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# Synthesis and biological evaluation of heterocyclic ring-substituted maslinic acid derivatives as novel inhibitors of protein tyrosine phosphatase 1B

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ABSTRACT

A series of maslinic acid derivatives have been synthesized by introducing various fused heterocyclic rings at C-2 and C-3 positions. Their inhibitory effects on PTP1B, TCPTP and related PTPs are evaluated. Most of the compounds exhibited a dramatic increase in inhibitory potency and selectivity, the two most potent PTP1B inhibitors **20** ($IC_{50}$ = 0.61 μM) and **29** ($IC_{50}$ = 0.64 μM) showed about 10-fold more potent than lead compound maslinic acid. More importantly, **29** possesses the best selectivity of 6.9-fold for PTP1B over TCPTP.



The protein tyrosine phosphatase (PTP) superfamily comprises more than 100 enzymes.[1] These protein tyrosine phosphatases (PTPs) are expressed in insulin-sensitive tissues and can function as negative modulators in insulin signal transduction by dephosphorylation of tyrosyl residues.[2–5] The aberrant of PTP activity contributes to several human pathologies, such as diabetes, obesity, cancer and immune disorders.[6–9] Protein tyrosine phosphatase 1B (PTP1B), one of the PTPs, is a key member in the down-regulation of the insulin and leptin signaling pathway by dephosphorylating the insulin receptor (IR),[10] insulin receptor substrates (IRS)[11] and Janus kinase 2 (JAK2),[12,13] which is downstream of leptin receptor. Hereby, inhibition of PTP1B results in antidiabetes by increasing insulin sensitivity[14] and resistance to obesity.[15] In recent years PTP1B inhibitors are recognized as potential therapeutics for treatment of type-2 diabetes and obesity.[16,17] However two major drawbacks prevent most of these inhibitors from further development to clinical trials. Firstly, lack of sufficient cell permeability due to the presence of highly negative charged residues mimicking the phosphate group in IRS.[18] Secondly, because of their high conserved catalytic domain, the selectivity for PTP1B over the most homogeneous T-cell protein tyrosine phosphatase (TCPTP) is low.[19]

Natural products play a major role in drug discovery and nearly half of the new drugs introduced into the market in the last two decades are natural products or their derivatives.[20,21] In searching for new types of PTP1B agonists, researchers have found pentacyclic triterpenoids including oleanolic acid (OA) are moderate PTP1B inhibitors. Based on these results, some potent inhibitors with good cell permeability were obtained by modifying with long hydrophobic chains at C-3 and C-28 positions. However, these synthetic compounds shared poor water solubility and had no obvious selectivity between PTP1B and TCPTP.[22,23]

Maslinic acid (MA), a natural pentacyclic triterpene acid, which presents in many plant species, especially in olive. It has various pharmacological activities, such as anti-tumor,[24–28] antioxidant,[29,30] anti-HIV,[29] antimicrobial[31] and anti-diabetic activities by moderate to low inhibition to glycogen phosphorylase ($IC_{50}$ = 28 μM).[32,33] Recently we first screened the inhibitory effects of MA (**1**), its isomers 3-*epi*-maslinic acid (**2**) and augustic acid (**3**)[32,34] on PTP1B. Among them MA exhibited good PTP1B inhibition ($IC_{50}$ = 5.93 μM). Considering the effect of hypoglycemic[33] we postulate that the anti-diabetic pharmacological effect in KK-$A^y$ mice not only due to the glycogen phosphorylase inhibition but also maybe mainly or partly caused by PTP1B inhibition. The inhibitory activity data of TCPTP revealed MA has 3.3-fold selectivity for PTP1B over TCPTP (Table 1), therefore we speculate that modification of C-2 and C-3 positions simultaneously may increase the selectivity and MA is selected as a promising lead compound for developing potent and high selective PTP1B inhibitors as therapeutic agents for type-2 diabetes and obesity. It is well-known that pentacyclic triterpenes including OA are too hydrophobic to have reasonable water solubility and related pharmacokinetic

