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Synthesis and biological evaluation of chepraecoxin A derivatives as α-glucosidase inhibitors

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ABSTRACT

The *ent*-kaurane diterpenoid chepraecoxin A (CA) obtained in our previous study showed a potential inhibitory activity on α-glucosidase (IC_{50} 274.5 ± 12.5 μM). In order to figure out the structure-activity relationships (SARs), twenty-two derivatives of chepraecoxin A were synthesized by modifying the ester, allyl, double bond and carboxyl groups, and assayed for their α-glucosidase inhibitory activity. Of them, eight compounds (**14**–**17**, **19**–**22**) significantly increased activity with IC_{50} values ranging from 16.1 to 71.4 μM, even higher than the positive control, acarbose (IC_{50} 130.3 μM). Especially, compounds **17**, **19** and **21** could inhibit α-glucosidase with IC_{50} values of 16.9 ± 3.4, 16.1 ± 1.2, and 17.1 ± 0.6 μM, 17-fold higher than CA. The most active compound **19** was proven to be a non-competitive inhibitor with a K_i value of 19.4 μM based on enzyme kinetics study. The primary SARs of CA derivatives were summarized for exploring antidiabetic candidates.

α-Glucosidase is a key enzyme for hydrolyzing carbohydrates to absorbable glucose and other monosaccharides in small intestines. Inhibitors of α-glucosidase can retard the digestion of carbohydrates, and thus, control the postprandial blood glucose.[1,2] Currently, three α-glucosidase inhibitors, acarbose, voglibose, and mioglitol, are available in the market for treating type 2 diabetes, whereas their application is hindered by the side effects of hepatotoxicity and gastrointestinal symptoms.[3]

Natural products are interesting resources for searching new α-glucosidase inhibitors. In our previous investigation, different types of compounds involving *ent*-atisane and *ent*-kaurane diterpenes from *Sapium insigne* and *Chelonopsis praecox*,[4,5] monoterpenes and triterpenes from *Mentha haplocalyx*,[6] and oligostilbenes from *Paeonia lactiflora*,[7] have been revealed with α-glucosidase inhibitory effects. Of these compounds, diterpenes attract our attention due to their diverse structures and antidiabetic potency.[8–14]

Our previous study showed that chepraecoxin A could inhibit α-glucosidase.[5] Structurally, CA consists of the functional groups of carboxyl, terminal double bond, and ester groups. In order to explore the structure-activity relationships and search new antidiabetic candidates, a series of CA derivatives were synthesized and evaluated for the inhibition on α-glucosidase.

To explore the role of the group on C-1 position as well as the carboxyl group (C-19) for inhibiting α-glucosidase, a series of derivatives were synthesized as shown in Scheme 1. Derivatives **1**–**3** were synthesized to reveal the effect of acetoxyl group on C-1 position. Hydrolysis of CA with NaOH delivered chepraecoxin B (**1**) in excellent yield. Subsequent oxidation of **1** using PCC afforded the ketone derivative **2** in moderate yield. When an EtOH/H_2O solution of **2** and hydroxyl -amine hydrochloride was heated in the presence of sodium acetate trihydrate, the oxime derivative **3** was obtained in 91% yield.[15] To clarify the effect of carboxyl group, the methyl ester derivative **4** was obtained in 96% yield by methyl esterification.[16]

As shown in Scheme 2, modification on the allyl position (C-15) and double bond ($\Delta_{16,17}$) gave derivatives **5**–**10**. Oxidation of CA with SeO_2 furnished allylic alcohol derivative **5**,[17] which was further oxidized to α,β-unsaturated ketone derivative **6** with Dess-Martin periodinane.[18] Compounds **7**–**9** were synthesized via epoxidation, dihydroxylation, and Lemieux-Johnson oxidation.[19–21] Alkene hydrogenation (H_2, Pd/C) of CA gave **10** as a single diastereomer.[22]

As shown in Table 1, hydrogenation product **10** showed higher activity against α-glucosidase than that of CA. Thus, further modification on **10** was conducted to develop more potent α-glucosidase inhibitors (Scheme 3). Esterification of carboxyl group yielded the methyl ester **11**; deacylation of compound **10** gave compound **12**, which was further converted to the ketone derivative **13** using PCC oxidation. In order to evaluate the influence of ester side chain on their α-glucosidase

