m, 1 H); 2.89-2.92 (m, 1 H); 1.19-2.11 (m, 19 H); 1.16 (s, 3 H); 1.12 (s, 3 H); 1.09 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.85 (s, 3 H); 0.61 (s, 3 H).

**Compound 4.** Hydroxylamine hydrochloride (165 mg, 2.35 mmol) and 3 (450 mg, 0.78 mmol) were dissolved in pyridine (20 mL). After refluxing for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 3:1) to give 4 (345 mg, 75%) as a white solid. ¹H NMR (400 MHz, CDCl₃): 7.32-7.35 (m, 5 H); 5.33 (s, 1 H); 5.04-5.12 (m, 2 H); 3.10-3.15 (m, 1 H); 2.90-2.93 (m, 1 H); 1.21-2.05 (m, 19 H); 1.18 (s, 3 H); 1.12 (s, 3 H); 1.07 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.85 (s, 3 H); 0.63 (s, 3 H).

**Compound 5.** KOH (32 mg, 0.58 mmol) and 4 (345 mg, 0.58 mmol) were dissolved in ethylene glycol (10 mL) and dioxane (5 mL). After refluxing for 5 h, the reaction mixture was poured into H₂O (30 mL) and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give 5 (200 mg, 60%) as a white solid. ¹H NMR (400 MHz, CDCl₃): 7.32-7.36 (m, 5 H); 5.36 (s, 1 H); 5.04-5.12 (m, 2 H); 3.06-3.10 (m, 1 H); 2.94-2.96 (m, 1 H); 2.13-2.17 (m, 1 H); 1.46-1.99 (m, 18 H); 1.41 (s, 3 H); 1.33 (s, 3 H); 1.12 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.81 (s, 3 H); 0.65 (s, 3 H).

**Compound 6.** General procedure C, afforded 6 (168 mg, 92%) as a white solid. Mp 243-245°C. ¹H NMR (400 MHz, CDCl₃): 5.35 (s, 1 H); 3.08-3.12 (m, 1 H); 2.86-2.89 (m, 1 H); 2.15-2.19 (m, 1 H); 1.44-2.02 (m, 18 H); 1.41 (s, 3 H); 1.30 (s, 3 H); 1.17 (s, 3 H); 0.93 (s, 3 H); 0.91 (s, 3 H); 0.84 (s, 3 H); 0.82 (s, 3 H). ¹³C NMR (CDCl₃, 100 MHz): 184.3; 159.6; 150.5; 143.6; 122.0; 52.9; 46.6; 45.9; 45.8; 41.8; 41.0; 39.3; 38.4; 35.2; 33.8; 33.2; 33.0; 32.4; 31.7; 31.3; 30.7; 29.7; 27.7; 25.7; 24.7; 23.5; 22.9; 19.0; 16.6; 15.4. ESI-HRMS (m/z) [M+Na]⁺ calc'd for C₃₀H₄₄N₂NaO₃ 503.3244; found 503.3284.

**Compound 7.** Zn dust (70 mg, 1.1 mmol) and 3 (200 mg, 0.35 mmol) were dissolved in AcOH (10 mL) and Ac₂O (5 mL). After stirring for 12 h at rt, the reaction mixture was filtered with diatomite, the pH of filtrate was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 1:1) to give 7 (170 mg, 86%) as a white solid. ¹H NMR (400 MHz, CDCl₃): 7.27-7.32 (m, 5 H); 6.58 (d, J = 6.0 Hz, 1 H); 5.26 (s, 1 H); 5.05 (d, J = 12.0 Hz, 1 H); 5.00 (d, J = 12.0 Hz, 1H); 4.84-4.88 (m, 2H); 2.87-2.90 (m, 1H); 2.54-2.61 (m, 1H); 2.04 (s, 3H); 1.19-2.02 (m, 19 H); 1.14 (s, 3 H); 1.11 (s, 3 H); 1.07 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.74 (s, 3 H); 0.57 (s, 3 H).
Compound 8. POCl₃ (0.5 mL) and 7 (170 mg, 0.28 mmol) were dissolved in pyridine (10 mL). After stirring for 12 h at rt, H₂O (20 mL) was added to quench the reaction and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give 8 (125 mg, 75%) as a white solid. ¹H NMR (400 MHz, CDCl₃): 7.22-7.27 (m, 5 H); 5.27 (s, 1 H); 5.01 (d, J = 12 Hz, 1 H); 2.85-2.88 (m, 1 H); 2.32-2.36 (m, 1 H); 2.31 (s, 3 H); 1.19-2.02 (m, 19 H); 1.12 (s, 3 H); 1.10 (s, 3 H); 1.03 (s, 3 H); 0.85 (s, 3 H); 0.83 (s, 3 H); 0.82 (s, 3 H); 0.59 (s, 3 H).

Compound 9. General procedure C, afforded 9 (83 mg, 82%) as a white solid. Mp 267-268°C. ¹H NMR (500MHz, CDCl₃): 5.32 (s, 1 H); 2.84-2.86 (m, 1 H); 2.39 (s, 3 H); 1.24-2.08 (m, 20 H); 1.17 (s, 3 H); 1.13 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 3 H); 0.81 (s, 3 H). ¹³C NMR (100 MHz, CDCl₃): 183.4; 159.8; 151.9; 143.6; 129.8; 122.4; 53.5; 53.4; 46.5; 46.2; 45.9; 41.7; 41.0; 39.4; 39.0; 38.5; 33.8; 33.0; 32.4; 31.9; 30.6; 28.8; 27.7; 25.7; 23.5; 22.9; 21.0; 18.8; 16.8; 15.7; 14.0. ESI-HRMS (m/z) [M+H]⁺ calcd for C₃₂H₄₈NO₃ 494.3629; found 494.3695.

Compound 10. General procedure A, afforded 10 (1.0 g, 90%) as a white solid and used without further purification.