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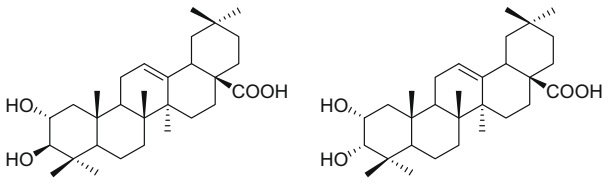
**Table 1**
Inhibitory activity of maslinic acid derivatives against PTP1B and TCPTP

| Compounds | $IC_{50}$ (μM) | | TCPTP/PTP1B[b] |
|---|---|---|---|
| | PTP1B | TCPTP | |
| **1** | 5.93 ± 0.08 | 19.47 ± 1.33 | 3.3 |
| **2** | 14.33 ± 0.65 | nd[a] | |
| **3** | 4.93 ± 0.17 | nd | |
| **9** | 1.43 ± 0.23 | 5.88 ± 0.52 | 4.1 |
| **11** | 1.79 ± 0.14 | 8.31 ± 1.30 | 4.6 |
| **15** | 5.44 ± 1.11 | nd | |
| **16** | 1.78 ± 0.10 | 5.51 ± 0.77 | 3.1 |
| **18** | 2.73 ± 0.23 | 8.19 ± 0.31 | 3.0 |
| **19** | 2.61 ± 0.46 | 6.50 ± 0.65 | 2.5 |
| **20** | 0.61 ± 0.04 | 1.60 ± 0.21 | 2.6 |
| **22** | 1.92 ± 0.12 | 6.44 ± 0.55 | 3.4 |
| **24** | 2.60 ± 0.62 | 8.44 ± 0.54 | 3.3 |
| **25** | 1.39 ± 0.19 | 3.80 ± 0.04 | 2.7 |
| **26** | 1.65 ± 0.23 | 5.99 ± 0.35 | 3.6 |
| **27** | 1.48 ± 0.08 | 4.93 ± 0.15 | 3.3 |
| **28** | 1.75 ± 0.10 | 5.56 ± 0.55 | 3.2 |
| **29** | 0.64 ± 0.03 | 4.39 ± 0.31 | 6.9 |
| **30** | 0.81 ± 0.08 | 3.62 ± 0.12 | 4.5 |
| **31**[c] | 3.89 ± 0.08 | 6.24 ± 0.19 | 1.6 |

[a] nd, not determined.
[b] TCPTP/PTP1B, the ratio of $IC_{50}$ of TCPTP and PTP1B.
[c] Positive control.

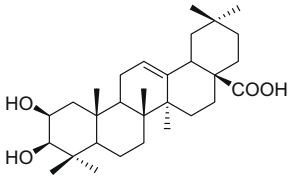
properties. In this paper, a series of heterocyclic ring-substituted maslinic acid derivatives at C-2 and C-3 have been synthesized and biologically evaluated in order to find more potent and high selective PTP1B inhibitors with improved water solubility.



maslinic acid (**1**)



3-*epi*-maslinic acid (**2**)



augustic acid (**3**)

