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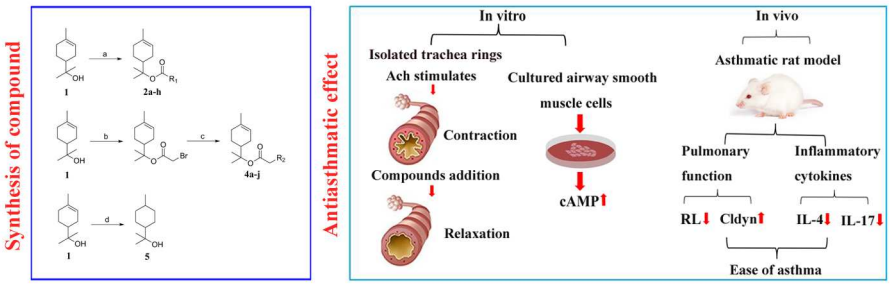


Discovery of a novel series of α-terpineol derivatives as promising anti-asthmatic agents: their design, synthesis, and biological evaluation

Wanping Zhu [a,b], Xia Liu[a], Yuji Wang[a], Yeling Tong[a], Yongzhou Hu[b*]

[a]Zhejiang Academy of Traditional Chinese Medicine, Hangzhou 310007, China

[b]ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang University, Hangzhou 310058, China

* Corresponding author. Tel./fax: 86 571 88208460.

E-mail addresses: huyz@zju.edu.cn (Y. Hu).

Abstract

A series of novel α-terpineol derivatives were designed and synthesized through structural derivatization of the tertiary hydroxyl moiety or reduction of the double bond. Of the resulting compounds, eight compounds enhanced relaxation of airway smooth muscle (ASM) compared to the α-terpineol precursor, and four compounds（ **4a**, **4d**, **4e,** and **4i**）were superior or comparable to aminophylline at a concentration of 0.75mmol/L. Assays for 3'-5'-Cyclic adenosine monophpsphate (cAMP) activation revealed that some representative α-terpineol derivatives in this series were capable of upregulating the level of cAMP in ASM cells. Further *in vivo* investigation using the asthmatic rat model, illustrated that treatment with the compounds **4a** and **4e** resulted in significantly lowered lung resistance (RL) and enhanced dynamic lung compliance (Cldyn), two important parameters for lung fuction. Moreover, treatment with **4e** downregulated the levels of both IL-4 and IL-17. Due to its several favorable physiological functions, including ASM relaxation activity, cAMP activation capability, and *in vivo* anti-asthmatic efficacy, **4e** is a promising remedy for bronchial asthma, meriting extensive development.

Key words:α-terpineol derivatives, ASM relaxation activity, cAMP activation assay, asthmatic rat model

1. Inroduction

Bronchial asthma is a common, chronic respiratory disease affecting 1-18% of the population in different countries [1], and is characterized by bronchoconstriction, airway hyperresponsiveness, mucus secretion, and chronic inflammation. Of these symptoms, the decreased lung function observed

in bronchial asthma is due to the contraction of airway smooth muscle (ASM) and chronic inflammation [2-4]. It is not only a serious influence on people's normal life, even a life threatening to patients. However, there's no a radical cure means for asthma treatment so far. The treatment and prevention of asthma is only by effective drug to improve symptoms [5].

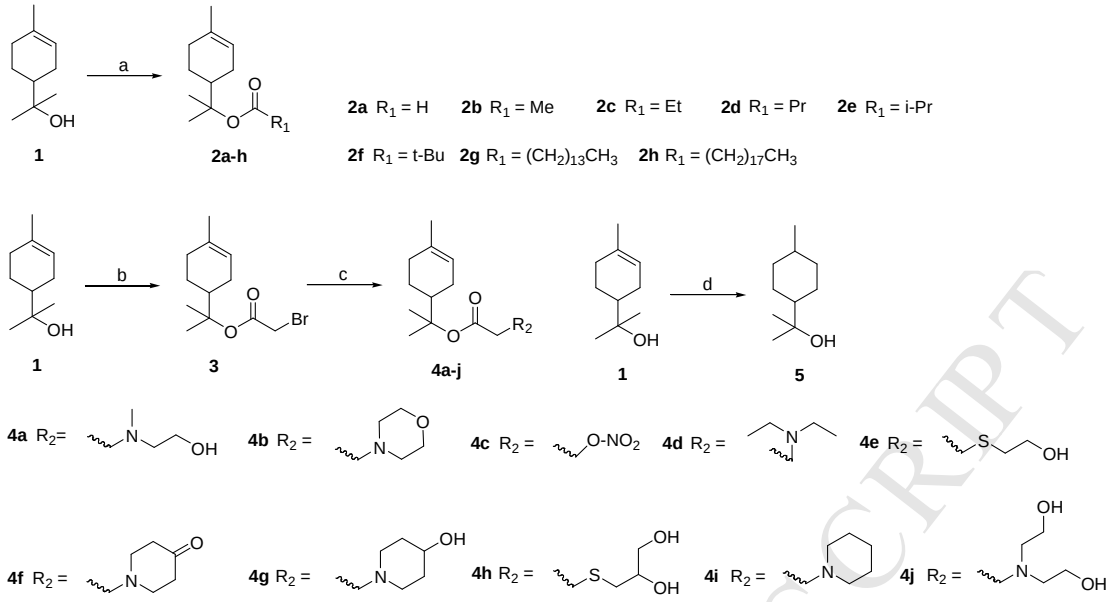
Therefore, it is common to combine an anti-inflammatory inhaled corticosteroid and an antispasmodic β-adrenergic agonist for battling the symptoms or exacerbations of bronchial asthma.