Compound 11. MeONa (595 mg, 11 mmol) and 10 (1.0 g, 2.2 mmol) were dissolved in dry toluene (30 mL). After stirring for 1 h at rt, HCO₂Et (0.89 mL, 11 mmol) was added. After stirring for 6 h, the reaction mixture was concentrated under reduced pressure. The residue was diluted with H₂O (30 mL) and adjusted pH to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give 11 (743 mg, 70%) as a pink solid. ¹H NMR (500 MHz, CDCl₃): 14.92 (d, J = 4.0 Hz, 1 H), 8.57 (d, J = 4.0 Hz, 1 H), 5.34 (m, 1 H), 2.90 (m, 1 H), 1.20-2.87 (m, 21 H); 1.19 (s, 3 H), 1.14 (s, 3 H), 1.10 (s, 3 H), 0.94 (s, 3 H), 0.90 (s, 3 H), 0.89 (s, 3 H), 0.79 (s, 3 H).

Compound 12. General procedure B, CC (PE/AcOEt 5:1), afforded 2 (430 mg, 78%) as a pink solid. ¹H NMR (500 MHz, CDCl₃): 15.71 (s, 1H, CO₂H); 5.33 (s, 1H, H-12); 2.83-2.86 (m, 1H, H-18); 2.50-2.53 (m, 1H); 0.82-2.04 (m, 20H); 1.25 (s, 3 H); 1.17 (s, 3 H); 1.10 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.81 (s, 3 H).

Compound 13. General procedure B, CC (PE/AcOEt 5:1), afforded 13 (410 mg, 78%) as a white solid. ¹H NMR (500 MHz, CDCl₃): 12.57 (s, 1H, CO₂H); 5.32 (s, 1H, H-12); 4.14-4.27 (m, 2H, CH₂O); 2.82-2.85 (m, 1H, H-18); 2.32-2.35 (m, 1H); 0.85-2.03 (m, 23H); 1.16 (s, 3H); 1.13 (s, 3H); 1.07 (s, 3H); 0.92 (s, 6H); 0.90 (s, 3H); 0.85 (s, 3H).

Compound 14. General procedure B, CC (PE/AcOEt 5:1), afforded 14 (410 mg, 78%) as a white solid.
H NMR (400 MHz, CDCl$_3$): 5.24 (s, 1 H); 4.25-4.32 (m, 2 H); 2.86-2.89 (m, 1 H); 2.32-2.36 (m, 1 H); 1.27-1.91 (m, 26 H); 1.24 (s, 3 H); 1.14 (s, 3 H); 1.08 (s, 3 H); 0.83 (s, 3 H); 0.77 (s, 3 H); 0.60 (s, 3 H).

**Compound 15.** Hydroxylamine hydrochloride (210 mg, 3.0 mmol) and 11 (500 mg, 1.0 mmol) were dissolved in pyridine (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 4:1) to give 15 (370 mg, 74%) as a white solid. Mp 159-162°C.

1H NMR (400 MHz, CDCl$_3$): 8.03 (s, 1 H); 5.33 (s, 1 H); 2.84-2.86 (m, 1 H); 2.65-2.69 (m, 1 H); 1.25-2.04 (m, 19 H); 1.36 (s, 3 H); 1.23 (s, 3 H); 1.15 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.81 (s, 3 H); 0.78 (s, 3 H).

13C NMR (100 MHz, CDCl$_3$): 184.1; 167.7; 153.5; 143.6; 122.3; 113.0; 53.2; 46.6; 45.9; 45.8; 41.8; 41.0; 39.2; 38.1; 34.0; 33.8; 33.6; 33.0; 32.3; 31.9; 31.4; 30.6; 27.7; 25.7; 24.7; 23.5; 23.3; 22.9; 19.0; 16.7; 14.9. ESI-HRMS (m/z) [M+H]$^+$ calcld for C$_{31}$H$_{46}$NO$_3$ 480.3478; found 480.3448.

**Compound 16.** 85% hydrazine hydrate (270 mg, 4.5 mmol) and 12 (500 mg, 0.91 mmol) were dissolved in dry EtOH (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 40:1) to give 16 (400 mg, 81%) as a white solid. Mp 254-256°C.

1H NMR (400 MHz, DMSO-$_d$6): 13.2 (bs, 1 H); 12.1 (bs, 1 H); 5.21 (s, 1 H); 2.75-2.78 (m, 1 H); 2.25-2.28 (m, 1 H); 1.31-1.97 (m, 24 H); 1.15 (s, 3 H); 1.10 (s, 3 H); 1.05 (s, 3 H); 0.86 (s, 6 H); 0.79 (s, 3 H); 0.76 (s, 3 H).

13C NMR (100 MHz, DMSO-$_d$6): 178.5; 147.8; 143.6; 123.8; 121.4; 121.2; 110.2; 52.1; 45.6; 45.5; 45.4; 41.5; 40.9; 40.1; 37.8; 34.9; 33.3; 32.8; 32.6; 32.0; 31.6; 30.3; 30.2; 27.2; 25.3; 23.2; 23.0; 22.8; 22.6; 18.4; 16.5; 15.1. ESI-HRMS (m/z) [M+Na]$^+$ calcld for C$_{32}$H$_{45}$F$_3$N$_2$NaO$_2$ 569.3325; found 569.3344.

**Compound 17.** 85% hydrazine hydrate (140 mg, 2.4 mmol) and 12 (300 mg, 0.56 mmol) were dissolved in dry EtOH (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 20:1) to give 17 (194 mg, 67%) as a white solid. Mp 265-266°C.

1H NMR (400 MHz, DMSO-$_d$6): 5.22 (s, 1 H); 2.75-2.78 (m, 1 H); 2.25-2.28 (m, 1 H); 1.31-1.97 (m, 24 H); 1.15 (s, 3 H); 1.10 (s, 3 H); 1.05 (s, 3 H); 0.86 (s, 6 H); 0.79 (s, 3 H); 0.76 (s, 3 H).

13C NMR (100 MHz, DMSO-$_d$6): 178.7; 158.6; 146.8; 143.6; 123.8; 121.7; 95.5; 52.7; 45.7; 45.5; 41.5; 40.9; 40.1; 38.8; 38.1; 34.6; 33.4; 33.0; 32.9; 32.1; 31.8; 30.4; 30.3; 27.3; 25.4; 23.3; 22.9; 22.7; 18.6; 16.6; 15.1; 14.1. ESI-HRMS (m/z) [M+H]$^+$ calcld for C$_{31}$H$_{47}$N$_2$O$_3$ 495.3581; found 495.3587.