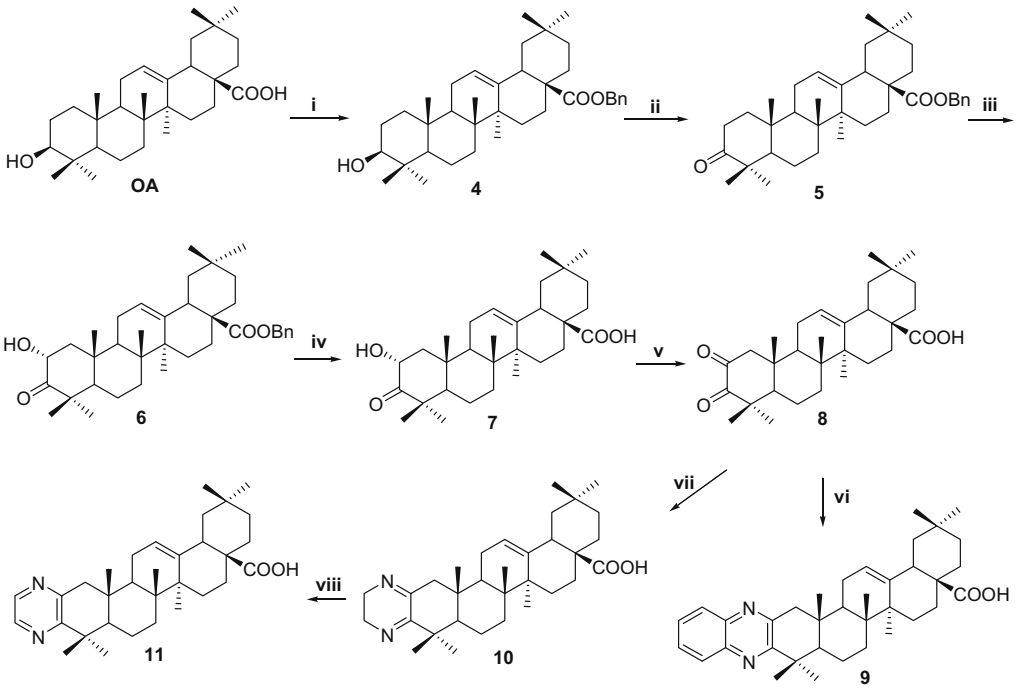
A series of maslinic acid derivatives with heterocyclic rings (pyrazine, pyrimidine, indole, thiazole, isoxazole and pyrazole) at C-2 and C-3 positions were synthesized (Scheme 1–3). Benzylation of OA followed by PCC oxidation[35] afforded ketone **5**. Stereoselective hydroxylation of **5** with *m*CPBA gave 2α-hydroxyl ketone **6**. Deprotection of **6** afforded **7**, which was then treated with KOH in MeOH to give diketone **8**.[32] As a common intermediate, **8** reacted with phenylenediamine to give quinoxaline derivative **9** and reacted with ethylene diamine to give **10**. **10** underwent dehydro-aromatization with $MnO_2$ to produce pyrazine derivative **11** as shown in Scheme 1.[36,37]

OA was oxidized with PCC to afford ketone **12**, which reacted with *N*,*N*-Dimethylformamide dimethyl acetal in presence of TEA to afford enamine derivative **13**.[38] Condensation of **13** with guanidine hydrochloride in the presence of NaOEt produced **14**. Demethylation of C-28 methyl ester with LiI in refluxing anhydrous DMF[39] produced a complex, and compound **15** was obtained with a yield of 12% after separation with column, which has a methylated pyrimidine moiety. To avoid the undesired methylation, *n*-octylamine was added. Indeed, compound **16** was successfully synthesized in the presence of this MeI scavenger. Compounds **18** and **19** were obtained in a similar process as **16**, the only difference is the demethylation of C-28 methyl ester was smoothly carried out in presence of LiI in anhydrous DMF without *n*-octylamine as shown in Scheme 2.

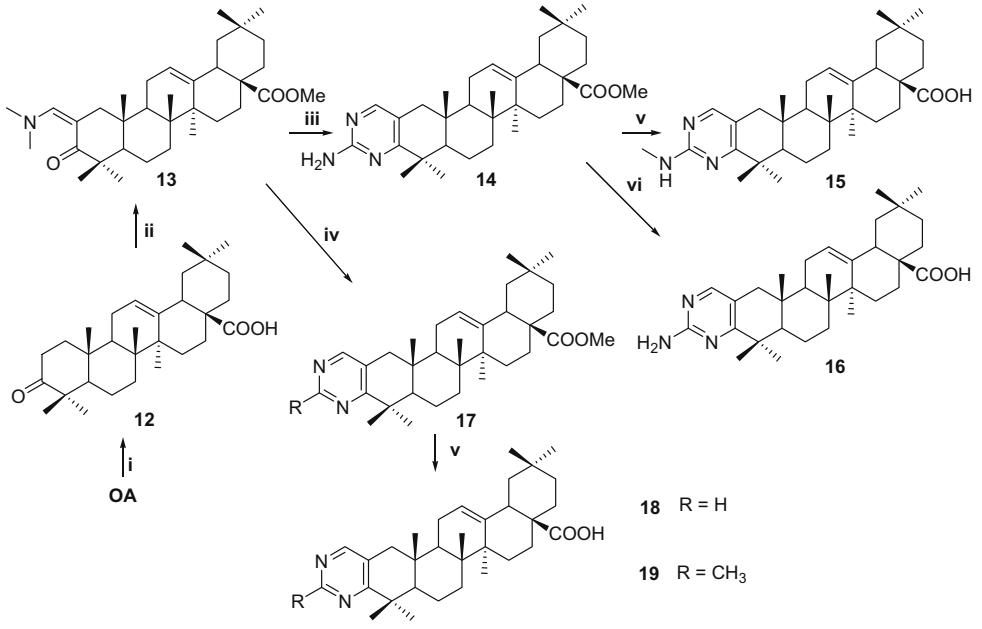
Compound **20** was prepared by Fischer indole synthesis of **12** with phenylhydrazine.[40] Bromination of **12** with pyridinium tribromide in HOAc and DCM gave **21**, which then condensed with thiourea in refluxing EtOH to afford thiazole derivative **22**.[41,42] **12** was formylated with HCOOEt in presence of MeONa to produce **23**, which reacted with hydroxylamine hydrochloride in EtOH to give isoxazole derivative **24**.[43] Pyrazole derivatives **25** and **26** were synthesized by treatment of **23** with phenylhydrazine hydrochloride and hydrazine hydrochloride, respectively.[44] Compound **27**–**30**[45] were prepared by reacting **26** with acetyl chloride, hexanoyl chloride, nicotinoyl chloride and isonicotinoyl chloride, respectively, as shown in Scheme 3.

