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Synthesis and Biological Evaluation of Ginsenoside Compound K Analogues as a Novel Class of Anti-asthmatic Agents

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ABSTRACT

Ginsenoside Compound K (CK) showed potent activity against IgE for the treatment of asthma. A series of CK analogues were then synthesized by straightforward procedures. The *in vivo* anti-IgE activity evaluations using the OVA-induced asthmatic mouse model revealed preliminary SARs of the CK analogues, which showed that the sugar type, modifications on A-ring and the C20 side chain of CK all affected much on the activities. Primary SARs optimization led to the discovery of compounds **T1**, **T2**, **T3**, **T8** and **T12**, which displayed superior or comparable anti-asthmatic effects (IgE value = 1237.11±106.28, 975.82±160.32, 1136.96±121.85, 1191.08±107.59 and 1258.27±148.70 ng/mL, respectively) in comparison with CK (1501.85±184.66 ng/mL). These potent compounds could serve as leads for further development.

Keywords: Ginsenoside Compound K, asthma, glycosylation, saponin, IgE

Asthma is a common, chronic inflammatory disease, characterized by lung inflammation, airway hyperresponsiveness (AHR) and mucus hypersecretion.[1] It is considered to be an important public health issue which affects more than 300 million people with a strong personal, social and economic impact.[2] However, there is no cure developed for asthma so far, which invariably relapse on ceasing the treatment. [3] The goal of asthma treatment is to achieve good control of symptoms, reduce exacerbations and improve quality of life. Now the Inhaled corticosteroids is still the pharmacotherapeutic cornerstone, [4] while systemic exposure to corticosteroids suffers from some adverse effects, including diabetes, hypertension, myopathies, and osteoporosis.[5] Therefore, it is necessary to find new drugs for the treatment of asthma. Allergic inflammation in asthma is associated with T-helper (Th2) cells and their cytokines (IL-4, -5, and -13). Both IL-4 and IL-13 induce the secretion of IgE by B cells, which mediates the type 1 hypersensitivity reactions and the orchestration of local chronic allergic inflammation.[6] 80% of childhood asthma and more than half of adult asthma were potentially mediated by IgE.[7] And anti-IgE omalizumab has been successfully used for more than 10 years in the treatment of asthma.[8] These facts validate IgE as an ideal target in the treatment of asthma.

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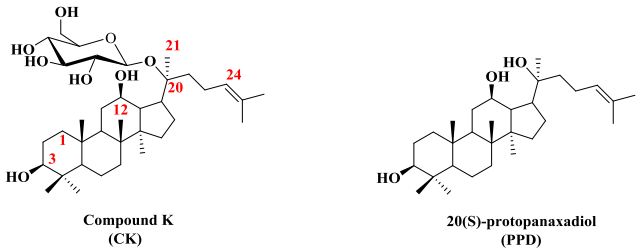


Figure 1. The structures of compound K and *20S*-protopanaxadiol

The current anti-IgE drugs are all biological agents, while there is no small molecule one reported. Fortunately, we recently discovered that a saponin, Ginsenoside Compound K (CK) (Figure 1), could significantly reduce IgE level in the serum and decrease the airway resistance on OVA (ovalbumin)-induced mouse model with asthma. CK, one of the active metabolites of *Panaxnotoginseng* saponins, has been reported to exhibite potent anti-inflammatory effect.[9, 10] In 2008, Yang *et al* reported that CK was a novel agonist of glucocorticoid receptor, which could compete with the synthetic glucocorticoid dexamethasone for binding to glucocorticoid receptor and activated glucocorticoid responsive element-containing reporter plasmids in a dose-dependent manner.[11] In addition, Ling *et al* reported that elevating glucocorticoid receptor level and promoting glucocorticoid receptor translocation by increasing the glucocorticoid effect may be the common mechanism of ginsenosides effects.[12] The potent anti-asthmatic mechanism of CK and its analogous may be related to glucocorticoid receptor. These findings encouraged us to make a systematic structure and activity relationship (SAR) studies on the potential effects of the CK analogues as anti-asthmatic agents. Herein we described our efforts on this project.