**Compound 18.** Hydroxylamine hydrochloride (78 mg, 1.14 mmol), AcONa (93 mg, 1.14 mmol) and 13 (120 mg, 0.23 mmol) were dissolved in dry EtOH (15 mL). After refluxing for 24 h, the reaction mixture was diluted with H$_2$O (30 mL) and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 30:1) to give 18 (76 mg, 68%) as a white solid. Mp 217-218°C.

1H NMR (400 MHz, DMSO-$_d$6): 5.27 (s, 1 H); 2.81-2.84 (m, 1 H); 2.10-2.14 (m, 1 H); 1.23-2.03 (m, 23 H);
1.26 (s, 3 H); 1.16 (s, 3 H); 1.13 (s, 3 H); 0.93 (s, 6 H); 0.88 (s, 3 H); 0.82 (s, 3 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 178.6; 171.8; 169.7; 143.6; 121.5; 63.3; 52.1; 48.6; 45.7; 45.5(×2); 41.5; 40.9; 37.5; 34.1; 33.6; 33.4; 32.8; 32.1; 31.6; 30.4; 28.9; 27.3; 25.4; 23.3; 22.7; 21.6; 18.3; 16.5; 15.0; 14.1. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{31}$H$_{45}$NNaO$_4$ 518.3241; found 518.3263.

Compound 19. 85% hydrazine hydrate (100 mg, 1.71 mmol), and 14 (190 mg, 0.34 mmol) were dissolved in AcOH (15 mL). After stirring for 12 h at rt, the reaction mixture was diluted with H$_2$O (30 mL) and adjusted pH to 6-7 with NaHCO$_3$ and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 20:1) to give 19 (150 mg, 78%) as a white solid. Mp 311-312 °C. $^1$H NMR (400 MHz , DMSO-$d_6$): 5.23 (s, 1 H); 4.19 -4.21 (m, 2 H); 2.78 -2.85 (m, 2 H); 1.25-2.03 (m, 25 H); 1.23 (s, 3 H); 1.20 (s, 3 H); 1.11 (s, 3 H); 1.10 (s, 6 H); 0.85 (s, 3 H); 0.77 (s, 3 H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$): 178.6; 169.2; 150.5; 143.7; 138.9; 121.5; 119.2; 59.6; 52.4; 45.7; 45.5; 41.5; 40.9; 40.1; 38.8; 37.7; 36.6; 33.4; 32.8; 32.1; 31.7; 30.6; 30.4; 27.3; 27.2; 25.4; 23.3; 23.2; 22.9; 22.7; 18.6; 16.5; 15.3; 14.3. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{34}$H$_{50}$N$_2$NaO$_4$ 573.3663; found 573.3663.

Compound 20. General procedure D, CC (DCM/MeOH 10:1), afforded 20 (277 mg, 53%) as a white solid. Mp 274-276 °C. $^1$H NMR (500 MHz, DMSO-$d_6$): 12.45 (bs, 3 H); 5.24 (s, 1H); 2.92 (d, $J$ = 16.0 Hz, 1 H); 2.78 (d, $J$ = 16.0 Hz, 1 H); 1.33-2.08 (m, 19 H); 1.32 (s, 3 H); 1.17 (s, 3 H); 1.13 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 6 H); 0.80 (s, 6 H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): 178.6; 163.3; 150.5; 143.6; 138.9; 121.6; 115.3; 52.4; 45.7; 45.5; 41.5; 40.9; 40.0; 39.0; 38.9; 37.7; 36.6; 33.4; 32.9; 32.8; 32.1; 31.8; 30.8; 30.4; 27.2; 25.4; 23.4; 23.3; 23.0; 22.7; 16.6; 15.3. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{32}$H$_{46}$N$_2$NaO$_4$ 545.3350; found 545.3355.

Compound 21. Hydroxylamine hydrochloride (123 mg, 1.8 mmol) and 14 (200 mg, 0.36 mmol) were dissolved in dry EtOH (15 mL). After refluxing for 5 h, the reaction mixture was diluted with H$_2$O (30 mL) and extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 40:1) to give 21 (150 mg, 75%) as a white solid. Mp 170-172 °C. $^1$H NMR (500 MHz , DMSO-$d_6$): 5.32 (s, 1 H); 4.37 -4.41 (m, 2 H); 2.82 -2.85 (m, 1 H); 2.77 -2.76 (m, 1 H); 1.37-2.09 (m, 22 H); 1.32 (s, 3 H); 1.17 (s, 3 H); 1.13 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 3 H); 0.86 (s, 3 H); 0.79 (s, 3 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 183.2; 175.0; 159.7; 153.0; 142.3; 121.2; 109.7; 60.5; 59.2; 52.2; 51.8; 45.4; 45.0; 44.6; 40.6; 39.8; 38.2; 37.3; 34.5; 33.8; 32.7; 31.9; 31.3; 30.7; 29.5; 27.6; 26.5; 24.6; 21.7; 19.8; 17.5; 15.6; 14.3; 13.0. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{34}$H$_{49}$N$_2$NaO$_5$ 574.3503; found 574.3550.

Compound 22. General procedure D, CC (DCM/MeOH 10:1), afforded 22 (288 mg, 55%) as a white
solid. Mp 163-164°C. $^1$H NMR (500 MHz, DMSO-$d_6$): 5.24 (s, 1 H); 2.78-2.80 (m, 1 H); 2.65-2.69 (m, 2H); 1.28-2.09 (m, 18 H); 1.24 (s, 3 H); 1.09 (s, 3 H); 1.07 (s, 3 H); 0.89 (s, 6 H); 0.83 (s, 3 H); 0.80 (s, 3 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 178.5; 175.2; 161.6; 154.8; 143.7; 121.3; 110.3; 59.7; 52.1; 45.7; 45.5; 45.4; 41.5; 37.9; 35.1; 34.4; 33.1; 32.8; 32.0; 31.4; 30.3; 28.4; 27.2; 25.3; 23.3; 22.9; 22.6; 20.7; 18.1; 16.5; 15.1; 14.0. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{32}$H$_{45}$NNaO$_5$ 546.3190; found 546.3182.