In spite of their therapeutic effects, both corticosteroids and β-adrenergic agonists suffer from some inevitable shortcomings. For instance, the adminstration of corticosteroids frequently culminates in oral candidiasis, dysphonia, adrenal suppression, and growth suppression [6]. Meanwhile, in addition to the tremor that may be caused by stimulation of the β_2 receptor in skeletal muscle, treatment with β-adrenergic agonists results in increased heart rate and palpitations, arrhythmias, myocardial ischemia, and hypomagnesemia[7]. Moreover, nearly 30% of patients in the Gaining Optimal Asthma control (GOAL) study failed to maintain asthma control despite regular administration of high-dose fluticasone and salmeterol [8].

As the active ingredient derived from the dry leaves of *Artemisia argyi* Levl. et Vant., α-terpineol (α-T) has multiple pharmacological functions, including relaxation of tracheal smooth muscle, as well as the expectorant, antitussive, anti-inflammatory, and anti-allergic effects [9]. Although α-terpineol didn't show the adverse effects of β_2-agonists byaerosol inhalation for the treatment of asthma, the short half-life limited its application at clinic. Herein, a novel series of ester derivatives of α-terpineol were designed and prepared with the goal of pursuing more favorable biological properties, such as improved bioavailability and elongated elimination half-life. All of the α-terpineol derivatives were evaluated biologically for their ASM relaxation activity. Furthermore, some were investigated for their capability to upregulate cAMP as well as their efficacy *in vivo* using an asthmatic rat model.

2.Results and disscussion

2.1 Chemistry



Scheme 1. (a) corresponding anhydride, TsCl, rt, 1.5 h or corresponding acyl chloride, pyridine, DCM, rt, 8 h; (b) bromoacetyl bromide, pyridine, DCM, rt, 7 h; (c) corresponding secondary amine, mercaptan or nitric acid, acetonitrile, K_2CO_3, rt, 5 h; (d) Pd/C, H_2, EtOH, 12 h.

The preparation of compounds **2a-h**, **4a-j,** and **5** was outlined in scheme 1. Condensation of α-T **1** with different anhydrides or acyl chlorides provided the alkyl carboxylate derivatives **2a-h**. Compounds **4a-j** were synthesized through the condensation of compound **1** with bromoacetyl bromide, followed by nucleophilic substitution at the α-position of the ester carbonyl functionality of **3,** using the corresponding secondary amine, mercaptan, or nitric acid as a reactant. Additionally, catalytic hydrogenation was used to reduce the double bond of **1** resulting in target compound **5**.

2.2 ASM relaxation assay

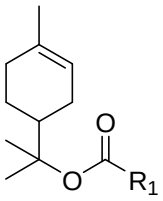
The hyperresponsiveness of airway, namely the excessive contraction of airway, is a risk factor for the development of asthma, and is also an important endpoint evaluation index for the efficacy of asthma. Therefore, medication by alleviating the excessive contraction of the bronchial smooth muscle (i.e., the high reactivity of airway) that is one of the targets to treat asthma. The relaxation efficacy of all of the target compounds were evaluated *via* ASM relaxation assay using aminophylline, a anti-asthmatic agent in clinic, and α-terpineol as the references. The results are

summarized in Table 1,Fig. 1 and 2.

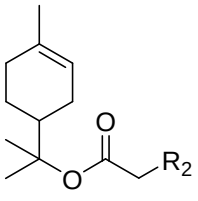
As shown in Table 1, Fig. 1 and 2 these α-terpineol derivatives exhibited relaxation activity in a dose-dependent manner. Among them, half of the tested compounds showed relaxation effects with similar or more potency to that of α-terpineol. Four compounds（**4a**, **4d**, **4e** and **4i**） displayed superior or comparable relaxation effects to that of aminophylline.

In general, saturated fatty acid esters of α-terpineol (**2a** and **2c-h**) displayed unfavorable activity, with the exception of the acetic ester **2b**. Introduction of some hydrophilic substituents at the acid fragment , which boosted the relaxation effect. For example, compounds **4a** and **4e** with a hydroxyethyl amino or hydroxyethyl sulfide moiety at the end of the acid fragment showed the most favorable effect on enhancing ASM relaxant activity, whereas compounds 4c and 4d with a nitrooxy or diethylamino moiety at the end of the acid fragment showed weak relaxant activities. Compounds **4h** and **4j** with two hydroxyl at the acid fragment didn't exhibit enhanced activity much further. On the other hand, among the alicyclic heterocyclic derivatives of α-terpineol, compounds **4g** with 4-hydroxypiperidine at the end of acid fragment showed preferable relaxant activity . When the hydroxyl group of **4g** was oxidized to a carbonyl group (**4f**), a significant decrease in relaxant activity was observed. Compounds **4b, 4i** with morpholine or piperidine ring at the end of acid fragment displayed better relaxant activities. These results suggested that one hydroxy at the end of acid fragment had a significant effect on relaxant activity. Because of the anti-asthmatic active fragment of α-terpineol is not clear, compound **5,** a reduction product of α-terpineol, was synthesized. It showed the similar relaxation activity as that of α-terpineol . This result seemed that the existence of the ethylenic bond has little influence on the relaxation of the tracheal smooth muscle of the guinea pig.