*Results and discussion*: Initial SAR analysis for modified OA analogs with various side chains on C-3 and C-28 positions indicated a strong preference for the hydrophobic group and these derivatives have no obvious selectivity for PTP1B over TCPTP.[23] So in this paper we focus on improving water solubility, increasing inhibitory activity and selectivity between the two homogenous enzymes by introducing a series of heterocyclic rings at C-2 and C-3 positions. All the synthetic maslinic acid derivatives were evaluated in the enzyme inhibition assay against PTP1B by the method of *p*-nitrophenyl phosphate using compound **31** as reference compound.[46] Homogeneous TCPTP inhibitory activities were investigated simultaneously by the same method for further selectivity studying (Table 1). 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 2). The assay results indicated that most of the synthetic compounds exhibited more potent inhibition of PTP1B than that of lead compound MA. All these compounds showed 2.5–6.9-fold selectivity for PTP1B over TCPTP.

We tested the $IC_{50}$ of MA (**1**) and its isomers 3-*epi*-maslinic acid (**2**) and augustic acid (**3**) on PTP1B. The results indicated that the stereo configuration of C-3 β-hydroxyl is important because the C-3 epimer (**2**) with α-hydroxyl group has dramatically decreased activity. However, the stereo configuration of C-2 has no significant impact on PTP1B inhibition no matter the compound bears either α-hydroxyl or β-hydroxyl group. For comparison, MA has 3.3-fold selectivity for PTP1B over TCPTP. According to previous study, modifying of C-3 and C-28 of triterpenoids couldn't increase the selectivity between the two most homogeneous PTPs.[23] We postulate that modification on both C-2 and C-3 positions maybe helpful to increase the selectivity. In this Letter, SAR analysis of A-ring fused heterocyclic derivatives showed that six-member heterocyclic pyrazine-substituted and pyrimidine-substituted maslinic acid derivatives could significantly increase PTP1B inhibitory potency (**9**, **11**, **16**, **18** and **19**) with the exception of *N*-methyl pyrimidinyl derivative **15**. Most of the five-member heterocyclics have higher inhibitory potency on PTP1B than six-member heterocyclics do, with the exception of thiazole-substituted derivative **22** and isoxazole-substituted derivative **24**. Among these five-member heterocyclic compounds, **20** ($IC_{50}$ = 0.61 μM) and **29** ($IC_{50}$ = 0.64 μM) are the best two PTP1B inhibitors, about 10-fold more potent than lead compound MA. In addition, **29** is the most selective inhibitor (6.9-fold) for PTP1B over TCPTP. Therefore, modifying the five-member heterocyclics may provide the possibility to further improve inhibitory activity and selectivity.



