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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel 2-(*E*)-substituted benzylidene-6-(*N*-substituted aminomethyl)cyclohexanones and cyclohexanols as analgesic and anti-inflammatory agents

Dan Liu · Weishe Yu · Jingjing Li · Cong Pang · Linxiang Zhao

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Abstract Twenty-two new 2-(*E*)-substituted benzylidene-6-(*N*-substituted aminomethyl)cyclohexanones (**6a–6j**) and cyclohexanols (**7a–7l**) were designed and synthesized. Target compounds were obtained through Stork enamine, Mannich, and Grignard reactions taking cyclohexanone as starting material. The structures were confirmed by the application of IR, ^{1}H NMR, MS, and HR-MS data. The analgesic activities were evaluated by acetic acid-induced writhing test and hot plate method. The anti-inflammatory activities were assayed by xylene-induced ear swelling and carrageenan-induced paw edema in mice model. All tested compounds showed analgesic and anti-inflammatory capacities in oral administration. Some compounds (**6a, 6c, 6h, 6i, 7c, 7h**, and **7i**) displayed the moderate analgesic activity compared with positive control ibuprofen, and some compounds (**6a, 6b, 6d, 6h, 7a,** and **7d**) exhibited more anti-inflammatory activity than ibuprofen. Among them, compound **6a** could be a potential nonsteroidal anti-inflammatory agent with significant analgesic activities and remarkable anti-inflammatory activities. Further research is being conducted.

Keywords 2-(*E*)-Substituted benzylidene-6-(*N*-substituted aminomethyl)cyclohexanones · 2-(*E*)-Substituted benzylidene-6-(*N*-substituted aminomethyl)-1-aryl-cyclohexanols · Analgesia · Anti-inflammation

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are common medication for treatment of pain, inflammation, and fever. However, the significant side effects have been shown with a long-term usage of NSAIDs including serious gastrointestinal lesions, kidney injury, and cardiovascular risk. Cycloketo compounds with exo-cyclic α,β-unsaturated double bond have multifunctional pharmacological bioactivities, such as anti-inflammatory, antiviral, and antitumor (Tham *et al*., 2011; Li *et al*., 2011; Wang *et al*., 2005). In our previous research, we found that cyclopentanones (cyclohexanones) with 2-alkylaminomethyl, the simplified structure of bioactivity diterpenes and sesquiterpenes, showed powerful inhibiting effects on inflammation and pain, as well as cancer cell growth, and explored the preliminary antitumor mechanisms (Wang *et al*., 2004, 2005; Zhang *et al*., 2006). The stability of cyclohexanones is more powerful than the cyclopentanones. So, in view of these studies, introduction of substituted phenyl aminomethyl group into the ortho position of cyclohexanones and cyclohexanols is expected to increase the stability and enhance the anti-inflammatory and analgesic activity. A series of novel 2-(*E*)-substituted benzylidene-6-(*N*-substituted aminomethyl) cyclohexanones and cyclohexanols were designed and synthesized. Their analgesic and anti-inflammatory effects were measured in mice model.

Results and discussion

Chemistry

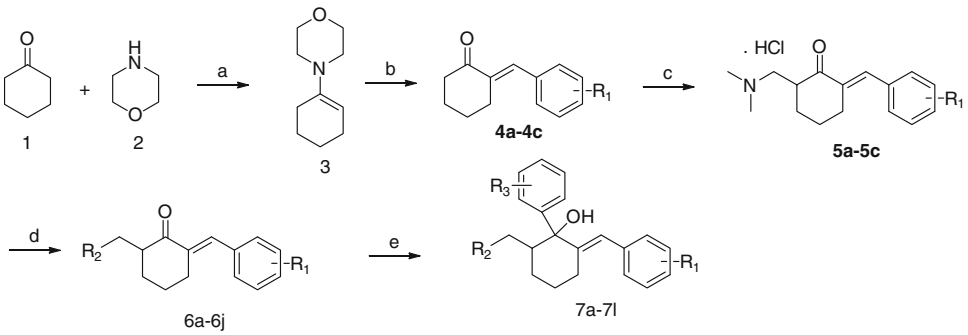
The synthetic route to the 2-(*E*)-substituted benzylidene-6-(*N*-substituted aminomethyl) cyclohexanones **6a–6j** and