A series of CK analogues that all with a sugar head were designed as shown in Figure 2. To show if the sugar type plays a role on the activity, **T1**-**T5** that bearing different sugar rings were prepared. In addition, it's also easy to modify on the 3-OH, 12-OH and C20 side chain of CK, resulting designed compounds **T6**-**T14**. We believe that the syntheses and evaluations of these CK analogues would give us a preliminary but useful body of knowledge to tell if these kinds of compounds are worthy of further studies.

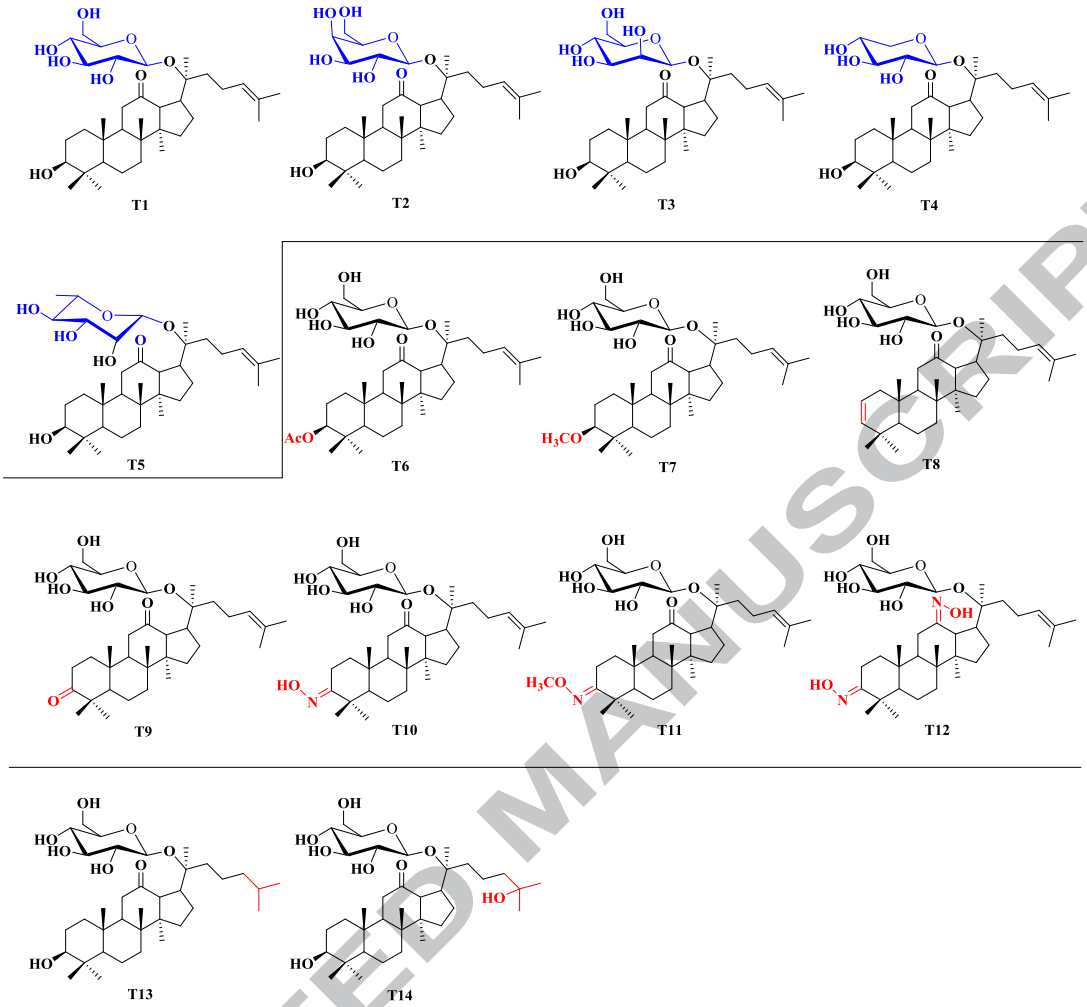
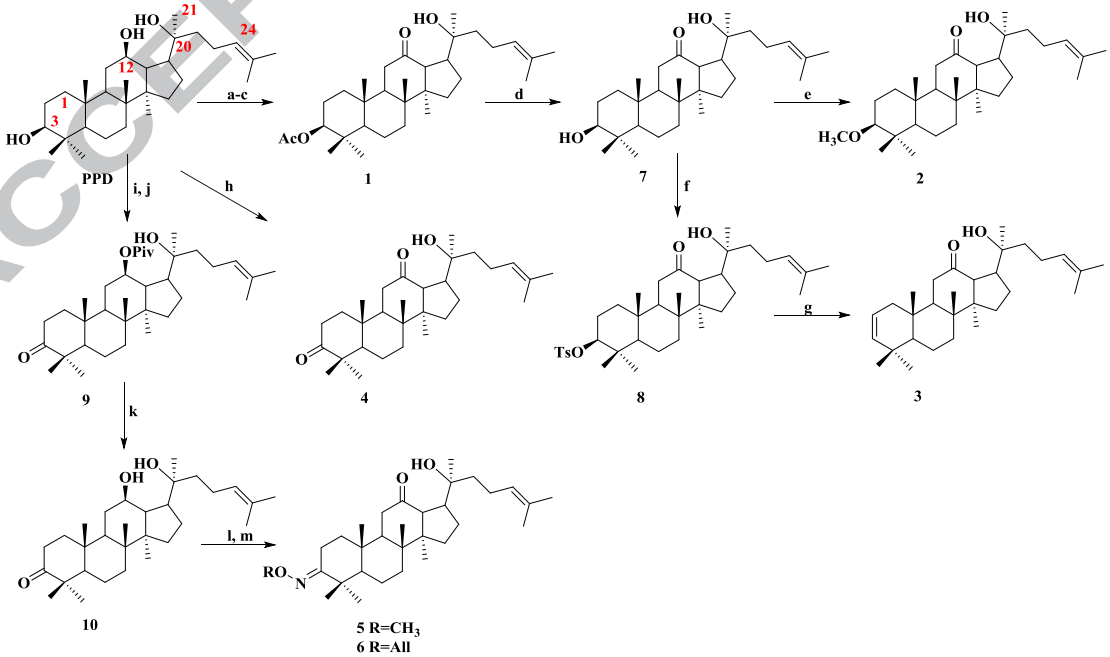