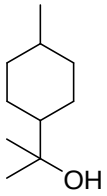
Table 1. The effect of target compounds on the relaxation of isolated trachea rings



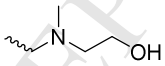
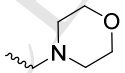
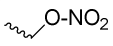
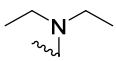
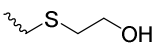
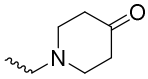
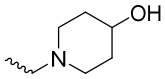
2a-h

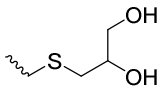
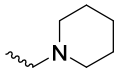
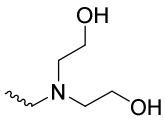


4a-j



5

Cpd.	R_1 or R_2	Contraction%	Relaxation%	
			0.75 mmol/L	1.25 mmol/L
Control		188.73±75.91	7.73±4.81	13.59±9.14
Aminophylline		197.16±91.98	43.67±10.20	56.70±9.26
α-T		166.15±52.48	28.40±16.13	38.35±13.62
2a	H	175.74±72.17	14.62±4.24	21.57±7.04
2b	Me	142.94±26.13	34.35±14.72	47.72±15.94
2c	Et	147.23±62.18	18.18±6.26	23.22±4.39
2d	Pr	124.72±51.92	21.20±9.24	25.47±8.69
2e	iPr	162.62±59.82	19.88±8.82	22.79±8.27
2f	tBu	131.91±40.22	25.49±6.53	28.02±8.83
2g	$(CH_2)_{13}CH_3$	171.05±73.10	17.56±2.89	16.55±14.47
2h	$(CH_2)_{17}CH_3$	184.01±99.32	15.4±9.83	10.63±13.77
4a		152.33±86.19	50.24±7.30$^{\triangle}$	76.85±7.70$^{**\triangle\triangle}$
4b		157.28±30.13	36.60±13.79	59.35±14.66$^{\triangle}$
4c		122.40±83.88	17.13±6.98	10.84±4.46
4d		107.29±28.93	42.33±10.45	46.71±3.56
4e		138.31±52.89	48.29±9.36$^{\triangle}$	81.09±10.15$^{**\triangle\triangle}$
4f		181.84±94.98	20.85±7.55	43.81±10.16
4g		174.77±74.96	35.17±13.87	71.53±15.45$^{*\triangle\triangle}$

4h		173.96±71.23	34.84±19.74	65.29±13.43$^{\Delta}$
4i		174.20±52.52	42.49±6.14	52.21±6.98$^{\Delta}$
4j		103.55±15.95	27.74±2.08	61.01±5.07$^{\Delta}$
5		165.78±96.91	27.16±14.10	41.28±13.17

The values are presented as means±S.D (n=10)

* p<0.05, compared to aminophylline;

** p<0.01, compared to aminophylline;

Δp<0.05, compared to α-terpineol;

$\Delta\Delta$ p<0.01, compared to α-terpineol;

Contraction (%) = $(N_{ACH} - N_e)/N_e \times 100\%$;

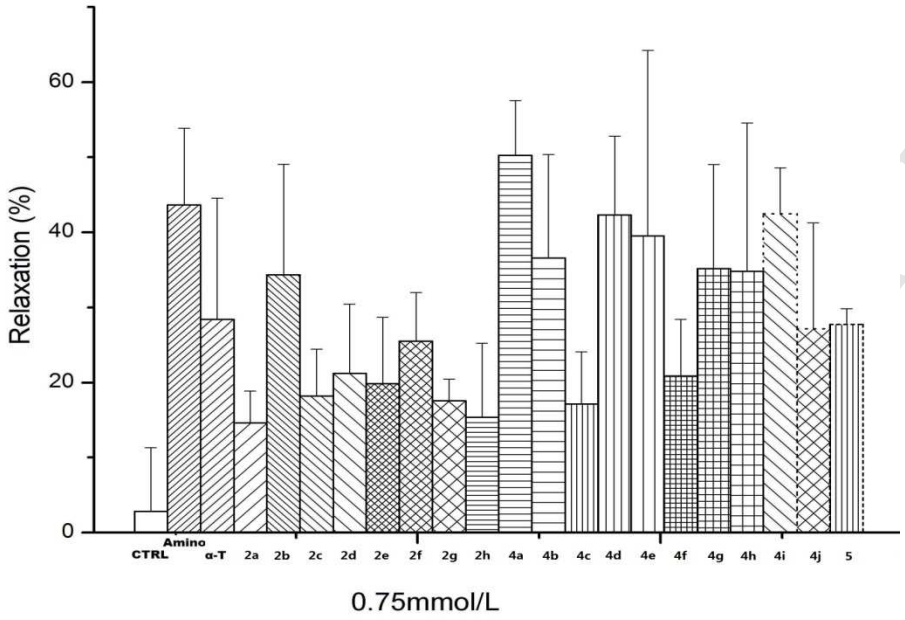
Relaxation (%) = $(N_{ACH} - N_{drug})/N_{ACH} \times 100\%$;

N_e (g): the average tension of the tracheal ring after equilibration;

N_{ACH} (g): the average tension of the tracheal ring after acetylcholine (ACH) exposure;