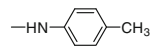
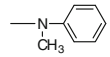
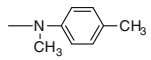
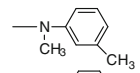
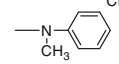
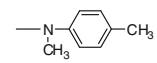
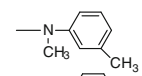
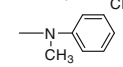
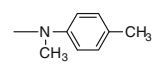
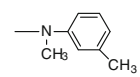
2-(*E*)-substituted benzylidene-6-(*N*-substituted aminomethyl)-1-aryl cyclohexanols **7a–7l** are presented in Scheme 1. The substituents of compounds **6a–6j** and **7a–7l** are also listed in Scheme 1. Enamine **3** was produced as a colorless liquid through Stork enamine synthesis by commercially available cyclohexanone condensed with morpholine (Hunig *et al*., 1973). 2-Benzylidene cyclohexanone **4a–4c** were synthesized by fresh enamine **3** refluxing in toluene with corresponding benzaldehyde (Stork *et al*., 1963). Compounds **4a–4**c were reacted with fresh Eschenmoser's salt (*N*,*N*-dimethylmethylene-ammonium chloride), synthesized based on the literature procedure (Brand *et al*., 2003), to yield the Mannich base hydrochlorides **5a–5c**. After amine exchange reaction of compounds **5a–5c** with aromatic amine, target compounds **6a–6j** were produced. α,β-Unsaturated ketones **6a–6j** were reacted with appropriate Grignard reagent to get the target compounds **7a–7l** as the racemic mixture with the specific rotator power 0° in 1 % MeOH.

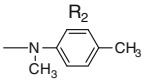
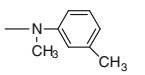
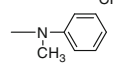
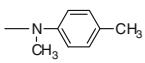
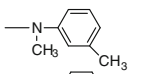
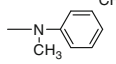
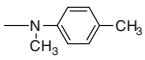
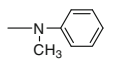
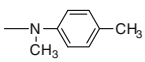
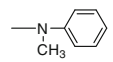
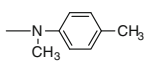
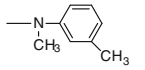
Biologic results and discussion

The synthesized compounds, **6a–6j** and **7a–7l**, were evaluated for analgesic and anti-inflammatory, and the results are summarized in Tables 1 and 2. The results indicated that all the tested compounds exhibited different degree of analgesic and anti-inflammatory capacities under the experimental conditions.

The analgesic capacities were evaluated by acetic acid-induced writhing test and hot plate test using Kunming male mice, ibuprofen as position drug. As shown in Table 1, all the tested compounds exhibited moderate



	R_1	R_2
6a	H	
6b	H	
6c	H	
6d	H	
6e	4-CH_3	
6f	4-CH_3	
6g	4-CH_3	
6h	4-SO_2CH_3	
6i	4-SO_2CH_3	
6j	4-SO_2CH_3	

	R_1	R_2	R_3
7a	H		H
7b	H		H
7c	H		3-OCH_3
7d	H		3-OCH_3
7e	H		3-OCH_3
7f	H		4-OCH_3
7g	H		4-OCH_3
7h	4-CH_3		3-OCH_3
7i	4-CH_3		3-OCH_3
7j	4-CH_3		4-OCH_3
7k	4-CH_3		4-OCH_3
7l	4-CH_3		4-OCH_3

Scheme 1 The synthetic pathway of target compounds. Reagents and conditions: *a* toluene, *p*-toluenesulfonic acid, reflux, 20 h; *b* substituted benzaldehyde, toluene, reflux, 36 h; *c* Eschenmoser's salt, dry acetonitrile, r.t., 5 h; *d* substituted aromatic amine, 50 % ethanol, 30 °C, 12 h; *e* grignard reagent, THF, r.t., 24 h

Table 1 The analgesic activity of the target compounds **6a–7l**

Code	Acetic acid-induced writhing test		Hot plate test	
	Writhing number (/15′)	Inhibition rate (%)	Pain threshold (s)	Increasing rate (%)
Control	47.83 ± 13.84	0	16.80 ± 5.08	4.20
Ibuprofen	7.60 ± 3.21**	84.11	41.11 ± 7.98**	158.56
6a	8.21 ± 1.80**	82.82	43.88 ± 3.55**	166.19
6b	14.50 ± 6.17**,#	69.69	35.66 ± 6.98**	109.59
6c	9.07 ± 3.12**	81.04	41.62 ± 8.69**	146.12
6d	11.14 ± 5.22**	76.70	33.84 ± 3.43#	108.45
6e	15.21 ± 9.80**,#	68.19	28.68 ± 6.34**,##	79.04
6f	11.21 ± 6.38**	76.55	26.97 ± 4.76**,##	71.60
6g	11.57 ± 6.29**	75.81	27.51 ± 8.38*	75.50
6h	9.07 ± 3.74**	81.04	38.41 ± 4.71**	142.70
6i	7.40 ± 3.07**	84.53	40.61 ± 8.01**	155.12
6j	13.00 ± 7.47**	72.82	33.80 ± 5.65**	109.98
7a	17.14 ± 5.76**,##	64.16	26.84 ± 7.41**,##	70.08
7b	14.07 ± 6.57**	70.58	29.57 ± 10.64*,#	83.22
7c	8.50 ± 3.27**	82.23	42.63 ± 2.73**	162.71
7d	10.64 ± 6.10**	77.75	33.79 ± 3.89**	110.54
7e	9.60 ± 4.67**	79.93	34.62 ± 7.63**	116.52
7f	11.64 ± 4.79**	75.66	35.68 ± 9.07**	121.45
7g	10.21 ± 5.25**	78.65	34.99 ± 3.49**	117.46
7h	9.14 ± 2.09**	80.89	39.75 ± 4.12**	153.01
7i	8.78 ± 2.48**	81.63	35.58 ± 6.25**	125.34
7j	13.36 ± 5.59**	72.08	32.83 ± 10.46**	106.84
7k	18.07 ± 5.38**,##	62.22	31.50 ± 3.64**,#	93.41
7l	12.36 ± 4.40**	74.17	31.22 ± 4.40**,#	93.52