**Compound 23.** DMAP (0.45 g, 3.66 mmol), 1 (10 g, 18.3 mmol), Ac$_2$O (3.73 g, 36.6 mmol) and pyridine (4.4 mL, 55 mmol) were dissolved in dry DMF (50 mL). After stirring at rt for 12 h, the reaction mixture was poured into ice water (100 mL) and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with aqueous NaHCO$_3$, 5% HCl and brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure afforded 23 (9.7 g, 90%) as a white solid and used without further purification.

**Compound 24.** Chromium anhydride (1.02 g, 10.2 mmol) was dissolved in DCM (20 mL). To the soln. pyridine (1.64 mL, 20.4 mmol) was slowly added at 0°C and the reaction mixture was stirred for 0.5 h, then 23 (300 mg, 0.51 mmol) was added. After stirring for 12 h at rt, the solution was filtered through silica gel, the filtrate was concentrated under reduced pressure and dissolved in AcOEt (100 mL), washed with saturated aqueous NaHSO$_3$ and brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give 24 (153 mg, 50%) as a white solid.

**Compound 25.** General procedure D and C, afforded 25 (377 mg, 80%, over two steps) as a white solid. Mp 279-280°C. $^1$H NMR (400 MHz, CDCl$_3$): 12.33 (bs, 1 H); 5.43 (s, 1 H); 4.24 (s, 1 H); 3.17 (m, 1 H); 3.0-3.02 (m, 1 H); 2.84-2.86 (m, 1 H); 2.59-2.62 (m, 1 H); 2.29 (s, 1 H); 1.34-2.08 (m, 17 H); 1.33 (s, 3 H); 1.01 (s, 3 H); 0.90 (s, 6 H); 0.88 (s, 3 H); 0.87 (s, 3 H); 0.66 (s, 3 H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): 199.2; 178.1; 169.1; 126.8; 76.6; 61.1; 54.2; 45.1; 44.6; 43.8; 43.2; 41.2; 38.7; 38.5; 36.9; 36.8; 33.1; 32.5; 31.2; 30.3; 28.1; 27.3; 26.9; 23.2; 23.1; 22.4; 18.8; 17.1; 16.0; 15.9. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{30}$H$_{46}$NaO$_4$ 493.3288; found 493.3268.

**Compound 26.** mCPBA (1 g, 5.1 mmol) and 23 (1 g, 1.7 mmol) were dissolved in CHCl$_3$ (20 mL). After stirring for 24 h at rt, the reaction mixture was diluted with H$_2$O (30 mL) and extracted with CHCl$_3$ (2×30 mL). The combined organic layer was washed with saturated aqueous Na$_2$SO$_3$ and brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give 26 (616 mg, 60%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): 7.31-7.34 (m, 5 H); 5.19 (d, $J = 12.2$ Hz, 1 H); 5.06 (d, $J = 12.2$ Hz, 1 H); 4.45 (dd, $J = 5.1, 12.0$ Hz, 1 H); 2.80-2.84 (m, 1 H); 2.43 (d, $J = 5.2$ Hz, 1 H); 2.18-2.19 (m, 1 H); 2.15 (s, 3 H); 1.15-2.08 (m, 21 H); 1.12 (s, 3 H);
0.98 (s, 3 H); 0.92 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.61 (s, 3 H).

**Compound 27.** General procedure D and C, afforded 27 (364 mg, 77%, over two steps) as a white solid. Mp 223-224 °C. ¹H NMR (500 MHz, CDCl₃): 3.16-3.20 (m, 1 H); 2.67-2.69 (m, 1 H); 2.42-2.45 (m, 1 H); 2.19 (s, 1 H); 1.14-2.15 (m, 20 H); 1.12 (s, 3 H); 0.98 (s, 3 H); 0.92 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.61 (s, 3 H). ¹³C NMR (100 MHz, DMSO-d₆): 209.3; 177.5; 75.0; 52.9; 49.4; 47.6; 44.4; 39.8; 39.2; 36.8; 36.4; 35.9; 34.8; 34.2; 31.7; 31.0; 30.0; 29.8; 29.0; 28.7; 26.4; 25.5; 25.3; 21.5; 20.8; 18.5; 16.4; 14.2; 13.3. ESI-HRMS (m/z) [M+Na]^+ calcd for C₃₀H₄₈NaO₄ 495.3445; found 495.3457.

**Compound 28.** 2 M solution of bromine in AcOH (0.5 mL), 2₆ (300 mg, 0.49 mmol) and several drops of hydrobromic acid were added to glycial AcOH (15 mL). The reaction mixture was heated for 4 h at 60 °C. The reaction mixture was quenched with H₂O (30 mL) and adjusted pH to 7-8 by adding saturated aqueous NaHCO₃ solution, then extracted with AcOEt (3 ×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give 28 (230 mg, 78%) as a white solid. ¹H NMR (500 MHz, CDCl₃): 7.28-7.36 (m, 5 H); 5.71 (s, 1 H); 5.14 (d, J = 12.0 Hz, 1 H); 5.10 (d, J = 12.0 Hz, 1 H); 4.46 (dd, J = 5.0, 12.0 Hz, 1 H); 3.05-3.08 (m, 1 H); 2.73 (d, J = 4.4 Hz, 1 H); 2.06 (s, 3 H); 0.79-1.94 (m, 19 H); 1.16 (s, 3 H); 1.0 (s, 3 H); 0.94 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.86 (s, 3 H).

**Compound 29.** General procedures D and C, afforded 29 (348 mg, 74%, over two steps) as a white solid. Mp 299-301 °C. ¹H NMR (500 MHz, CDCl₃): 5.75 (s, 1 H); 3.22 (dd, J = 5.0, 11.7 Hz, 1 H); 3.22 (dd, J = 5.0, 11.7 Hz, 1 H); 2.98-3.01 (m, 1 H); 2.89-2.90 (m, 1 H); 1.18-1.95 (m, 19 H); 1.17 (s, 3 H); 1.03 (s, 3 H); 1.00 (s, 3 H); 0.94 (s, 3 H); 0.89 (s, 3 H); 0.87 (s, 3 H); 0.83 (s, 3 H). ¹³C NMR (100 MHz, DMSO-d₆): 197.6; 177.2; 176.9; 120.1; 74.3; 48.1; 47.0; 44.3; 43.2; 39.5; 38.5; 37.3; 34.4; 33.7; 32.3; 31.5; 30.8; 30.7; 29.4; 28.6; 26.4; 26.0; 25.6; 22.0; 21.9; 21.3; 20.6; 19.5; 15.9; 14.3. ESI-HRMS (m/z) [M+Na]^+ calcd for C₃₀H₄₆NaO₄ 493.3194; found 493.3174.