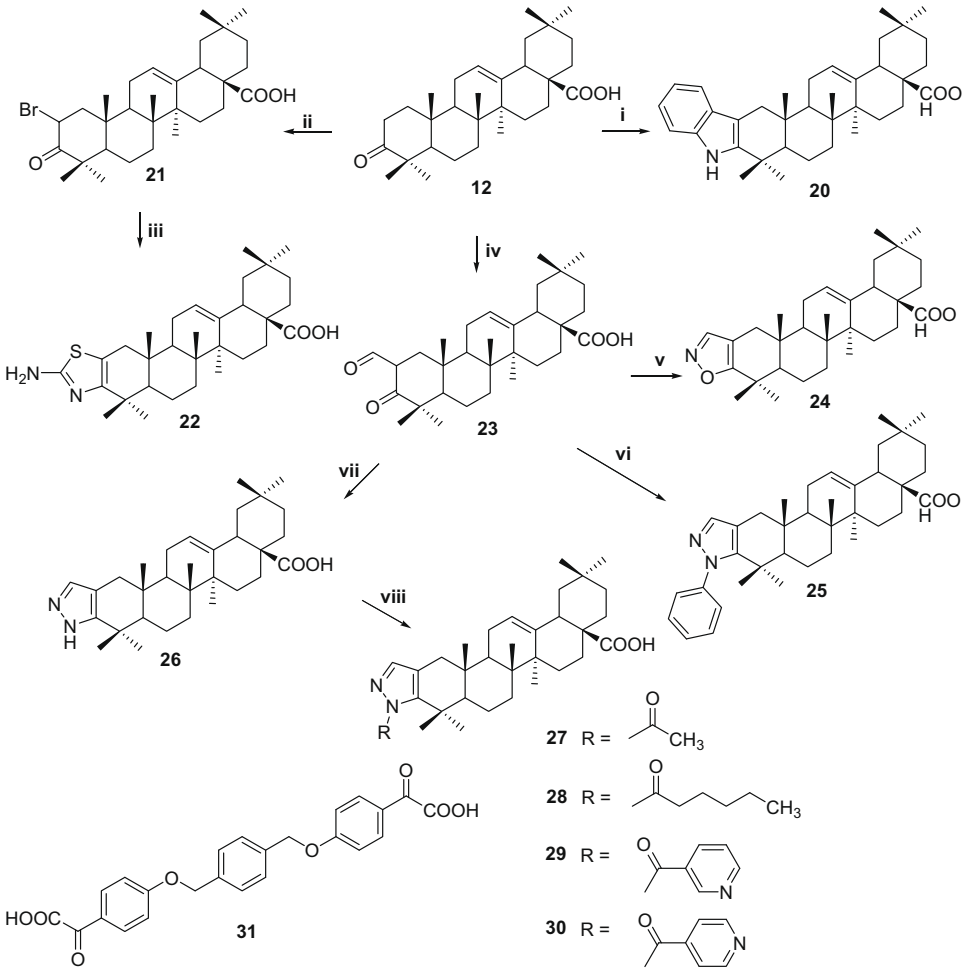

**Scheme 1.** Reagents and conditions: (i) BnCl, $K_2CO_3$, DMF, 100 °C, 3 h, (92%); (ii) PCC, DCM, rt, 12 h, (90%); (iii) *m*CPBA, concd $H_2SO_4$ (cat), MeOH-DCM, 10 °C, 20 h, (80%); (iv) $H_2$, Pd/C, MeOH, rt, 5 h, (98%); (v) NaOH, MeOH, rt, 12 h, (97%); (vi) phenylenediamine, EtOH, reflux, 3 h, (56%); (vii) ethylene diamine, EtOH, rt, 3 h, (95%); (viii) $MnO_2$, KOH, DMF, 120 °C, 6 h, (28%).




**Scheme 2.** Reagents and conditions: (i) PCC, DCM, rt, 12 h, (85%); (ii) *N*,*N*-dimethylformamide dimethyl acetal, TEA, toluene, reflux, 12 h, (78%); (iii) guanidine hydrochloride, NaOEt, EtOH, reflux, 3 h, 70%; (iv) methanimidamide acetic acid or ethanimidamide hydrochloride, NaOEt, EtOH, reflux, 3 h, R = H, (78%); R = Me, (74%); (v) LiI, anhydrous DMF, reflux, 48 h, **15** (12%); **18**, R = H, (74%); **19**, R = Me, (80%); (vi) LiI, *n*-octylamine, anhydrous DMF, reflux, (78%).