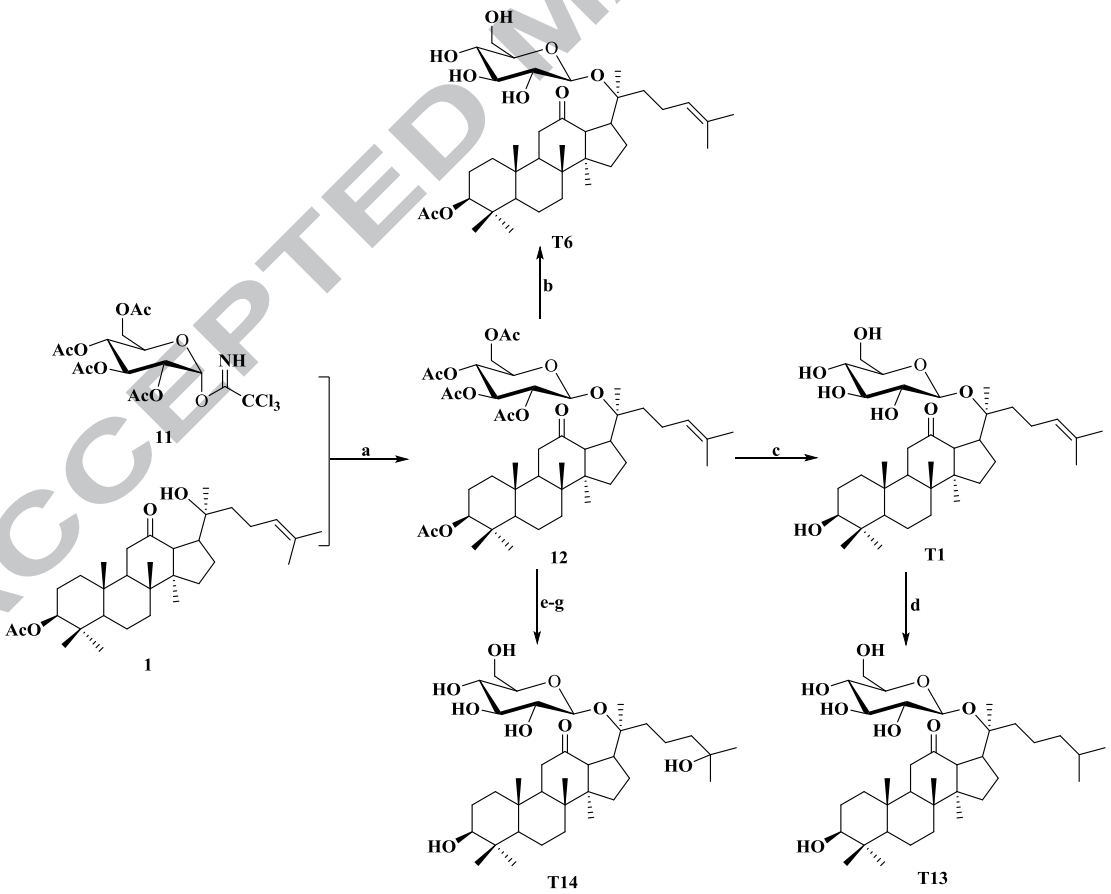
Figure 2. Designed CK analogues for biological evaluations.