Values are expressed as mean ± SEM. $n = 10$

* $p < 0.05$, ** $p < 0.01$ compared with control; # $p < 0.05$, ## $p < 0.01$ compared with ibuprofen

analgesic capacity. Cyclohexanones with methylsulfonyl group at the para-position of benzylidene group (**6h–6j**) increased the analgesic activity, in which compound **6i** was the best analgesic one with inhibition of 84.53 % (ibuprofen 84.11 %) and improvement pain threshold (>150 %). Cyclohexanones with no substituent at the para-position of benzylidene group (**6a–6d**) could also increase the pain threshold (>100 %). Cyclohexanols with methoxy group at the meta-position of benzene ring (**7c–7e, 7h, 7i**) showed better analgesic activity than that of no or para-position substituent.

The anti-inflammatory activities were assayed in mice model of xylene-induced ear swelling using Kunming male mice and carrageenan-induced paw edema on Wistar rats, ibuprofen as position drug. As a whole, cyclohexanols (**7a–7l**) exhibited less anti-inflammatory activity than cyclohexanones (**6a–6j**), whereas compound **7a** and **7d** had the most potent activity during both experiments. The activity of cyclohexanones was altered by the substitution of the aromatic amines, and the order was methyl group at the para-position (R_2: 4-CH_3) < meta-position (R_2: 3-CH_3) < no substituent (R_2: H).

In summary, the synthesized compounds had certain analgesic and anti-inflammatory activity. It looked that all tested compounds exhibited preferable analgesic activity, pain threshold increasing rate was more than 70 %. And, they did not display the more powerful anti-inflammatory activity, except compounds **6a**, **6b, 7a, and 7d**. So, we will do the further study to focus their analgesic activity.

Experimental

Chemistry

^{1}H NMR spectra were recorded on a BRUKER ARX-300 instrument in $CDCl_3$ solution with Me_4Si as internal standard. MS were determined on Waters Quattro micro API LC–MS mass spectrometer. HR-MS were obtained on Finnigan MAT-711 mass spectrometer in ESI mode. Infrared spectra were recorded on a BRUKER IFS-55 FTIR spectrometer. Optical rotation was recorded on Pekin-Elmer 241 instrument. The melting points were determined on an electrically heated X4 digital visual melting point apparatus and were uncorrected. Unless specified, otherwise, all reagents and solvents were used as supplied by the manufacturer.

Table 2 The anti-inflammatory activity of the target compounds **6a–7l**

Code	Ear swelling test		Paw edema test	
	Swelling (mg)	Inhibition rate (%)	Edema (mL)	Inhibition rate (%)
Control	21.00 ± 4.74	0	0.47 ± 0.05	0
Ibuprofen	12.57 ± 2.30**	40.14	0.22 ± 0.03**	53.19
6a	9.17 ± 4.02**	56.35	0.13 ± 0.03**,##	72.34
6b	10.57 ± 3.46**	49.66	0.11 ± 0.03**,##	76.60
6c	17.17 ± 3.19	18.25	0.35 ± 0.04**,##	25.53
6d	11.57 ± 5.56**	44.90	0.25 ± 0.05**	46.81
6e	12.71 ± 3.99**	39.45	0.26 ± 0.01**,##	44.68
6f	15.20 ± 6.76*	27.62	0.29 ± 0.03**,##	38.30
6g	13.57 ± 8.02**	35.37	0.30 ± 0.03**,##	36.17
6h	11.14 ± 5.81**	46.94	0.23 ± 0.08**	51.06
6i	15.33 ± 2.81*	26.98	0.32 ± 0.06**,##	31.91
6j	13.29 ± 5.41*	36.73	0.30 ± 0.07**,##	36.17
7a	7.00 ± 4.62**,#	66.67	0.19 ± 0.03**,#	59.57
7b	16.14 ± 6.74	23.13	0.30 ± 0.08**,##	36.17
7c	13.71 ± 7.34*	34.69	0.29 ± 0.09**,#	38.30
7d	12.29 ± 2.50**	41.50	0.20 ± 0.07**	57.45
7e	13.43 ± 6.78*	36.05	0.26 ± 0.08**	44.68
7f	15.43 ± 4.54*	26.53	0.29 ± 0.05**,##	38.30
7g	15.43 ± 3.69*	26.53	0.37 ± 0.08**,##	21.28
7h	17.21 ± 7.50	18.04	0.35 ± 0.10**,##	25.53
7i	16.20 ± 5.63	22.86	0.37 ± 0.02**,##	21.28
7j	17.57 ± 5.38	16.33	0.39 ± 0.09*,##	17.02
7k	14.71 ± 4.61*	29.92	0.33 ± 0.01**,##	29.79
7l	15.86 ± 7.11*	24.49	0.33 ± 0.05**,##	29.79