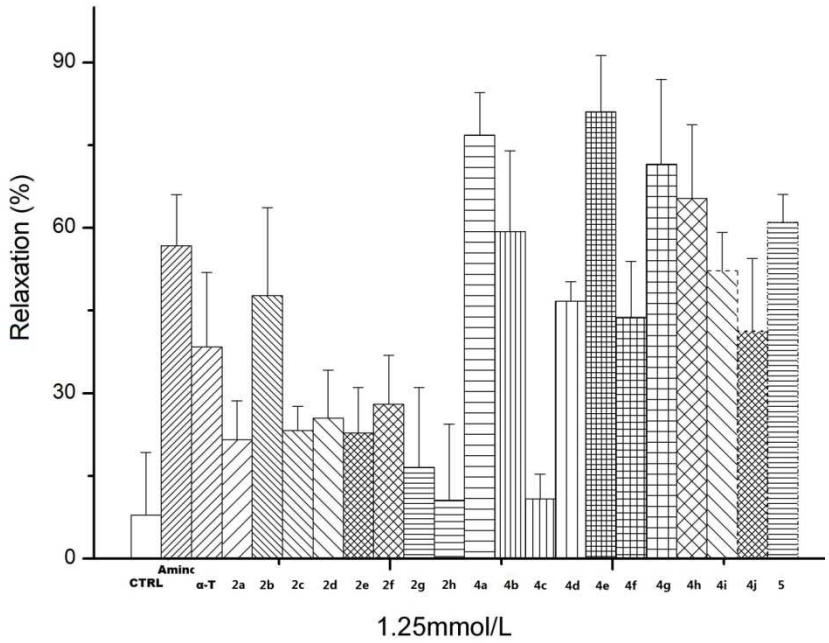
N_{drug} (g): the average tension of the tracheal ring after compound treatment

Fig. 1. Relaxation activity at the concentration of 0.75 mmol/L



The values are presented as means±S.D (n=10)

Fig. 2. Relaxation activity at the concentration of 1.25 mmol/l



The values are presented as means±S.D (n=10)

2.3 cAMP activation assay

cAMP is the second messenger in the cell, which play key roles in bronchial smooth muscle relaxation, stability of mast cell, and the bronchial cilia movement [10-11]. Based on the results of the ASM relaxation assay, the representative α-terpineol derivatives **4a**, **4b**, **4d**, **4e**, **4g**, **4h** and **4j,** were further assessed for their capability of upregulating cAMP in ASM cells. As illustrated in Table 2, all of these compounds increased the release of cAMP in ASM cells, at levels significantly higher than that of the model group (p<0.05). Contrastingly, neither α-terpineol nor aminophylline treatment led to a remarkable release of cAMP.

Based on the above experimental results, it is noteworthy that the ASM relaxation effect of tested compounds is possible by promoting the release of cAMP.

Table 2. The effect of some representative compounds on the cAMP level in rat bronchial smooth muscle cells

Groups	cAMP (nmol/L)
Model	8.402±0.041
Control	9.003±0.071
α-T	8.510±0.095
4a	9.012±0.056$^{\triangle}$
4b	8.815±0.186$^{\triangle}$
4d	9.532±0.094$^{\triangle}$
4e	8.824±0.121$^{\triangle}$
4g	8.985±0.219$^{\triangle}$
4h	8.761±0.071$^{\triangle}$
4j	8.941±0.135$^{\triangle}$
aminophylline	8.600±0.108

The values are presented as means±S.D (n=6)

Significant differences compared to model group are designated as $^{\triangle}P$<0.05.

2.4 Anti-asthmatic assay

The two most promising compounds in the ASM relaxation assay, **4a** and **4e**, were investigated extensively for their anti-asthmatic effect in the ovalbumin-induced asthmatic rat model.α-Terpineol and dexamethasone acetate were used as positive control. The smooth wheezing effect of compounds was assessed via two lung function parameters, airway resistance (RL) and the dynamic compliance of the airway (Cldyn). While the former reflects the resistance of the respiratory tract to airflow during inspiration and expiration, the latter is involved with the lung's ability to stretch and expand during actual movement of air. In addition, the assay also evaluated the levels of IL-4 and IL-17, which serve as asthma proinflammatory cytokines and play vital roles in recruiting eosinophils and neutrophils, respectively.

As shown in Table 3, treatment with **4a** or **4e** led to significantly lowered RL and enhanced Cldyn than that ofα-terpineol and the model group. Moreover, IL-4 and IL-17 levels were down regulated following **4e** treatment in contrast to the model group. Therefore, compound **4e** displayed similar or more potent anti-asthmatic effect in comparison with dexamethasone acetate and α-terpineol.

Table 3. The anti-asthmatic effect of compounds 4a and 4e

Groups	Cldyn	RL	IL-14 (pg/mL)	IL-17 (pg/mL)
Model	0.46±0.17	67.33±10.54	16.04±1.41	16.85±1.13
Control	0.79±0.29	41.97±12.85	14.49±1.52	15.52±1.22
a-T	0.53±0.08	60.57±15.73	15.50±1.48	16.19±0.92
4a	0.84±0.35$^{\triangle}$	51.69±21.27$^{\triangle}$	15.66±2.68	16.35±0.97
4e	0.88±0.29$^{\triangle}$	41.69±8.48$^{\triangle\triangle}$	14.00±2.06$^{\triangle}$	15.09±1.45$^{\triangle\triangle}$
Dexamethasone Acetate	0.66±0.04$^{\triangle}$	41.51±18.75$^{\triangle\triangle}$	14.31±0.90$^{\triangle\triangle}$	16.06±1.25