**Compound 30.** Hydroxylamine hydrochloride (58 mg, 0.83 mmol) and 2₆ (100 mg, 0.17 mmol) were dissolved in dry pyridine (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure and diluted with H₂O (30 mL), then extracted with AcOEt (3 ×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure afforded 30 (100 mg, 97%) as a white solid and used without further purification.

**Compound 31.** General procedure D and C, afforded 31 (370 mg, 76%, over two steps) as a white solid. Mp 184-185 °C. ¹H NMR (400 MHz, CDCl₃): 3.18 (dd, J = 5.0, 15.0 Hz, 1 H); 3.0-3.03 (m, 1 H); 2.79-2.82 (m, 1 H); 2.53-2.55 (m, 1 H); 1.23-1.97 (m, 21 H); 1.21 (s, 3 H); 0.98 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.85 (s, 3 H); 0.73 (s, 3 H). ¹³C NMR (100 MHz, DMSO-d₆): 179.5; 157.0; 120.1; 74.3; 48.1; 47.0; 44.3; 43.2; 39.5; 38.5; 37.3; 34.4; 33.7; 32.3; 31.5; 30.8; 30.7; 29.4; 28.6; 26.4; 26.0; 25.6; 22.0; 21.9; 21.3; 20.6; 19.5; 15.9; 14.3. ESI-HRMS (m/z) [M+Na]^+ calcd for C₃₀H₄₆NaO₄ 493.3194; found 493.3174.
28.0; 27.0; 23.1; 22.4; 20.0; 19.3; 18.0; 15.8; 15.5; 15.0. ESI-HRMS (m/z) [M+Na]+ calcd for C_{30}H_{49}NNaO_{4} 510.3629; found 510.3633.

**Compound 32.** Compound 28 (250 mg, 0.4 mmol) was dissolved in dry THF (20 mL). To the soln. SOCl\textsubscript{2} (0.29 mL, 4.0 mmol) was added. After stirring for 12 h at rt, the reaction mixture was concentrated under reduced pressure and diluted with H\textsubscript{2}O (30 mL), then extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 30:1) to give 32 (215 mg, 86%) as a white solid.

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): 7.33-7.37 (m, 5 H); 5.48 (d, \textit{J} = 5.0 Hz, 1 H); 5.22 (d, \textit{J} = 12.2 Hz, 1 H); 5.09 (d, \textit{J} = 12.2 Hz, 1 H); 4.47 (dd, \textit{J} = 5.0, 11.7 Hz, 1 H); 3.89-3.92 (m, 1 H); 2.52-2.55 (m, 1 H); 2.25-2.28 (m, 1 H); 2.03 (s, 3 H); 1.01-1.99 (m, 21 H); 0.94 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.77 (s, 3 H); 0.74 (s, 3 H); 0.70 (s, 3 H).

**Compound 33.** General procedure D and C, afforded 33 (332 mg, 68%, over two steps) as a white solid. Mp 172-173\textdegree C. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}: 6.12 (d, \textit{J} = 5.0 Hz, 1 H); 4.06-4.09 (m, 1 H); 3.15 (dd, \textit{J} = 5.0, 11.5 Hz, 1 H); 2.52-2.54 (m, 1 H); 2.26-2.29 (m, 1 H); 0.95-1.93 (m, 20 H); 0.90 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.77 (s, 3 H); 0.70 (s, 3 H). \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6): 176.9; 173.9; 74.9; 52.2; 48.5; 46.8; 44.5; 43.0; 40.7; 37.2; 36.9; 36.7; 36.6; 34.4; 32.9; 32.7; 31.7; 31.5; 31.3; 30.8; 28.9; 26.4; 25.2; 21.2; 19.7; 16.1; 15.6; 15.1; 14.2; 13.8. ESI-HRMS (m/z) [M+Na]+ calcd for C_{30}H_{49}NNaO_{4} 510.3554; found 510.3572.

**Compound 34.** Imidazole (1.3 g, 18.3 mmol), 1 (1.0 g, 1.83 mmol) and TBSCl (1.38 g, 9.16 mmol) were dissolved in dry DMF (25 mL). After stirring for 6 h at 60\textdegree C, the reaction mixture was poured into H\textsubscript{2}O (30 mL), then extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under reduced pressure. The residue was recrystallized with AcOEt to give 34 (1.0 g, 83%) as a white solid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): 7.29-7.30 (m, 5 H); 5.24 (s, 1H); 5.04 (d, \textit{J} = 12.4 Hz, 1 H); 4.99 (d, \textit{J} = 12.4 Hz, 1 H); 3.11 -3.14 (m, 1 H); 2.84-2.87 (m, 1 H); 1.13-2.15 (m, 21 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.87 (s, 3 H); 0.82 (s, 3 H); 0.69 (s, 3 H); 0.50 (s, 3 H); -0.02 (s, 6H).

**Compound 35.** mCPBA (2.36 g, 6.82 mmol) and 34 (1.5 g, 2.27 mmol) were dissolved in CHCl\textsubscript{3} (30 mL). After stirring for 12 h at rt, the reaction mixture was poured into saturated aqueous NaHSO\textsubscript{3} solution (30 mL), and then extracted with AcOEt (3×30 mL). The combined organic layer was washed with saturated aqueous Na\textsubscript{2}CO\textsubscript{3} solution and brine, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give 35 (800 mg, 67%) as a white solid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): 7.31 -7.33 (m, 5 H); 5.05-5.21 (m, 2 H); 3.12-3.14 (m, 1 H); 2.77-2.80 (m, 1 H); 2.42-2.45 (m, 1 H); 2.17-2.19 (m, 1 H); 1.13-2.15 (m, 21 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.87 (s, 12 H); 0.77 (s, 3 H); 0.61 (s, 3 H); 0.03 (s, 6 H).
**Compound 36 and 37.** Compound 35 (900 mg, 1.33 mmol) was dissolved in THF (20 mL) and H$_2$O (2 mL). To this soln. NaBH$_4$ (150 mg, 4 mmol) was added. After stirring for 5 h, the pH of the reaction mixture was adjusted to 6-7 by adding 5% HCl, and then the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 15:1) to give 36 and 37 (804 mg, 89%, 36/37 = 2:3).