Values are expressed as mean ± SEM. $n = 10$

* $p < 0.05$, ** $p < 0.01$ compared with control; # $p < 0.05$, ## $p < 0.01$ compared with ibuprofen

*N-(1-Cyclohexen-1-yl)morpholine (**3**)*

A mixture of cyclohexanone **1** (58.8 g, 0.6 mol), morpholine **2** (87.2 g, 1.0 mol), and *p*-toluenesulfonic acid (0.5 g, 0.003 mol) in toluene (200 mL) was heated to boiling 20 h in a 500 mL round-bottomed flask to which a water separator under the reflux condenser was attached. Cooled and distilled at atmospheric pressure to remove the most toluene. The residue was evaporated under reduced pressure. Compound **3** was obtained as a colorless liquid at 117–119 °C/10 mmHg (118–120 °C/10 mmHg, Hunig *et al*., 1973). Yield: 85.5 %.

*General procedure for the synthesis of **4a–4c***

The corresponding benzaldehyde (0.20 mol) was dissolved in toluene (80 mL) and fresh *N*-(1-cyclohexen-1-yl)morpholine **3** (50.2 g, 0.30 mol) was added. The solution was heated to boiling 36 h in a 250 mL round-bottomed flask attached a water separator under the reflux condenser. Cooled and added 6 mol/L HCl (40 mL). The organic layer was washed with 10 % $NaHCO_3$ solution, H_2O, and dried by Na_2SO_4. It was filtered, and the filtrate was evaporated to dryness. The solid was recrystallized from methanol or ethanol.

*2-Benzylidene cyclohexanone (**4a**)* Yield: 55 %, pale yellow powder; m.p.: 52–53 °C (52–54 °C, Falck *et al*., 2006).

*2-(4-Methylbenzylidene) cyclohexanone (**4b**)* Yield: 64 %, pale yellow powder; m.p.: 66–68 °C (71 °C, Das *et al*., 2008).

*2-(4-Methylsulfonylbenzylidene) cyclohexanone (**4c**)* Yield: 70 %, pale yellow powder; m.p.: 94–95 °C (97–98 °C, Ao *et al*., 2004).

*General procedure for the synthesis of **5a–5c***

Fresh *N*,*N*-dimethylmethylene-ammonium chloride (0.02 mol), synthesized based on a literature procedure (Gaudry *et al*., 1988), was added to a solution of 2-benzylidene cyclohexanone **4a**, **4b,** or **4c** (0.01 mol) in acetonitrile at 80 °C. The mixture was reacted under reflux monitored by TLC using a solvent system of chloroform–

methanol (7:1). After 5 h, the mixture was cooled and filtered. The residue was crystallized from chloroform-tetrahydrofuran to give **5a–5c** as white solid.

*2-Dimethylaminomethyl-6-benzylidene-cyclohexanone hydrochloride (**5a**)* Yield: 61.2 %; m.p.: 152–154 °C; MS (ESI) *m/z*: 243.2 (M^+); ^{1}H NMR (300 MHz, DMSO-*d6*) δ: 10.31 (brs, 1H), 7.46–7.42 (m, 5H), 7.34 (s, 1H), 3.57–3.55 (q, $J = 7.6$ Hz, 1H), 3.09–3.06 (d, $J = 6.4$ Hz, 2H), 2.94 (brs, 1H), 2.78 (s, 6H), 2.71 (s, 1H), 2.31 (m, 1H), 1.86 (m, 1H), 1.69 (m, 2H).

*2-Dimethylaminomethyl-6-[(4-methyl)benzylidene]-cyclohexanone hydrochloride (**5b**)* Yield: 59.9 %; m.p.: 163–166 °C; MS (ESI) *m/z*: 258.2 $(M+H)^+$.

*2-Dimethylaminomethyl-6-[(4-methylsulfonyl)benzylidene]-cyclohexanone hydrochloride (**5c**)* Yield: 71.2 %; m.p.: 142–144 °C; MS (ESI) *m/z*: 322.4 $(M+H)^+$.

*General procedure for the synthesis of **6a–6j***

To a solution of substituted cyclohexanone hydrochlorides **5a–5c** (0.01 mol) in 50 % (v/v) ethanol (30 mL), substituted aromatic amines (0.02 mol) was added and reacted for 12 h at 30 °C. The reaction was cooled and filtered. The residue was purified by crystallization from acetone or by a silica gel column with $CHCl_3$:MeOH (v/v) = 60:1 to give **6a–6j** as yellow solid.