Scheme 1. Synthesis of aglycones **1-6**. Reagents and conditions: (a) Ac_2O, DMAP, pyridine; (b)

$NaOCH_3$, CH_3OH-CH_2Cl_2; (c) PDC, Ac_2O, CH_2Cl_2, 49% for three steps; (d) $NaOCH_3$, CH_3OH-CH_2Cl_2, 50 °C, 96%; (e) CH_3I, NaH, DMF, 78%; (f) 4-Toluene sulfochloride, CH_2Cl_2, pyridine, 80 °C, 88%; (g) LiBr, Li_2CO_3, DMF, 153 °C, 84%; (h) PDC, Ac_2O, CH_2Cl_2, 61%; (i) PivCl, Et_3N, CH_2Cl_2; (j) PDC, Ac_2O, CH_2Cl_2; (k) $NaOCH_3$, CH_3OH-CH_2Cl_2, 50 °C, 40% for three steps; (l) O-Methylhydroxylamine hydrochloride or O-Allylhydroxylamine hydrochloride, pyridine, 80 °C; (m) PDC, Ac_2O, CH_2Cl_2, 46% for two steps for **5**, 65% for two steps for **6**.

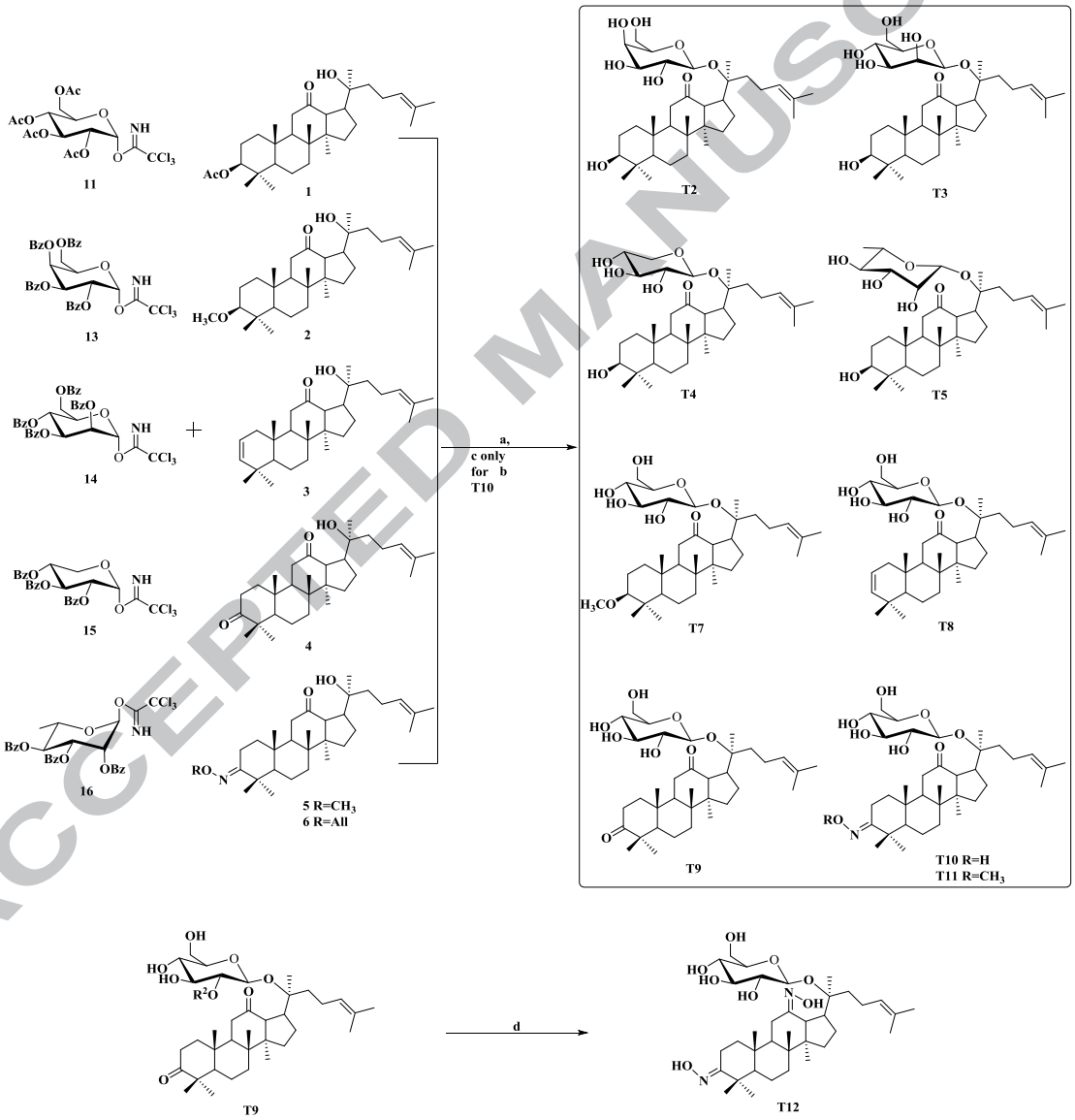
Our synthetic work commenced from (*20S*)-protopanaxadiol (PPD) which is much cheaper than CK and is more flexible to be modified. Scheme 1 described the syntheses of several aglycons that modified on the 3-OH of A-ring (compounds **1**-**6**). The common feature of compounds **1**-**6** is that they all have the 12-keto group, which is the key for the successful 20-OH glycosylation in the later stage. First, PPD was transformed into compound **1** by blocking the 3-OH with an acetyl group followed by oxidation of the 12-OH. [13] Replacing of the 3-*O*-acetyl group by methyl afforded compound **2**. Compound **7** was selectively tosylated on 3-OH, which was then eliminated to provide compound **8**. On the other hand, blocking of the 12-OH of PPD first with pivaloyl (Piv) followed by oxidation afforded compound **9**. Removal of the Piv group gave **10**. The 3-keto group of **10** further reacted with *O*-methylhydroxylamine or *O*-allylhydroxylamine to provid oxime derivative **5** or **6**, respectively. [14]