36: $^1$H NMR (500 MHz, CDCl$_3$): 7.31-7.35 (m, 5 H); 5.17 (d, $J = 12.3$ Hz, 1 H); 5.07 (d, $J = 12.3$ Hz, 1 H); 3.99 (m, 1 H); 3.17 (dd, $J = 4.1$, 15.5 Hz, 1 H); 2.47-2.50 (m, 1 H); 1.29-2.38 (m, 23 H); 1.28 (s, 3 H); 0.93 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 12 H); 0.80 (s, 3 H); 0.72 (s, 3 H); 0.61 (s, 3H); 0.03 (s, 6 H).

$^{13}$C NMR (100 MHz, CDCl$_3$): 178.1; 136.4; 128.5(×2); 128.2; 128.1(×2); 79.3; 74.0; 55.6; 48.5; 42.0; 40.9; 40.4; 39.4; 39.2; 38.8; 36.6; 34.1; 33.5; 33.4; 33.2; 32.0; 30.7; 30.6; 28.4; 27.4; 27.3; 25.9; 23.7; 23.6; 22.7; 20.8; 18.4; 18.1; 16.5; 15.9; 15.8; 15.1; -3.8; -4.9.

**Compound 37.**

$^1$H NMR (400 MHz, CDCl$_3$): 7.31-7.40 (m, 5 H); 5.21 (d, $J = 12.2$ Hz, 1 H); 5.08 (d, $J = 12.2$ Hz, 1 H); 3.67 (m, 1 H); 3.14-3.16 (m, 1 H); 2.71-2.74 (m, 1 H); 1.01-2.69 (m, 23 H); 0.93 (s, 3 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 12 H); 0.78 (s, 3 H); 0.72 (s, 3 H); 0.03 (s, 6 H).

$^{13}$C NMR (100 MHz, CDCl$_3$): 177.9; 136.5; 128.5(×2); 128.4; 128.1(×2); 79.3; 68.3; 66.0; 55.3; 49.1; 47.4; 43.1; 41.5; 40.5; 39.4; 38.6; 36.8; 36.1; 34.5; 33.4; 33.1; 32.6; 31.9; 31.5; 30.5; 28.7; 28.4; 27.7; 25.9(×3); 23.4; 23.2; 18.4; 18.1; 17.8; 16.2; 15.9(×2); -3.8; -4.9.

**Compound 39.** General procedure E afforded 39 (474 mg, 84%) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$): 7.30-7.39 (m, 5 H); 5.18 (d, $J = 12.0$ Hz, 1 H); 5.06 (d, $J = 12.0$ Hz, 1 H); 3.67 (m, 1 H); 3.14-3.16 (m, 1 H); 2.71-2.74 (m, 1 H); 1.01-2.69 (m, 23 H); 0.93 (s, 3 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 12 H); 0.78 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3H); 0.03 (s, 6 H).

**Compound 40.** General procedure E and C afforded 40 (370 mg, 78%, over two steps) as a white solid. Mp 246-247 °C. $^1$H NMR (400 MHz, CDCl$_3$): 3.77-3.80 (m, 1 H); 2.96 -3.00 (m, 1 H); 2.24 -2.27 (m, 1 H); 2.08-2.11 (m, 1 H); 1.07-1.97 (m, 27 H); 0.87 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.76 (s, 3 H); 0.64 (s, 3 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$): 179.8; 76.8; 71.6; 55.2; 47.3; 44.8; 41.6; 40.4; 38.5; 38.4; 38.3; 36.3; 33.9; 33.5; 33.0; 31.9; 30.6; 30.2; 30.0; 28.9; 28.7; 28.1; 27.0; 23.4; 20.1; 18.6; 18.0; 16.3; 16.0; 15.8. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{30}$H$_{50}$NaO$_4$ 497.3601; found 497.3624.

**Compound 41.** General procedure E and C afforded 41 (356 mg, 75%, over two steps) as a white solid. Mp 320-321 °C. $^1$H NMR (400 MHz, CDCl$_3$): 3.73-3.76 (m, 1 H); 3.18-3.20 (m, 1 H); 2.70-2.76 (m, 1 H); 1.04-1.88 (m, 27 H); 0.93 (s, 6 H); 0.90 (s, 3 H); 0.83 (s, 3 H); 0.76 (s, 3 H); 0.68 (s, 3 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$): 179.7; 77.1; 66.4; 55.2; 49.0; 46.5; 42.3; 41.4; 40.6; 38.9; 38.7; 36.9; 35.9; 34.6; 33.8; 33.4; 32.7; 32.0; 31.2; 30.6; 28.9; 28.5; 27.5; 23.6; 23.5; 18.4; 18.0; 16.5; 16.1; 16.0. ESI-HRMS (m/z) [M+Na]$^+$ calcd for C$_{30}$H$_{50}$NaO$_4$ 497.3601; found 497.3624.
**Compound 42.** 60% NaH (35 mg, 0.9 mmol) and 37 (200 mg, 0.3 mmol) were dissolved in dry DMF (15 mL). To this soln., MeI (0.06 mL, 0.9 mmol) was added. After stirring for 3 h at rt, the reaction mixture was poured into H₂O (30 mL), and then the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **42** (174 mg, 85%). 

**1H NMR** (500 MHz, CDCl₃): 7.29-7.40 (m, 5 H); 5.17 (d, J = 12.5 Hz, 1 H); 5.11 (d, J = 12.5 Hz, 1 H); 3.30 (s, 3 H); 3.21-3.24 (m, 1 H); 3.15 (dd, J = 4.4, 15. 7 Hz, 1 H); 0.95 -1.93 (m, 24 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 9 H); 0.87 (s, 3 H); 0.79 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6H).