*2-(E)-Benzylidene-6-(p-toluidinomethy)cyclohexanone (**6a**)* Yield: 58 %. m.p.: 83–85 °C; MS (ESI, *m/z*): 305.3 (M^+); IR (KBr) cm^{-1}: 3,412, 2,933, 1,669, 1,608, 1,131, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43 (s, 1H), 7.38–7.30 (m, 5H), 7.01–7.00 (d, $J = 8.2$ Hz, 2H), 6.62–6.59 (d, $J = 8.3$ Hz, 2H), 4.45 (brs, 1H), 3.54–3.48 (q, $J = 20.2$ Hz, 1H), 3.29–3.23 (q, $J = 18.4$ Hz, 1H), 3.02–2.97 (d, $J = 16.0$ Hz, 1H), 2.73–2.68 (m, 2H), 2.24 (s, 3H), 2.12 (m, 1H), 1.95–1.91 (m, 1H), 1.79–1.69 (m, 2H); HR-MS: *m/z*, calcd, $C_{21}H_{23}NO$ (M^+): 305.1780, found: 305.1778.

*2-(E)-Benzylidene-6-(N-methyl-N-phenylaminomethyl)cyclohexanone (**6b**)* Yield: 53 %. m.p.: 129–131 °C; MS (ESI, *m/z*): 305.2 (M^+); IR (KBr) cm^{-1}: 3,426, 2,943, 1,692, 1,622, 1,134, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37–7.34 (m, 5H), 7.26–7.15 (m, 5H), 6.83 (s, 1H), 3.00–2.93 (m, 1H), 2.87–2.82 (m, 1H), 2.66–2.60 (q, $J = 17.5$ Hz, 2H), 2.29–2.19 (m, 2H), 2.13–2.09 (m, 2H), 2.02–1.96 (m, 2H), 1.78–1.66 (m, 2H); HR-MS: *m/z*, calcd, $C_{21}H_{23}NO$ (M^+): 305.1780, found: 305.1782.

*2-(E)-Benzylidene-6-(N-methyl-N-(p-tolyl)aminomethyl)cyclohexanone (**6c**)* Yield: 56 %. m.p.: 130–131 °C; MS (ESI, *m/z*): 319.6 (M^+); IR (KBr) cm^{-1}: 3,434, 2,944, 1,694, 1,622, 1,134, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36–7.14 (m, 9H), 6.82 (s, 1H), 2.95–2.86 (m, 2H), 2.64–2.62 (d, $J = 6.0$ Hz, 2H), 2.28–2.18 (m, 3H), 2.10–2.08 (m, 3H), 2.05–2.00 (m, 4H), 1.68–1.64 (m, 4H); HR-MS: *m/z*, calcd, $C_{22}H_{25}NO$ (M^+): 319.1936, found: 319.1948.

*2-(E)-Benzylidene-6-(N-methyl-N-(m-tolyl)aminomethyl) cyclohexanone (**6d**)* Yield: 51 %. m.p.: 128–130 °C; MS (ESI, *m/z*): 319.5 (M^+); IR (KBr) cm^{-1}: 3,428, 2,942, 1,692, 1,578, 1,133, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36–7.12 (m, 9H), 6.82 (s, 1H), 2.96 (m, 1H), 2.85 (m, 1H), 2.64–2.62 (d, $J = 6.6$ Hz, 2H), 2.26–2.21 (m, 3H), 2.10–2.18 (m, 3H), 2.00 (m, 1H), 1.79–1.66 (m, 4H),; HR-MS: *m/z*, calcd, $C_{22}H_{25}NO$ (M^+): 319.1936, found: 319.1939.

*2-(E)-(4-Methyl)benzylidene-6-(N-methyl-N-phenylaminomethyl)cyclohexanone (**6e**)* Yield: 47 %. m.p.: 103–105 °C; MS (ESI, *m/z*): 319.6 (M^+); IR (KBr) cm^{-1}: 3,444, 2,940, 1,681, 1,578, 1,149, 808; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.29–7.25 (m, 3H), 7.18–7.16 (m, 2H), 7.10–7.06 (m, 4H), 6.79 (s, 1H), 2.94 (m, 1H), 2.87 (m, 1H), 2.63–2.61 (m, 2H), 2.37 (m, 1H), 2.32 (s, 3H), 2.26–2.18 (m, 3H), 1.78–1.65 (m, 4H); HR-MS: *m/z*, calcd, $C_{22}H_{25}NO$ (M^+): 319.1936, found: 319.1939.