The values are presented as means±S.D (n=10)

Significant differences compared to model group are designated as $^{\triangle}P<0.05$ and $^{\triangle\triangle}p<0.01$

3. Conclusions

A novel series ofα-terpineol derivatives were designed and syntheized *via* prodrug formation. Eight of the resulting compounds demonstrated enhanced ASM relaxation activity over that of α-terpineol, and four compounds, including **4a**, **4d**, **4e,** and **4i**, were superior or comparable to aminophylline at the concentration of 0.75mmol/L. In addition, the results of cAMP activation assays from some representative α-terpineol derivatives illustrated that all the tested compounds were capable of upregulating cAMP in ASM cells. We further investigated the anti-asthmatic properties of these compounds using the ovalbumin-induced asthmatic rat model, and found that treatment with **4a** or **4e** led to a significant decrease in RL and enhanced Cldyn. Moreover, both IL-4 and IL-17 levels were downregulated following the adminstration of **4e**. By virtue of its favorable ASM relaxation activity, cAMP activation capability, and anti-asthmaticeffect, **4e** merits extensive development as a promising remedy for bronchial asthma.

4. Experimental

4.1.Chemistry

^{1}H NMR spectra were recorded on a 500 MHz and 400 MHz spectrometer.^{13}CNMR were recorded on a 125 MHz and 100 MHz spectrometer (chemical shifts are given in ppm (δ) relative to TMS as the internal standard, coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet, etc.). Mass spectral data were obtained on an Esquire-LC-00075 spectrometer.

4.1.1 General procedure for the synthesis of compounds 2a-f

A mixture ofα-terpineol (**1**, 2 mmol), acid anhydride (3 mL) and TsCl (0.2 mmol) was stirred at room temperaturefor 1.5 h, and then neutralized to pH7-8 with 10% NaOH aqueous solution, washed with water (2×10 mL) and brine (10 mL). The organic layer was separated and dried over anhydrous Na_2SO_4, then concentrated and purified by silica gel column chromatography (petroleum ether/ethyl acetate=1:5 v/v) to afford the desired products.

4.1.1.1 2-(4-methylcyclohex-3-enyl)propan-2-yl formate (2a)

colorless oily liquid (15.5%).^{1}H NMR (500 MHz, $CDCl_3$)δ 8.04 (s, 1H), 5.36 (s, 1H), 2.05-1.96 (m, 4H), 1.84-1.80 (m, 2H), 1.64 (s, 3H), 1.47 (s,3H),1.46(s,3H) 1.34-1.26 (m,1H);

^{13}C NMR (125 MHz, $CDCl_3$)δ 160.82, 134.17, 120.18, 86.28, 43.02, 30.9, 26.45,23.95, 23.87, 23.49,23.43.ESI-MS: m/z=205 $[M+Na]^+$

4.1.1.2 2-(4-methylcyclohex-3-enyl)propan-2-yl acetate (2b)

colorlessoil (63.9%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 2.08-1.97 (m, 4H), 1.92 (s, 3H), 1.76-1.86 (m, 2H), 1.64 (s, 3H), 1.44 (s, 3H), 1.41 (s, 3H), 1.33-1.25 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.45, 133.90, 120.38, 84.84, 42.64, 30.93, 26.42, 23.91, 23.35, 23.31, 23.13, 22.45.ESI-MS: m/z=219 $[M+Na]^+$

4.1.1.3 2-(4-methylcyclohex-3-enyl)propan-2-yl propionate (2c)

colorless oil (57.2%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 2.23 (q, J = 7.6 Hz, 2H), 2.05-1.91(m, 4H), 1.85-1.76 (m, 2H),1.63 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H), 1.33-1.24 (m, 1H), 1.08 (t, J = 7.6 Hz, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ 173.90, 134.04, 120.48, 84.58, 42.88, 31.04, 29.02, 26.48, 24.00, 23.48, 23.42, 23.32, 9.45.ESI-MS: m/z=233 [M+Na]+

4.1.1.4 2-(4-methylcyclohex-3-enyl)propan-2-yl butyrate (2d)

colorlessoil (80.5%). ^{1}H NMR (500 MHz, $CDCl_3$):δ5.37 (s, 1H), 2.20 (t, J = 7.4 Hz, 2H), 2.09 – 1.90 (m, 4H), 1.77– 1.86 (m, 2H), 1.64 (s, 3H), 1.58-1.63 (m, 2H), 1.44 (s, 3H), 1.42 (s, 3H), 1.26-1.33 (m, 1H), 0.93 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.09, 133.99, 120.48, 84.55, 42.91, 37.74, 31.01, 26.47, 23.97, 23.46, 23.38, 23.27, 18.75, 13.74. ESI-MS(m/z): 247$[M+Na]^+$

4.1.1.5 2-(4-methylcyclohex-3-enyl)propan-2-yl isobutyrate(2e)

colorless oil (74.3%). ^{1}H NMR (500 MHz, CDCl3): δ 5.37 (s, 1H), 2.43 (p, J = 7.0 Hz, 1H), 2.02-1.95(m, 4H), 1.87-1.77(m, 2H), 1.63 (s, 3H), 1.43 (s, 3H), 1.41 (s, 3H), 1.32-1.28 (m, 1H), 1.11 (d, J = 7.0 Hz, 6H).