Scheme 2. Synthesis of compounds **T1**, **T6**, **T13 and T14**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, -40 °C; (b) $NaOCH_3$, CH_3OH-CH_2Cl_2, rt, 81% for two steps; (c) $NaOCH_3$, CH_3OH-CH_2Cl_2, 50 °C, 75% for two steps; (d) Pd-C, H_2, CH_3OH, 61%; (e) NBS, H_2O, THF; (f)

Pd-C, H_2, DIEA, EtOAc; (g) $NaOCH_3$, CH_3OH-CH_2Cl_2, 50 °C, 37% for four steps.

With the six aglycones in hand, the final products were synthesized according to the procedures as depicted in Schemes 2-3. The preparations of target compounds **T1**, **T6**, **T13** and **T14** were shown in Scheme 2. Glycosylation of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **11** catalyzed by TMSOTf led to **12** in a good yield. [15] Treated **12** with sodium methoxide in MeOH at room temperature only afforded compound **T6** with 3-*O*-acetyl intact, while prolonged reaction time at 50 °C further cleaved the 3-*O*-acetyl group, resulting **T1**. Hydrogenation of the double bond of **T1** gave **T13**. Treatment of **12** with NBS in water/THF mixture, debromination with Pd-C/H_2, and removal of all acetyl groups provided **T14**.



Scheme 3. Synthesis of compounds **T2-T12**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, -40 °C-0 °C; (b) $NaOCH_3$, CH_3OH-CH_2Cl_2, 50 °C, 70% for two steps for **T2**, 66% for two steps for **T3**, 63% for two steps for **T4**, 67% for two steps for **T5**; 52% for two steps for **T7**, 80% for two steps for **T8**, 39% for two steps for **T9**, 59% for two steps for **T11**; (c) $Pd(OAc)_2$, PPh_3, EtOH-H_2O, 40% for three steps; (d) Hydroxylamine hydrochloride, pyridine, 80 °C, 70%.

As shown in scheme 3, the syntheses of **T2-T11** are straightforward. In detail, aglycone **1** was glycosylated by 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate **13**, or the corresponding mannosyl, xylosyl and rhamnosyl type donor (**14-16**), providing **T2**, or **T3-T5**, respectively. [16, 17] On the other hand, when trichloroacetimidate **11** was employed as the glycosyl donor to react with aglycone **2-6**, the expected glycosides were all furnished in good yields. Removal the acyl protecting groups with sodium methoxide and allyl group with $Pd(OAc)_2$ (if needed) gave the designed CK analogues **T7-T11**. Subsequent condensation of **T9** with hydroxylamine hydrochloride in pyridine provided **T12**.