Some of synthetic heterocyclic compounds were also evaluated on homogenous enzymes LAR, SHP-1 and SHP-2. No indication of inhibition on these PTPs was found. It has been reported that oleanolic acid and its derivatives (with the modification on C-28 and C-3 positions of OA) only have 2–10-folds selectivity for PTP1B over SHP-1.[23] Compared to these reported compounds, the heterocyclic ring-substituted maslinic acid derivatives developed by our group clearly have better selectivity between the two homogenous enzymes.

In summary, we reported a series of novel maslinic acid analogs with various fused heterocyclic rings on C-2 and C-3 positions. These compounds have improved inhibitory activity on PTP1B and especially selectivity for PTP1B over TCPTP. This Letter provides some important information for discovering specific potent inhibitors of PTP1B by modifying naturally occurring triterpenoids. A broader SAR research on A-ring fused heterocyclic analogues of PTP1B inhibitors and the further evaluation in cell models of




**Scheme 3.** Reagents and conditions: (i) $C_6H_5NHNH_2$, TFA, HOAc, 100 °C, 20 h, (55%); (ii) pyridinium tribromide, HOAc, DCM, 0 °C to rt, 1 h, (82%); (iii) thiourea, anhydrous EtOH, reflux, 12 h, (63%); (iv) HCOOEt, MeONa, benzene, rt, 5 h, (94%); (v) hydroxylamine hydrochloride, EtOH/$H_2O$, reflux, 1 h, (85%); (vi) phenylhydrazine hydrochloride, EtOH/$H_2O$, reflux, 1 h, (65%); (vii) hydrazine hydrochloride, EtOH/$H_2O$, reflux, 1 h, (80%); (viii) RCOCl, pyridine, anhydrous DMF, rt, 3 h, for **27** (82%), **28** (85%), **29** (77%), **30** (80%).

**Table 2**
Inhibitory activity of selected maslinic acid derivatives against related PTPs

| Compounds | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | LAR | SHP-1 | SHP-2 |
| **1** | >40 | >40 | >40 |
| **9** | >40 | >40 | >40 |
| **11** | >40 | >40 | >40 |
| **18** | >40 | >40 | >40 |
| **22** | >40 | >40 | >40 |
| **24** | >40 | >40 | >40 |
| **25** | >40 | >40 | >40 |
| **26** | >40 | >40 | >40 |

enhancing insulin receptor phosphorylation and stimulating glucose uptake is ongoing and will be reported in due time.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.017.

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45. *Analytical data for compound* **29**: $^{1}$H NMR ($CDCl_3$, 500 MHz): $\delta$ 9.38 (1H, s, pyridine H-2), 8.78 (1H, d, $J$ = 4.0 Hz, pyridine H-6), 8.49 (1H, d, $J$ = 8.0 Hz, pyridine H-4), 8.03 (1H, s, pyrazole N=CH), 7.45 (1H, dd, $J$ = 4.0, 8.0 Hz, pyridine H-5), 5.35 (1H, s, H-12), 2.88 (1H, m, H-18), 2.72 (1H, d, $J$ = 16 Hz, H-1$\alpha$), 2.06 (1H, d, $J$ = 16 Hz, H-1$\beta$), 1.31 (3H, s), 1.23 (3H, s), 1.18 (3H, s), 0.95 (3H, s), 0.92 (3H, s), 0.86 (3H, s), 0.84 (3H, s); $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ 183.2, 164.1, 164.0, 152.3, 152.1, 143.8, 139.4, 128.5, 126.5, 122.9, 122.2, 119.7, 53.1, 46.5, 46.0, 45.9, 41.8, 41.1, 39.3, 38.1, 36.1, 34.5, 33.9, 33.1, 32.4, 32.0, 31.4, 30.7, 27.7, 25.7, 24.7, 23.6, 23.3, 23.0, 19.4, 16.7, 15.2; ESI-HRMS ($m/z$): $[M+H]^+$ calcd for $C_{37}H_{50}N_3O_3$, 584.3852; found 584.3843.
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