^{13}C NMR (125 MHz, CDC_{l3}) δ 176.54, 134.05, 120.53, 84.25, 43.11, 35.27, 31.06, 26.47, 24.00, 23.43,23.42, 23.32, 19.23,19.23.ESI-MS: m/z=247 [M+Na]+

4.1.1.6 2-(4-methylcyclohex-3-enyl)propan-2-yl pivalate (2f)

colorless oil (80.5%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (s, 1H),2.03-1.91 (m, 4H),1.90-1.79 (m, 2H), 1.64 (s, 3H), 1.44 (s, 3H), 1.42 (s, 3H), 1.33-1.28 (m, 1H), 1.15 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.92, 134.04, 120.58, 84.04, 43.47, 39.64, 31.08, 27.37, 27.37, 27.12, 26.45, 24.02, 23.42,23.28,23.28.ESI-MS: m/z=261 $[M+Na]^+$

4.1.2 General procedure for the synthesis of compounds 2 g-2 h

Acyl chloride (6.0 mmol) was added to a solution of compound **1** (4.0 mmol) and anh ydrous pyridine (6.0 mmol) in dichloromethane (10 mL) at 0°C. The mixture was then st irred at room temperature for 8 h. The mixture was filtered and then concentrated in vacu

o. The crude product was purified by silica gel column chromatography (ethy acetate / pet roleum ether =1:5 v/v) to give the desired compounds.

4.1.2.1 2-(4-methylcyclohex-3-enyl)propan-2-yl tetradecanoate (2g)

colorless oil (93.5%), ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 2.20 (t, J = 7.3 Hz, 2H), 2.08 – 1.93 (m, 4H), 1.85-1.78(m, 2H), 1.64 (s, 3H), 1.61 – 1.53 (m, 2H), 1.43(m, 3H), 1.41 (m, 3H), 1.25 (s, 21H), 0.88 (dd, J = 4.7, 1.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$)δ 173.39, 134.09, 120.53, 84.61, 42.95, 35.89, 32.08, 29.83, 29.80, 29.80, 29.76, 29.66, 29.51, 29.46, 29.30, 26.53, 25.38, 25.35, 24.02, 23.53, 23.45, 23.34, 22.84, 14.26.ESI-MS: m/z=363 $[M-H]^-$

4.1.2.2 2-(4-methylcyclohex-3-enyl)propan-2-yl stearate (2h)

white solid (96.5%), mp: 28-32°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 2.20 (t, *J* = 7.5 Hz, 2H), 2.05-1.92 (m, 4H), 1.88-1.75 (m, 2H), 1.64 (s, 3H), 1.58-1.53(m, 2H), 1.44 (s, 3H), 1.41 (s, 3H), 1.33-1.28 (m,1H),1.25(s,28H), 0.88 (t, *J* = 7.0 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.41, 134.10, 120.52, 84.62, 42.93, 35.89, 32.08, 31.06, 29.85, 29.85, 29.85, 29.85, 29.85, 29.81, 29.81, 29.77, 29.66, 29.52, 29.46, 29.30, 26.52, 25.35, 24.01, 23.53, 23.46, 23.33, 22.85, 14.27.**ESI-MS:** m/z=459 $[M+K]^+$

4.1.3 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-bromoacetate (3)

Anhydrous pyridine (24 mmol) bromoacetyl bromide (24mmol) was added to a solution of compound **1** (1.85g, 12 mmol) and Anhydrous pyridine (24 mmol) in CH_2Cl_2 (30 mL) at 0°C.The reaction mixture was then stirred at room temperature for 7 h.The mixture was filtered and then concentrated in vacuo The crude product was purified by silica gel column chromatography (ethy acetate / Petroleum ether =1:4 v/v) to give compound **3** (1.62 g, 48.2%) as a colorlessoil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.39 (s, 1H), 3.77 (s, 2H), 2.13 – 1.94 (m, 4H), 1.92 – 1.76 (m, 2H), 1.67 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H),1.28-1.39 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.27,134.14,120.24,87.73, 42.91, 30.93, 27.75, 26.46, 23.94, 23.42, 23.27, 22.99.

ESI-MS: (m/z)=275 $[M+H]^+$

4.1.4 General procedure for the synthesis of 4a-j

A mixture of compound **3** (1.0 mmol), the corresponding secondary amine, mercaptan or nitric acid (2.0 mmol), and anhydrous K_2CO_3 (276.4 mg, 2 mmol) in CH_3CN(10 mL) was stirred at room temperature for 12 h.The mixture was filtered by suction filtration. The filtrate was then

concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Petroleum ether /ethy acetate = 15:2, v/v) to give the desired compounds.

4.1.4.1 2-(4-methylcyclohex-3-en-1-yl)propan-2-yl N-(2-hydroxyethyl)-N-methylglycinate (4a)

colorless oil (76.5%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.57 (m, 2H), 3.21 (s, 2H), 2.68 (m, 2H), 2.40 (s, 3H), 2.08-1.92(m,4H),1.84-1.74(m,2H), 1.63 (s, 3H), 1.45(s,3H),1.42 (s, 3H), 1.34 – 1.22 (m, 1H).