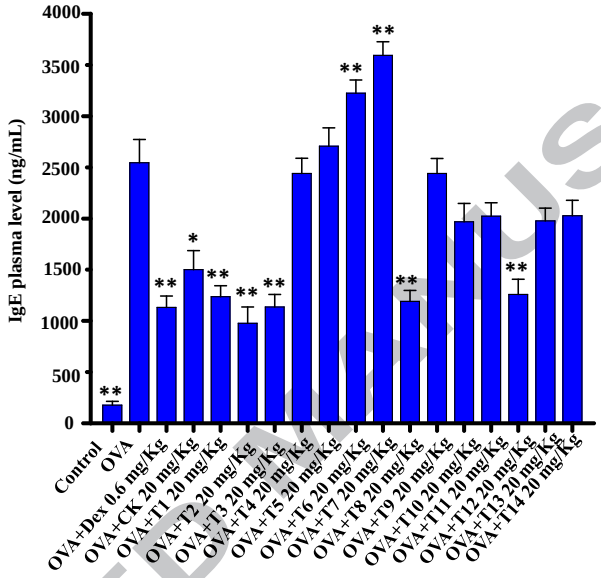


Figure 3. *In vivo* efficacies of all compounds on IgE level in plasma. CK and its analogues were orally administered daily for 7 consecutive days, Dex was orally given as a positive control. IgE level was measured 24 hours after the final OVA challenge. Results are expressed as the mean ± SEM (n = 5/group). SEM, standard error of the mean. *****p<0.05, ****** p<0.01 compared to OVA group.

The synthesized CK analogues were evaluated for their *in vivo* efficacies in mice that had been sensitized to an allergen (OVA). [18, 19] Dexamethasone acetate (Dex) was selected as the positive control. The results are summarized in Figure 3 (See details in ESI). It is worth to mention that compound **T1** with 12-carbonyl group exhibited enhanced potency of reducing IgE level in comparison with CK. Comparison of the activities of **T1-T5** bearing different sugar types revealed that the sugar moiety do play an important role. The galactopyranosyl and mannopyranosyl containing compounds **T2** and **T3** exhibited better activities than the glucopyranoside **T1**. While the rhamonopyranosy and xylopyranose containing compounds **T4** and **T5** showed almost no anti-IgE effect. In addition, modifications on A-ring also led to different activities. 3-*O*-acetyl and 3-*O*-methoxyl compounds **T6** and **T7** showed obviously increased effect on the IgE, while 3-keto (**T9**) and 3-oxime (**T10** and **T11**) analogues almost lost the activity. The Δ3 (**T8**) and dioxime (**T12**) compounds played a similar activity to CK. Furthermore, the importance of the double bond on the C20 side chain of CK was clearly revealed. Compounds **T13** and **T14** both displayed very weak anti-IgE activity.

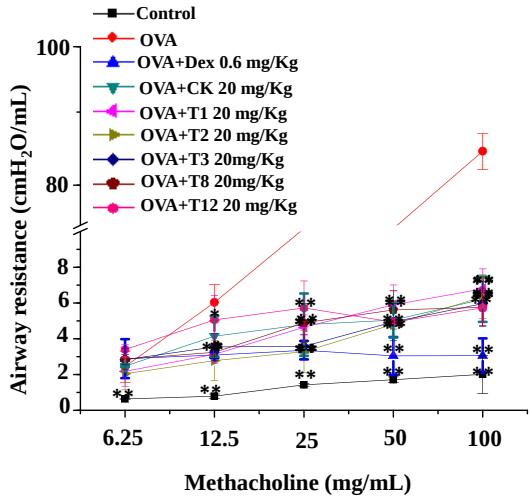


Figure 4. *In vivo* efficacies of some representative compounds on AHR to methacholine. Results are expressed as the mean ± SEM (n = 5/group). SEM, standard error of the mean. *****p<0.05, ****** p<0.01 compared to OVA group.

Airway hyperresponsiveness (AHR) is referred as one of the major hallmark features of asthma. Therefore, decreasing airway resistance is one of the goals to treat asthma.[20] Methacholine was used to induce AHR. The bronchomotor response to methacholine leads to contraction of distal airways and focal airway closure in select lung territories. As shown in Figure 4 (See details in ESI), the OVA group developed significantly greater airway resistance than the control group at methacholine doses of 25, 50 and 100 mg/mL. Based on the above ant-IgE evaluation, compounds **T1**, **T2**, **T3**, **T8**, **T12** as well as CK were selected to conduct the AHR experiment. To our delight, all of these compounds (20 mg/Kg) remarkably decreased airway resistance in mice as efficacious as 0.6 mg/Kg of DEX.