**Compound 43.** General procedure E and C, afforded **43** (362 mg, 74%, over two steps) as a white solid. Mp 274-276°C. 

**1H NMR** (500 MHz, CDCl₃): 3.36 (s, 3 H); 3.26-3.30 (m, 1 H); 3.19 (dd, J = 4.6, 16.2 Hz, 1 H); 2.72 -2.75 (m, 1 H); 0.98 -2.03 (m, 23 H); 0.97 (s, 3 H); 0.95 (s, 3 H); 0.91 (s, 3 H); 0.87 (s, 3 H); 0.83 (s, 3 H); 0.76 (s, 3 H).

**13C NMR** (100 MHz, DMSO-d₆): 183.1; 92.0; 78.9; 55.9; 55.3; 48.9; 47.2; 41.5; 41.2; 40.4; 38.9; 38.7; 37.2; 36.5; 34.5; 33.5; 32.5; 31.2; 30.6; 29.7; 28.8; 27.3; 26.0; 23.5; 23.1; 18.3; 17.9; 16.6; 15.9; 15.4. ESI-HRMS (m/z) [M+Na]⁺ calcd for C₃₁H₅₂NaO₄ 511.3758; found 511.3760.

**Compound 44.** Benzoyl chloride (0.35 mL, 3.0 mmol), 37 (200 mg, 0.3 mmol) and TEA (1.0 mL) were dissolved in DCM (15 mL). After stirring for 5 h at rt, the reaction mixture was poured into H₂O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **44** (180 mg, 78%) as a white solid.

**1H NMR** (400 MHz, CDCl₃): 7.96 (d, J = 7.3 Hz, 2 H); 7.46-7.48 (m, 1 H); 7.41 (d, J = 7.3 Hz, 2 H); 7.32-7.34 (m, 5 H); 5.24 (d, J = 12.2 Hz, 1 H); 5.11 (d, J = 12.2 Hz, 1 H); 3.16-3.18 (m, 1 H); 3.10-3.14 (m, 1 H); 2.70-2.72 (m, 1 H); 2.12-2.15 (m, 1 H); 1.13-2.11 (m, 22 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.87 (s, 12 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6 H).

**Compound 45.** Chloroacetyl chloride (0.23 mL, 3.0 mmol), 37 (200 mg, 0.3 mmol) and pyridine (1.0 mL) were dissolved in DCM (15 mL). After stirring for 5 h at rt, the reaction mixture was poured into H₂O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **45** (200 mg, 90%) as a white solid.

**1H NMR** (400 MHz, CDCl₃): 7.96 (d, J = 7.3 Hz, 2 H); 7.46-7.48 (m, 1 H); 7.41 (d, J = 7.3 Hz, 2 H); 7.32-7.34 (m, 5 H); 5.24 (d, J = 12.2 Hz, 1 H); 5.11 (d, J = 12.2 Hz, 1 H); 3.16-3.18 (m, 1 H); 3.10-3.14 (m, 1 H); 2.70-2.72 (m, 1 H); 2.12-2.15 (m, 1 H); 1.13-2.11 (m, 22 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.87 (s, 12 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6 H).

**Compound 46.** General procedure E and C, afforded **46** (432 mg, 75%, over two steps) as a white solid. Mp 195-197°C. 

**1H NMR** (500 MHz, DMSO-d₆): 12.25 (br, 1 H); 7.96-7.97 (m, 2 H); 7.60-7.63 (m, 1 H).
H); 7.48-7.51 (m, 2 H); 4.90-5.20 (m, 1 H); 2.62-2.65 (m, 1 H); 2.10-2.40 (m, 1 H); 1.11-2.23 (m, 20 H); 1.01 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 3 H); 0.75 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3 H); 0.45 (s, 3 H). 

\(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): 183.3; 165.4; 133.1; 130.2; 129.0 (×2); 128.5 (×2); 128.2; 54.7; 48.4; 46.1; 41.0; 40.3; 40.1; 39.9; 38.4; 38.2; 36.6; 36.1; 34.0; 33.2; 32.7; 32.1; 31.2; 30.1; 28.5; 28.1; 27.2; 27.0; 22.9; 17.9; 17.4; 16.2; 15.7; 15.6. ESI-HRMS (m/z) [M+Na]\(^+\) calcd for C\(_{37}\)H\(_{54}\)NaO\(_5\) 601.3863; found 601.3884.

**Compound 47.** General procedure E, afforded 47 (526 mg, 82%) as a white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)): 7.37-7.43 (m, 5 H); 5.08-5.28 (m, 2 H); 4.03 (s, 2 H); 3.17-3.20 (m, 1 H); 2.57-2.60 (m, 1 H); 1.25-2.43 (m, 24 H); 1.21 (s, 3 H); 0.99 (s, 3 H); 0.92 (s, 3 H); 0.85 (s, 3 H); 0.79 (s, 3 H); 0.77 (s, 3 H); 0.65 (s, 3 H).

**Compound 48.** Piperidine (0.27 mL, 3.1 mmol) and 47 (200 mg, 0.31 mmol) were dissolved in DCM (20 mL). After refluxing for 6 h, the reaction mixture was poured into H\(_2\)O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na\(_2\)SO\(_4\) and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give 48 (182 mg, 85%) as a white solid. \(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.33-7.38 (m, 5 H); 5.04-5.23 (m, 3 H); 3.62-3.75 (m, 2 H); 3.22-3.50 (m, 4 H); 3.15-3.17 (m, 1 H); 2.43-2.45 (m, 1 H); 1.21-2.03 (m, 29 H); 0.94 (s, 6 H); 0.87 (s, 3 H); 0.77 (s, 3 H); 0.75 (s, 3 H); 0.73 (s, 3 H); 0.60 (s, 3 H).