1^{13}C NMR (125 MHz, $CDCl_3$) δ 170.76, 134.13, 120.28, 86.22, 59.40, 58.90, 58.66, 42.80, 42.18, 30.97, 26.54, 24.06, 23.57, 23.41, 23.34. **ESI-MS:** m/z=270 $[M+H]^+$

4.1.4.2 2-(4-methylcyclohexyl)propan-2-yl 2-morpholinoacetate (4b)

colorless oil (84.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (s, 1H), 3.73 – 3.72 (m, 4H), 3.09 (s, 2H), 2.55 (m, 4H), 2.05-1.90 (m, 4H), 1.83-1.74(m, 2H), 1.62(s,3H),1.44 (s, 3H), 1.41 (s, 3H).1.31-1.23(m,1H) ^{13}C NMR (125 MHz, $CDCl_3$)δ 169.39, 134.06, 120.28, 86.05, 66.94, 66.94,60.33, 53.42,53.42, 42.80, 30.94, 26.50, 24.00, 23.52, 23.39, 23.31.ESI-MS: m/z=282 $[M+H]^+$

4.1.4.3 2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 2-(nitrooxy)acetate (4c)

yellow oil (74.8%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 3.75 (s, 2H), 2.04-1.97 (m, 4H), 1.88-1.78 (m, 2H),, 1.65 (s, 3H), 1.48 (s, 3H), 1.46 (s, 3H), 1.36 – 1.28 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 160.70,134.10,120.21,86.23,43.10,37.68,30.93,26.48,23.94,23.90,23.51,23.36.

ESI-MS: m/z=258 $[M+H]^+$

4.1.4.4 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(diethylamino)acetate (4d)

colorless oil (91.4%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (s, 1H), 3.20 (s, 2H), 2.65-2.61 (q, J = 7.2 Hz, 4H), 2.05-1.91 (m, 4H), 1.83-1.75 (m, 2H),1.62(s,3H),1.43 (s, 3H), 1.41 (s, 3H), 1.31 – 1.29 (m, 1H),1.03 (t, J = 7.2 Hz, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 170.90, 134.04, 120.38, 85.44, 54.87, 47.69, 47.69,42.83, 30.97, 26.52, 24.02, 23.56, 23.39, 23.30, 12.53,12.53.ESI-MS: m/z=268 $[M+H]^+$

4.1.4.5 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(2-hydroxyethylthio)acetate(4e)

colorless oil (83.9%) . ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.75 (t, J = 5.7 Hz, 2H), 3.18 (s, 2H), 2.81 (t, J = 5.7 Hz, 2H), 2.07-1.92(m, 4H), 1.85-1.77 (m, 2H), 1.63 (s, 3H),1.45(s,3H),1.44 (s,3H), 1.33– 1.25 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.32, 134.12, 120.23, 87.01, 60.69, 42.76, 36.41, 35.01, 30.93, 26.49, 23.97, 23.40, 23.40, 23.11.ESI-MS(m/z): 295 $[M+Na]^+$

4.1.4.6 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(4-oxopiperidin-1-yl)acetate (4f)

colorless oil (82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.24 (s, 2H), 2.87 (t, *J* = 6.0 Hz, 4H), 2.48 (t, *J* = 6.0 Hz, 4H), 2.07-1.91 (m, 4H), 1.85-1.74 (m, 2H), 1.63 (s, 3H), 1.46 (s, 3H), 1.43 (s, 3H),

1.33-1.25 (m, 1H)**. ^{13}C NMR (125 MHz, $CDCl_3$)** δ 208.69, 169.55, 134.12, 120.20, 86.33, 59.15, 52.94, 52.94, 42.75, 41.36, 41.36, 30.89, 26.47, 23.98, 23.52, 23.41, 23.31.ESI-MS: m/z=312 $[M+NH_4]^+$

4.1.4.7 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(4-hydroxypiperidin-1-yl)acetate (4g)

faint yellow oil (82.8%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (m, 1H), 3.71-3.68 (m, 1H), 3.11 (s, 2H), 2.84-2.79 (m, 2H), 2.37-2.31 (m, 2H), 2.07-1.95(m, 4H), 1.92-1.88(m, 2H),1.85-1.75 (m, 2H), 1.66-1.59 (m, 3H),1.63 (s, 2H), 1.45 (s, 3H), 1.42 (s, 3H), 1.33-1.26 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$)δ 169.89, 134.13, 120.31, 85.89, 67.74, 59.99, 50.86, 50.85, 42.77, 34.50, 34.50, 30.95, 26.50, 23.99, 23.55, 23.45, 23.32. ESI-MS: m/z=296 $[M+H]^+$

4.1.4.8 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(2,3-dihydroxypropylthio)acetate (4h)

colorless oil (87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ5.35 (s, 1H), 3.81 (s, 1H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.58-3.54 (m, 1H), 3.27-3.15 (m, 2H), 2.85-2.77 (m, 1H), 2.68 (dd, *J* = 14.0, 8.5 Hz, 1H), 2.09-1.90 (m, 4H), 1.86-1.75 (m, 2H), 1.63 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.33-1.25 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$)δ 170.42, 134.08, 120.15, 87.15, 70.59, 65.30, 42.68, 36.39, 35.45, 30.84, 26.40, 23.88, 23.39, 23.35, 23.03. ESI-MS: m/z=325 $[M+Na]^+$

4.1.4.9 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(piperidin-1-yl)acetate (4i)

colorless oil (87.3%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.06 (d, *J* = 1.0Hz, 2H), 2.49 – 2.47 (m, 4H), 2.07 – 1.90(m, 4H), 1.84 – 1.74 (m, 2H), 1.62 (s, 3H), 1.60-1.57(m, 4H), 1.44 (s, 3H), 1.41 (s, 3H), 1.40（m, 2H）1.31-1.23(m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.87,133.99, 120.29, 85.57, 60.75, 54.23, 54.23, 42.68, 30.88, 26.43, 25.88, 25.88, 24.01, 23.92, 23.47, 23.38, 23.24. ESI-MS: m/z=280 $[M+H]^+$