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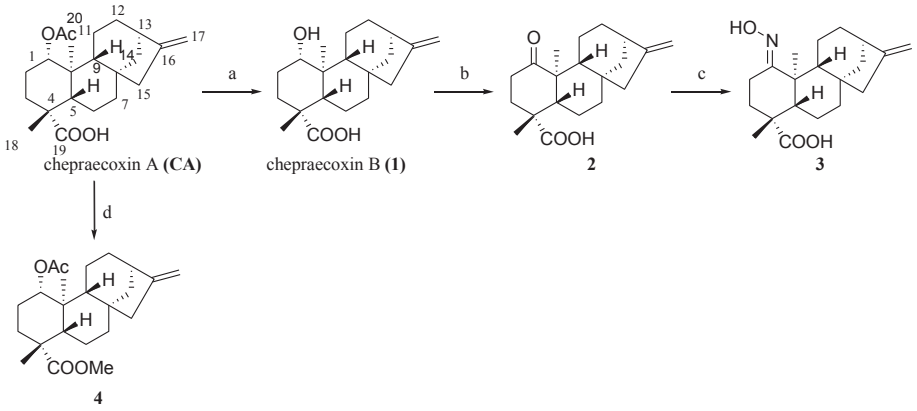
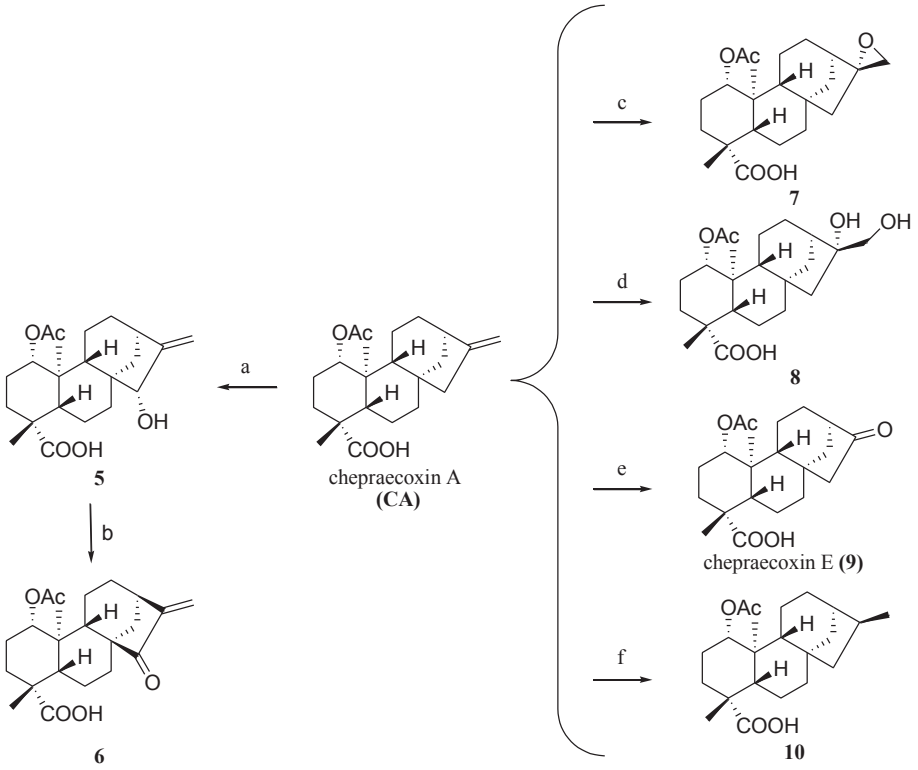


Scheme 1. Reagents and conditions: (a) 10% NaOH aqueous, MeOH, reflux, 97%; (b) PCC, CH_2Cl_2, r.t., 50%; (c) $NH_2OH{\cdot}HCl$, $CH_3COONa{\cdot}3H_2O$, EtOH/H_2O (10:1), reflux, 91%; (d) MeI, TBAF, dry THF, r.t., 96%.



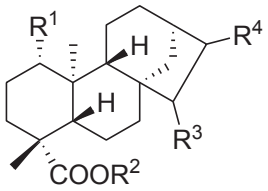
Scheme 2. Reagents and conditions: (a) SeO_2, *t*-BuOOH, CH_2Cl_2, r.t., 85%; (b) Dess-Martin periodinane, CH_2Cl_2, r.t., 90%; (c) *m*-CPBA, CH_2Cl_2, r.t., 86%; (d) OsO_4, NMO, acetone, r.t., 90% ; (e) OsO_4, $NaIO_4$, MeOH/H_2O (3:2), r.t., 68%; (f) Pd/C, H_2, EtOH, r.t., 89%.

inhibitory activity, nine derivatives (**14–22**) with different acyl groups were synthesized through the condensation of the alcohol **12** with various anhydrides or carboxylic acids.

The inhibitory activity of all the synthesized derivatives on *α*-glucosidase was tested with acarbose as the positive control. As depicted in Tables 1 and 2, the inhibition of derivatives was evaluated at the concentration of 500 μM, and if the inhibition rates were higher than 50%, the IC_{50} values were further measured by dose-response study at different concentrations.