**Compound 49.** Using the same procedure as preparation of 48 afforded 49 (181 mg, 85%) as a white solid. \(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.33-7.38 (m, 5 H); 5.06-5.23 (m, 3 H); 4.04 (m, 4 H); 3.62-3.75 (m, 2 H); 3.22-3.50 (m, 4 H); 3.15-3.23 (m, 1 H); 2.39-2.42 (m, 1 H); 1.25-1.94 (m, 24 H); 1.24 (s, 3 H); 0.95 (s, 3 H); 0.89 (s, 3 H); 0.87 (s, 3 H); 0.74 (s, 3 H); 0.64 (s, 3 H).

**Compound 50.** General procedure C, afforded 50 (516 mg, 86%) as a white solid. Mp 120-123°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): 4.86-4.88 (m, 1 H); 3.17 (d, \(J = 17.0\) Hz, 1 H); 3.00 (d, \(J = 17.0\) Hz, 1 H); 2.96-2.99 (m, 1 H); 2.44-2.46 (m, 4 H); 2.02-2.06 (m, 1 H); 1.11-1.80 (m, 29 H); 0.96 (s, 3 H); 0.87 (s, 3 H); 0.75 (s, 3 H); 0.65 (s, 3 H). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): 178.9; 169.7; 76.6; 71.1; 59.3; 54.8; 54.6; 53.2; 48.5; 48.2; 46.0; 41.0; 38.9; 38.4; 38.1; 36.5; 35.7; 33.8; 33.1; 32.7; 32.0; 30.9; 30.6; 30.1; 28.3; 28.0; 27.1; 27.0; 25.4; 23.5; 23.2; 22.6; 17.9; 17.3; 16.0; 15.7; 15.5. ESI-HRMS (m/z) [M+H]\(^+\) calcd for C\(_{37}\)H\(_{62}\)NO\(_5\) 600.4728; found 600.4705.

**Compound 51.** General procedure C, afforded 51 (512 mg, 85%) as a white solid. Mp 95-98°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): 12.25 (br, 1 H); 4.87-4.90 (m, 1 H); 4.26-4.28 (m, 1 H); 3.56 (t, \(J = 4.5\) Hz, 4 H); 3.25 (d, \(J = 16.9\) Hz, 1 H); 3.07 (d, \(J = 16.9\) Hz, 1 H); 2.96-2.99 (m, 1 H); 1.16-2.54 (m, 28 H); 0.96 (s, 3 H); 0.87 (s, 3 H); 0.85 (s, 3 H); 0.84 (s, 3 H); 0.76 (s, 3 H); 0.74 (s, 3 H); 0.64 (s, 3 H). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): 178.9; 169.5; 76.6; 71.2; 66.1; 58.7; 54.5; 52.5; 48.2; 46.0; 40.9; 40.2;
39.2; 39.1; 39.0; 38.4; 38.1; 36.5; 35.7; 33.7; 33.1; 32.7; 32.0; 30.9; 30.1; 28.3; 28.0; 27.1; 27.0; 23.2; 22.6; 17.9; 17.3; 16.0; 15.7; 15.6. ESI-HRMS (m/z) [M+H]$^+$ calcd for C$_{36}$H$_{60}$NO$_6$ 602.4415; found 602.4485.

**In vitro assay of PTP1B inhibitors**

The tested compounds were solubilized in Me$_2$SO at 5 mg/mL, and 2 μL samples was distributed to A2-H11 wells of 96-well clear polystyrene plate (Corning, Action, MA). The Me$_2$SO (2 μL) was distributed to A1-D1 and E12-H12 wells as the full enzyme activity, and compound 52 was distributed to E1-H1 and A12-D12 wells as the positive inhibition. After adding an assay mixture (80 μL), 10 μL of the GST-PTP1B (300 nM) was added to initiate the reaction. The high-throughput screening was carried out in a final 100 μL volume containing 50 mM MOPS, pH 6.5, 2 mM pNPP, 30 nM PTP1B and 2% Me$_2$SO, and the catalysis of pNPP was continuously monitored on SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C. For calculating IC$_{50}$, inhibition assays were performed with 30 nM recombinant enzyme, 2 mM pNPP in 50 mM MOPS at pH 6.5 and the inhibitors diluted around the estimated IC$_{50}$ values. IC$_{50}$ was calculated from the non-linear curve fitting of percent inhibition (% inhibition) vs. inhibitor concentration [I] by using the following equation 

$$\text{Inhibition} = 100 / \left(1 + \left(\frac{\text{IC}_{50}}{[I]}\right)^k\right)$$

where $k$ is the Hill coefficient.

**Crystal data and structure determination**

A single crystal of 39 with dimensions of 0.398 mm × 0.369 mm × 0.307 mm was chosen for X-ray diffraction analysis performed on a Bruker-AXS diffractometer, equipped with Mo Ka radiation ($\lambda = 0.71073$ Å) at 293(2) K by using a φ-ω scan mode. In the ranges of 1.73≤θ≤25.50°, a total of 35026 reflections were collected including 12334 unique ones ($R$ int = 0.0793), of which 12334 were observed with $I > 2\sigma(I)$. The structure was solved by direct methods using SHELXS program of the SHELXL-97 package and refined with SHELXL. The final refinement was performed by full-matrix least-squares method with Full-matrix least-squares on $F^2$ for the non-hydrogen atoms. 39 (C$_{37}$H$_{56}$O$_4$, $M_r$ = 564.82), crystallizes in the orthorhombic system, space group $P2_12_12_1$ with $a = 13.3536(9)$, $b = 19.8860(12)$, $c = 24.9647(16)$ Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 6629.4(7)$ Å$^3$, $Z = 8$, $D_c = 1.132$mg/m3, $\mu = 0.075$mm$^{-1}$, $F(000) = 2480$, the final $R_1 = 0.0705$ and $wR_2 = 0.1778$ for 12334 observed reflections ($I > 2\sigma(I)$). The hydrogen atoms were located from Fourier difference maps. The final $R_1 = 0.0705$, $wR_2 = 0.1778$ ($w = 1/\sigma^2(Fo^2) + (0.1038P)^2 + 0.0000P$), where $P = (Fo^2 + 2Fc^2)/3$, $S = 0.921$, ($\Delta\rho$) max = 0.592 and ($\Delta\rho$) min = -0.264 e/Å$^3$.

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REFERENCES

29. CCDC 815933 for compound 39. Free copy of the data can be obtained via http://www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 33.