*2-(E)-(4-Methyl)benzylidene-6-(N-methyl-N-(p-tolyl)aminomethyl) cyclohexanone (**6f**)* Yield: 49 %. m.p.: 105–107 °C; MS (ESI, *m/z*): 333.7 (M^+); IR (KBr) cm^{-1}: 3,445, 2,940, 1,681, 1,578, 1,149, 809; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28–7.25 (m, 2H), 7.16–7.13 (m, 2H), 7.10–7.06 (q, $J = 8.1$ Hz, 4H), 6.79 (s, 1H), 2.94–2.84 (m, 2H), 2.62–2.61 (d, $J = 3.6$ Hz, 2H), 2.36 (s, 3H), 2.31 (s, 3H), 2.26–2.22 (m, 2H), 2.13–2.09 (m, 2H), 2.10 (m, 2H), 1.78–1.73 (m, 2H), 1.67–1.63 (m, 2H); HR-MS: *m/z*, calcd, $C_{23}H_{27}NO$ (M^+): 333.2093, found: 333.2099.

*2-(E-(4-Methyl)benzylidene-6-(N-methyl-N-(m-tolyl)aminomethyl) cyclohexanone (**6g**)* Yield: 44 %. m.p.: 101–102 °C; MS (ESI, *m/z*): 333.5 (M^+); IR (KBr) cm^{-1}: 3,423, 2,940, 1,682, 1,578, 1,148, 810; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28–7.25 (m, 2H), 7.19–7.16 (m, 2H), 7.13–7.06 (q, $J = 20.6$ Hz, 4H), 6.79 (s, 1H), 2.94–2.86 (m, 2H), 2.63–2.61 (d, $J = 3.6$ Hz, 2H), 2.36 (s, 3H), 2.32 (s, 3H), 2.24–2.19 (m, 2H), 2.18–2.08 (m, 3H), 1.76–1.73 (m, 1H), 1.65–1.63 (m, 2H); HR-MS: *m/z*, calcd, $C_{23}H_{27}NO$ (M^+): 333.2093, found: 333.2097.

*2-(E)-(4-Methylsulfonyl)benzylidene-6-(N-methyl-N-phenylaminomethyl) cyclohexanone (**6h**)* Yield: 47 %. m.p.: 143–145 °C; MS (ESI, *m/z*): 383.2 (M^+); IR (KBr) cm^{-1}: 3,446, 2,925, 1,685, 1,587, 1,306, 1,145, 769; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.95–7.92 (d, $J = 8.3$ Hz, 2H), 7.85–7.83 (d, $J = 8.3$ Hz, 2H), 7.52–7.49 (d, $J = 8.2$ Hz, 2H), 7.39–7.36 (d, $J = 8.3$ Hz, 2H), 7.21 (s, 1H), 6.82 (s, 1H), 3.08–3.06 (d, $J = 5.3$ Hz, 3H), 2.87 (m, 1H), 2.64–2.62 (d, $J = 6.3$ Hz, 2H), 2.15 (m, 3H), 2.05–2.04 (m, 2H), 1.83–1.68 (m, 4H); HR-MS: *m/z*, calcd, $C_{22}H_{25}NO_3S$ (M^+): 383.1555, found: 383.1562.

*2-(E)-(4-Methylsulfonyl)benzylidene-6-(N-methyl-N-(p-tolyl) aminomethyl) cyclohexanone (**6i**)* Yield: 52 %. m.p.: 136–139 °C; MS (ESI, *m/z*): 397.6 (M^+); IR (KBr) cm^{-1}: 3,446, 2,925, 1,685, 1,587, 1,306, 1,145, 768; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.95–7.92 (d, $J = 8.1$ Hz, 2H), 7.85–7.83 (d, $J = 8.4$ Hz, 2H), 7.52–7.49 (d, $J = 8.4$ Hz, 2H), 7.39–7.36 (d, $J = 8.4$ Hz, 2H), 6.82 (s, 1H), 3.08–3.06 (d, $J = 5.4$ Hz, 3H), 2.87 (m, 2H), 2.65–2.63 (d, $J = 6.0$ Hz, 2H), 2.28–2.15 (m, 5H), 2.05–2.04 (m, 2H), 1.84–1.78 (m, 2H), 1.72–1.68 (t, $J = 12.0$ Hz, 2H); HR-MS: *m/z*, calcd, $C_{23}H_{27}NO_3S$ (M^+): 397.1712, found: 397.1722.

*2-(E-(4-Methysulfonyll)benzylidene-6-(N-methyl-N-(mtolyl) aminomethyl) cyclohexanone (**6j**)* Yield: 50 %. m.p.: 140–142 °C; MS (ESI, *m/z*): 397.1 (M^+); IR (KBr) cm^{-1}: 3,443, 2,925, 1,686, 1,588, 1,305, 1,146, 769; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.95–7.92 (d, $J = 8.4$ Hz, 2H), 7.85–7.83 (d, $J = 8.4$ Hz, 2H), 7.52–7.49 (d, $J = 8.1$ Hz, 2H), 7.39–7.36 (d, $J = 8.4$ Hz, 2H), 6.82 (s, 1H), 3.08–3.06 (d, $J = 5.1$ Hz, 3H), 2.86 (m, 2H), 2.64–2.62 (d, $J = 6.3$ Hz, 2H), 2.24–2.10 (m, 5H), 2.07–2.04 (m, 2H), 1.83–1.78 (m, 2H), 1.72–1.68 (t, $J = 12.3$ Hz, 2H); HR-MS: *m/z*, calcd, $C_{23}H_{27}NO_3S$ (M^+): 397.1712, found: 397.1718.