In summary, CK was first discovered to show potent activity against IgE for the treatment of asthma. A series of CK analogues were synthesized by straightforward procedures. The synthesized compounds were evaluated *in vivo* on the anti-IgE activities using OVA-induced asthmatic mouse model and the *in vivo* airway hyperresponsiveness experiment. We found that compounds **T1**, **T2**, **T3**, **T8** and **T12** led to a significant decrease in IgE and airway resistance. Mode of action and further optimization of this novel class of anti-asthmatic agents are currently under investigation.

Acknowledgements

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Supplementary data

Supplementary data is available on the publishers' web site along with the published article.

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Highlights

1. CK showed potent activity against IgE for the treatment of asthma.
2. Fourteen CK analogues were synthesized and evaluated for their *in vivo* ant i-asthmatic activity.
3. Their structure-activity relationships were discussed.
4. Five compounds exhibited excellent anti-asthmatic effects.

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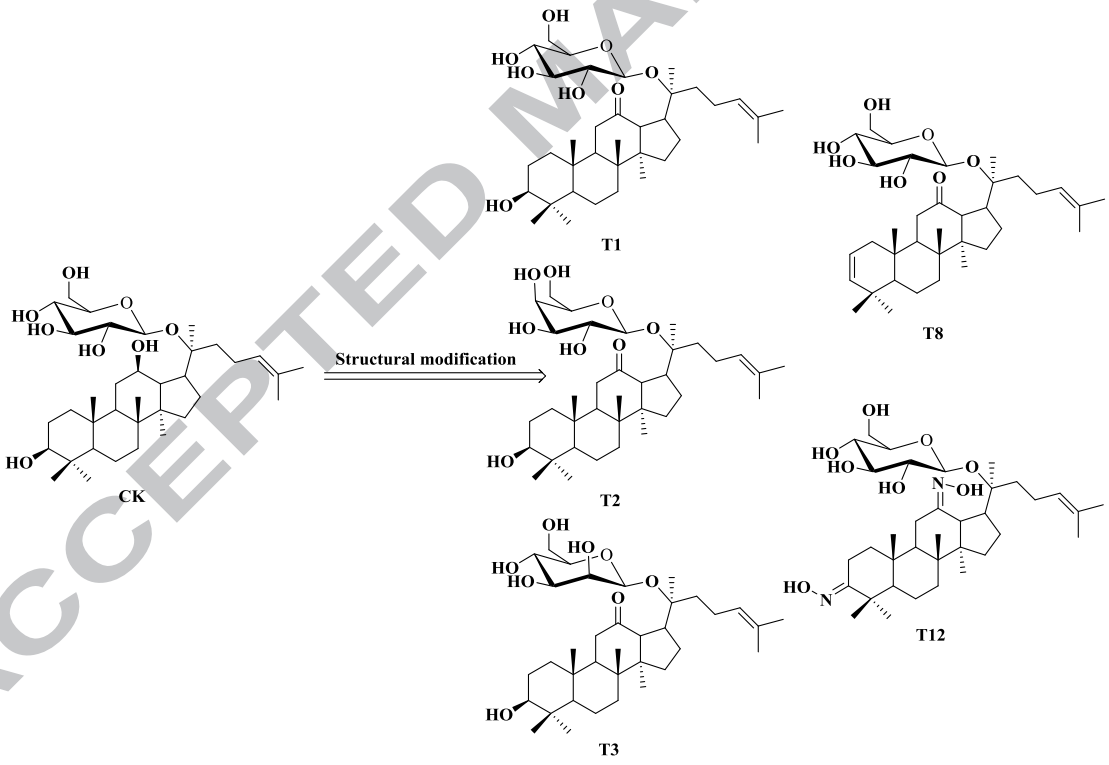

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