4.1.4.10 2-(4-methylcyclohex-3-enyl)propan-2-yl 2-(bis(2-hydroxyethyl)amino)acetate(4j)

faint yellow oil (63.5%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (s, 1H), 3.56-3.54 (m, 4H), 3.31 (s, 2H), 2.79 (t, *J* =5.5 Hz, 4H), 2.05-1.91 (m, 4H), 1.82-1.72(m, 2H), 1.61 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H), 1.30-1.22 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$)δ 172.39, 134.17, 120.20, 86.96, 59.91, 59.91, 57.87, 57.86, 56.66, 42.77, 30.92, 26.52, 24.05, 23.54, 23.40, 23.29.ESI-MS: m/z=300 $[M+H]^+$

4.1.5 Synthesis of 2-(4-methylcyclohexyl)propan-2-ol (5)

A mixture of compound **1** (2.6 mmol), methanol (10 mL), and 5% Pd/C (0.38 mmol) was stirred under an atmosphere of hydrogen for 12 h. The Pd/C was then removed by suction filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by

silica gel column chromatography (Petroleum ether /ethy acetate =20:1 v/v) to give compound **5** (380 mg, 93.5%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.71-1.80 (m, 4H), 1.52(m,1H), 1.23-1.20 (m, 1H), 1.13 (s, 6H), 1.03-1.00 (m, 2H), 0.87-0.90(m, 2H), 0.85-0.86 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 73.07, 48.98, 35.49, 35.49, 32.83, 27.54, 27.54, 27.13, 27.13, 22.70.ESI-MS: m/z=179 $[M+Na]^+$

4.2 Biological assay

4.2.1 ASM relaxation assay

Guinea pig tracheal smooth muscle rings were incubated in organ baths filled with Krebs-Henseleit solution and supplied with a plentiful mixture of 95% O_2 and 5% CO_2 at 36.5±0.5°C. Alterations in the tension of the isolated guinea-pig tracheal strips were input into the signal collection and processing system through a tension transducer (U/4C501H, Nanjing yi technology co., LTD, China). Before each experiment, a resting tension of 1.0 g was applied to each strip, followed by a 60-minute equilibration. Isometric tension measurements were obtained through observation of the alteration in the tension of isolated guinea pig tracheal rings contraction in response to acetylcholine (Ach) as described before [12-13], and calculated as the percentage change of smooth muscle tension for the tested compounds.

4.2.2 cAMP activation assays

Rat airway smooth muscle cells (ASMCs) cultured between passages four and six were plated at a density of 5×10^4/mL in 24-well plates and incubated for 48 hours as described [14]. With the exception of the normal group, cells were then stimulated for 4 hours with the contractile agonist Ach (0.33 mmol/L) followed by incubation with various compounds for 18 hours, with the exception of the control and model groups. Then, the cultured cells were passaged following trypsinization (0.25%, 2 min). At last, cells were collected and crushed with an utrasonic cell disruptor. Supernatants were later collected and used for future cAMP analysis by ELISA.

4.2.3 Anti-asthmatic assays

Sixty male Sprague-Dawley rats (200±20 g, purchased from the Animal Center of Zhejiang Academy of Traditional Chinese Medicine) were randomly divided into 6 groups (n=10 each): control group, model group, α-terpineol group, Dexamethasone Acetate group, compound **4a** group, and **4e** group. Rats were maintained in an animal facility for 5 days prior to

experimentation. All animals were provided water and standard chow ad libitum. All procedures in the experiment were performed in accordance with the Guide for Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

Animals were sensitized with an intraperitoneal injection of 100 mg OVA and 100 mg aluminum hydroxide in 1 mL normal saline (NS) on days 1 and 8, and then challenged from days 15 to 21 with OVA (1%, w/v, in NS) as previously described [15-16]. Meanwhile, the treatment was done on days 15 to 21, too. The **4a** and **4e** groups received compounds at a dosage of 1.6 g/kg through oral administration. The α-terpinene group was treated with an oral dosage of 1.0 g/kg. The positive group was treated with an oral dosage of 0.5 mg/kg of dexamethasone acetate tablets, while the control group and the model group received an equal volume of saline solution.

The challenge of all groups, with the exception of the normal group, was executed with OVA (1%, w/v, in NS) using a medical ultrasonic nebulizer (BSE2A, Beijing daya technology co., LTD, China) for 30min after the delivery as described [17]. Animals were narcotized immediately after the final challenge with 10% ethyl carbamate (1300 mg/kg) for lung function tests using a biological signal collecting and handling system (U/4C501H, Nanjing yi technology co., LTD, China). The abdominal venous blood was collected and later subjected to centrifugation (3000 rpm, 10 min) in order to obtain serum for assaying of IL-4 and IL-17.

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Highlights:

1. A novel series of α-terpineol derivatives were designed and syntheized.
2. Eight α-terpineol (α-T) derivatives demonstrated enhanced airway smooth muscle (ASM) relaxation activity.
3. Some representative α-T derivatives were capable of upregulating the level of cAMP in ASM cells.
4. Derivatives improved rat model lung function.
5. **4e** merits extensive development as a promising remedy for bronchial asthma.