*General procedure for the synthesis of **7a–7l***

In a 100-mL three-necked flask with thermometer, a dropping funnel and reflux condenser with calcium chloride tube, magnesium turnings (0.02 mol), and iodine (a grain) was covered with 10 mL purified tetrahydrofuran (THF) and treated with about 1/20 of a total of substituted bromobenzene (0.02 mol). After the reaction was started, the remaining bromobenzene, dissolved in 15 mL THF, was added drop wise with stirring under THF boiling gently. The mixture was boiled gently until almost all the magnesium was dissolved (about 30 min).

To a solution of substituted cyclohexanone **6a–6j** (0.01 mol) in 30 mL purified THF, fresh Grignard reagent (0.02 mol) was added dropwise under ice-water bath. After 24 h stirring at room temperature, the mixture was poured into 37 % (w/v) NH_4Cl solution and the solid was produced. The crude was purified using a silica gel column with $CHCl_3$–MeOH to give **7a–7j** as white solid.

*2-(E)-Benzylidene-6-(N-methyl-N-(p-tolyl)aminomethyl)-1-phenylcyclohexanol (**7a**)* Yield: 7.1 %. m.p.: 135–138 °C; [α]D 25 = 0° (*c* = 1 % MeOH); MS (ESI, *m/z*): 396 ($[M-H]^-$); IR (KBr) cm^{-1}: 3,492, 1,598, 1,448, 1,137, 751; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.57–7.55 (d, $J = 6.7$ Hz, 2H), 7.36–7.34 (d, $J = 4.3$ Hz, 4H), 7.29–7.27 (m, 2H), 7.24–7.23 (m, 4H), 7.10–7.08 (m, 2H), 2.63–2.39 (m, 2H), 2.32–2.22 (m, 2H), 2.18–2.05 (m, 3H), 1.98–1.87 (m, 3H), 1.64–1.47 (m, 5H); HR-ESI-MS: *m/z*, calcd, $C_{28}H_{31}NO$ ($[M-H]^-$): 397.2406, found: 397.2417.

*2-(E)-Benzylidene-6-(N-methyl-N-(m-tolyl)aminomethyl)-1-phenylcyclohexanol (**7b**)* Yield: 6.3 %. m.p.: 143–145 °C; MS (ESI, *m/z*): 396 ($[M-H]^-$); IR (KBr) cm^{-1}: 3,493, 1,597, 1,447, 1,138, 753; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.57–7.55 (d, $J = 6.8$ Hz, 2H), 7.36–7.34 (d, $J = 4.4$ Hz, 4H), 7.24–7.22 (m, 6H), 7.10–7.08 (d, $J = 7.5$ Hz, 2H), 2.63–2.44 (m, 2H), 2.39–2.32 (m, 2H), 2.25–2.12 (m, 3H), 1.98–1.86 (m, 3H), 1.62–1.47 (m, 5H); HR-ESI-MS: *m/z*, calcd, $C_{28}H_{31}NO$ ($[M-H]^-$): 397.2406, found: 397.2445.

*2-(E)-Benzylidene-6-(N-methyl-N-phenylaminomethyl)-1-(3methoxy)phenylcyclohexanol (**7c**)* Yield: 10.2 %. m.p.: 137–140 °C; MS (ESI, *m/z*): 413 (M^+); IR (KBr) cm^{-1}: 3,455, 2,831, 1,600, 1,437, 1,140, 759; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.36–7.35 (d, $J = 8.9$ Hz, 2H), 7.28–7.27 (m, 3H), 7.23–7.20 (m, 4H), 7.10–7.02 (m, 5H), 3.75 (s, 3H), 2.58 (m, 1H), 2.49–2.38 (m, 3H), 2.22–2.13 (m, 3H), 2.05 (brs, 2H), 1.63–1.52 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{28}H_{31}NO_2$ (M^+): 413.2355, found: 413.2356.

*2-(E)-Benzylidene-6-(N-methyl-N-(p-tolyl)aminomethyl)-1-(3-methoxy)phenylcyclohexanol (**7d**)* Yield: 11.0 %. m.p.: 143–145 °C; [α]D 25 = 0° (*c* = 1 % MeOH); MS (ESI, *m/z*): 427 (M^+); IR (KBr) cm^{-1}: 3,453, 2,831, 1,600, 1,437, 1,139, 757; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.34–7.32 (m, 4H), 7.28 (s, 1H), 7.13–7.11 (m, 4H), 7.09–7.07 (m, 4H), 3.60 (s, 3H), 2.61 (m, 1H), 2.50–2.39 (m, 3H), 2.31–2.22 (m, 3H), 2.05 (brs, 2H), 1.63–1.56 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{29}H_{33}NO_2$ (M^+): 427.2511, found: 427.2509.