When the acetoxyl group at C-1 position was converted to hydroxyl (**1**), carbonyl (**2**), and oxime (**3**) groups, their inhibitory activity for *α*-glucosidase significantly decreased, which indicated that the acetoxyl group at C-1 position was important for maintaining activity. Compared with CA, compound **4** exhibited weaker activity (45.4% *vs* 95.0%), suggesting the importance of carboxyl group. When different groups were introduced into the allyl position (C-15), the obtained compounds **5** and **6** were inactive at the concentration of 500 μM. The similar result was observed for the derivatives **7** and **8** synthesized by epoxidation and dihydroxylation. When the terminal double bond was changed to be carbonyl group, compound **9** showed similar activity with CA (78.8% *vs* 95.0%). Compound **10** was obtained by the reduction of double bond, and showed about a 2-fold increase in *α*-glucosidase inhibitory activity compared with CA. With the fact that compound **10** showed the most potent activity, 12 derivatives starting from **10** were further synthesized. When the carboxyl group was esterified, compound **11** lost the activity, further supporting the importance of carboxyl group. Similar with **1** and **2**, compounds **12** and **13** were inactive when the acetoxyl group at C-1 was changed to be hydroxyl or carbonyl group. By replacing the acetoxyl group with diverse acyloxys, all the derivatives **14–22** obviously increased activity, but with an exception

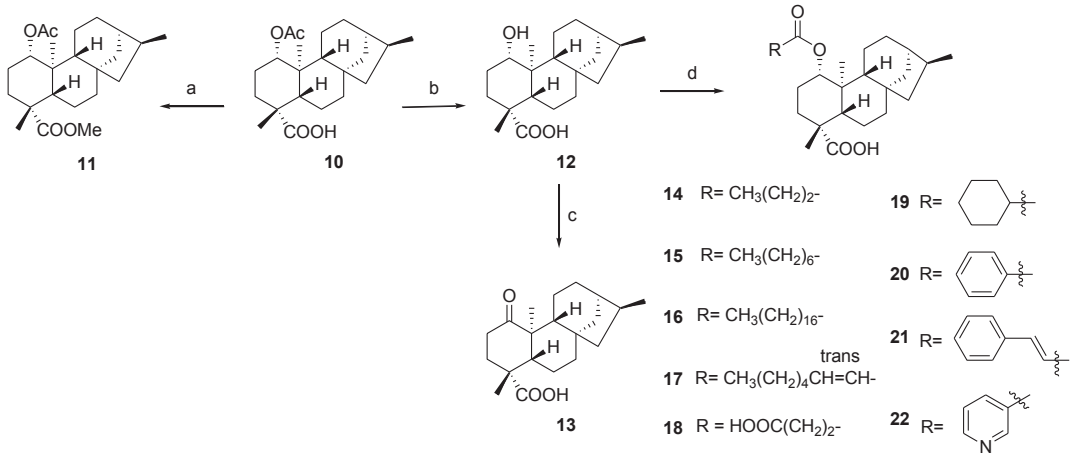
Table 1
α-Glucosidase inhibitory activity of derivatives **1–10**.[a]



chepraecoxin A**, 1-10**

Compounds	R^1	R^2	R^3	R^4	Inhibition (%)[b]	IC_{50} (μM)
chepraecoxin A	−OAc	H	H	$=CH_2$	95.0 ± 2.5	274.5 ± 12.5
1	−OH	H	H	$=CH_2$	52.0 ± 7.9	537.6 ± 16.1
2	=O	H	H	$=CH_2$	19.4 ± 0.5	–
3	=NOH	H	H	$=CH_2$	77.2 ± 6.7	329.9 ± 8.9
4	−OAc	Me	H	$=CH_2$	45.4 ± 5.6	–
5	−OAc	H	−OH	$=CH_2$	22.1 ± 4.4	–
6	−OAc	H	=O	$=CH_2$	18.6 ± 6.1	–
7	−OAc	H	H		9.6 ± 0.7	–
8	−OAc	H	H		7.5 ± 0.6	–
9	−OAc	H	H	=O	78.8 ± 2.5	261.5 ± 1.5
10	−OAc	H	H		98.4 ± 1.1	146.3 ± 11.8
acarbose[c]					79.8 ± 0.5	130.3 ± 1.5

[a] Data were expressed as means ± SD (n = 3).
[b] The tested concentration was 500 μM; [c] Acarbose was used as the positive control.



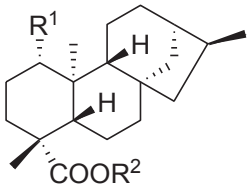
Scheme 3. Reagents and conditions: (a) MeI, TBAF, dry THF, r.t., 92%; (b) 10% NaOH aqueous, MeOH, reflux, 98%; (c) PCC, CH_2Cl_2, r.t., 61%; (d) DCC, DMAP, appropriate carboxylic acid, dry CH_2Cl_2, r.t., **15** (80%), **16** (74%), **17** (86%), **19** (74%), **21** (74%), **22** (56%) or appropirate anhydride, pyridine, r.t. or 80 °C, **14** (43%), **18** (51%), **20** (51%).

of **18**. Different from other derivatives, compound **18** maintained an additional carboxyl group, implying the polar group at C-1 was unfavorable for maintaining activity. Among these esterified derivatives, compounds **17**, **19** and **21** exhibited the most potent activity, with IC_{50} values of 16.9 ± 3.4, 16.1 ± 1.2, and 17.1 ± 0.6 μM, 17-fold higher than the substrate (IC_{50} 274.5 ± 12.5 μM). This investigation suggested that hydrophobic ester groups at C-1 position were important to increase activity, and were compatible for aliphatic, alicyclic, and aromatic side chains.

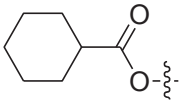
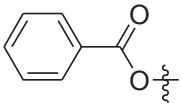
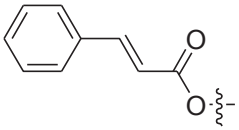
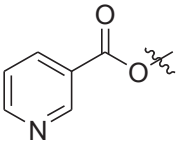
Compound **19** showed the highest activity against α-glucosidase, and thus, was performed enzyme kinetics study. As shown in the Lineweaver-Burk plot (Fig 1), the inhibition type of compound **19** was noncompetitive, for that V_m was decreased with the increasing of substrate concentration, but the k_m remained the same. The inhibition kinetic parameter (K_i) was determined to be 19.4 μM according to the Dixon plot.

In total, 22 derivatives of CA were synthesized and examined for their α-glucosidase inhibitory activity. Fourteen compounds (**1, 3–5, 9–10, 14–17, 19–22**) were active on α-glucosidase, of which eight compounds (**14–17, 19–22**) obviously increased activity, even higher than the positive control, acarbose. The primary structure-activity relationships were concluded as: (a) the carboxyl group (C-19) is crucial for maintaining α-glucosidase inhibitory activity; (b) hydrophilic groups on C-1 position result in the loss of activity; (c) additional

Table 2
. α-Glucosidase inhibitory activity of derivatives **10–22**.[a]



10-22

Compounds	R^1	R^2	Inhibition (%)[b]	IC_{50} (μM)
10	−OAc	H	98.4 ± 1.1	146.3 ± 11.8
11	−OAc	Me	11.4 ± 0.2	–
12	−OH	H	17.3 ± 0.2	–
13	=O	H	13.2 ± 3.1	–
14	$CH_3(CH_2)_2COO$–	H	98.0 ± 0.8	48.4 ± 2.2
15	$CH_3(CH_2)_6COO$-	H	95.5 ± 0.5	22.8 ± 2.4
16	$CH_3(CH_2)_{16}COO$-	H	94.2 ± 1.0	32.0 ± 0.4
17	(*E*) – $CH_3(CH_2)_4CH$ =CHCOO–	H	91.8 ± 0.8	16.9 ± 3.4
18	$HOOC(CH_2)_2COO$–	H	17.8 ± 5.5	–
19		H	99.5 ± 0.2	16.1 ± 1.2
20		H	96.4 ± 1.0	45.4 ± 7.4
21		H	97.4 ± 4.6	17.1 ± 0.6
22		H	93.0 ± 1.2	71.4 ± 0.7
acarbose[c]			79.8 ± 0.5	130.3 ± 1.5

[a] Data were expressed as means ± SD (n = 3).
[b] The tested concentration was 500 μM.
[c] Acarbose was used as the positive control.

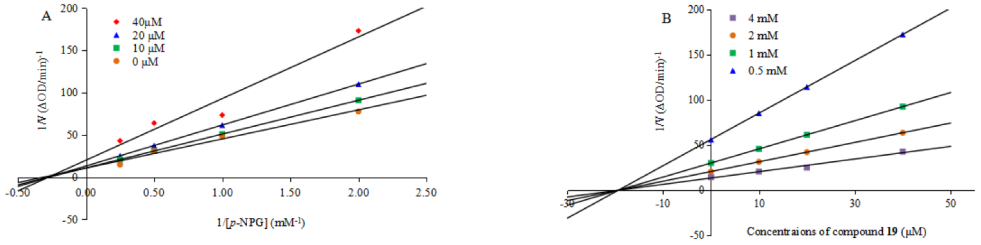


Fig. 1. α-glucosidase inhibition kinetics of compound **19.**

hydroxyl and carbonyl groups at the allyl position (C-15) are unfavorable; (d) the acyloxys at C-1 position are quite compatible for aliphatic, alicyclic, and aromatic side chains.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127020.

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