*2-(E)-Benzylidene-6-(N-methyl-N-(m-tolyl)aminomethyl)-1-(3-methoxy)phenylcyclohexanol (**7e**)* Yield: 10.8 %. m.p.: 141–144 °C; MS (ESI, *m/z*): 426 ($[M-H]^-$); IR (KBr) cm^{-1}: 3,452, 2,833, 1,597, 1,449, 1,138, 755; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.35–7.33 (m, 4H), 7.28–7.26 (m, 1H), 7.23–7.18 (m, 4H), 7.13–7.09 (m, 4H), 3.60 (s, 3H), 2.61 (m, 1H), 2.51–2.39 (m, 3H), 2.29–2.22 (m, 3H),

1.98 (brs, 2H), 1.65–1.52 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{29}H_{33}NO_2$ ($[M-H]^-$): 427.2511, found: 427.2523.

*2-(E)-Benzylidene-6-(N-methyl-N-phenyl)aminomethyl-1-(4-methoxy)phenylcyclohexanonol (**7f**)* Yield: 10.2 %. m.p.: 146–147 °C; MS (ESI, *m/z*): 413 (M^+); IR (KBr) cm^{-1}: 3,583, 2,825, 1,606, 1,443, 1,149, 753; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.84–7.82 (d, $J = 8.9$ Hz, 2H), 7.32–7.30 (m, 6H), 7.22–7.20 (m, 4H), 6.97 (s, 1H), 6.89–6.86 (d, $J = 8.9$ Hz, 2H), 3.80 (s, 3H), 2.88–2.83 (tt, $J = 15.1$ Hz, 1H), 2.56–2.52 (m, 3H), 2.03–1.99 (m, 3H), 180–1.73 (m, 3H), 1.61–1.56 (m, 3H); HR-ESI-MS: *m/z*, calcd, $C_{28}H_{31}NO_2$ (M^+): 413.2355, found: 413.2367.

*2-(E)-Benzylidene-6-(N-methyl-N-(p-tolyl))aminomethyl-1-(4-methoxy)phenylcyclohexanonol (**7g**)* Yield: 11.0 %. m.p.: 148–150 °C; MS (ESI, *m/z*): 427 (M^+); IR (KBr) cm^{-1}: 3,529, 2,827, 1,605, 1,443, 1,149, 753; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.84–7.81 (d, $J = 8.9$ Hz, 2H), 7.32–7.30 (m, 5H), 7.21–7.19 (m, 4H), 6.97 (s, 1H), 6.89–6.86 (d, $J = 8.9$ Hz, 2H), 3.81 (s, 3H), 2.83 (m, 1H), 2.60–2.52 (m, 3H), 2.17–2.10 (m, 2H), 2.03–1.99 (m, 3H), 1.80–1.73 (m, 3H), 1.61–1.55 (m, 3H); HR-ESI-MS: *m/z*, calcd, $C_{29}H_{33}NO_2$ (M^+): 427.2511, Found: 427.2532.

*2-(E)-(4-Methyl)benzylidene-6-(N-methyl-N-phenyl))aminomethyl-1-(3-methoxy)phenylcyclohexanol (**7h**)* Yield: 10.5 %. m.p.: 144–147 °C; [α]D 25 = 0° ($c = 1$ % MeOH); MS (ESI, *m/z*): 427 (M^+); IR (KBr) cm^{-1}: 3,451, 2,860, 1,602, 1,433, 1,145, 753; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.86–7.84 (d, $J = 8.9$ Hz, 2H), 7.32–7.29 (m, 5H), 7.21–7.18 (m, 4H), 6.95 (s, 1H), 6.86–6.84 (d, $J = 8.9$ Hz, 2H), 3.61 (s, 3H), 2.84–2.67 (m, 4H), 2.28–2.23 (m, 3H), 2.10–1.98 (m, 2H), 1.65–1.56 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{29}H_{33}NO_2$ (M^+): 427.2511, found: 427.2553.

*2-(E)-(4-Methyl)benzylidene-6-(N-methyl-N-(p-tolyl))aminomethyl-1-(3-methoxy)phenylcyclohexanol (**7i**)* Yield: 11.5 %. m.p.: 146–150 °C; MS (ESI, *m/z*): 441 (M^+); IR (KBr) cm^{-1}: 3,449, 2,862, 1,604, 1,438, 1,141, 755; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.25–7.18 (m, 4H), 7.16–7.11 (m, 6H), 6.92–6.89 (d, $J = 7.9$ Hz, 2H), 6.75–6.73 (m, 1H), 3.61 (s, 3H), 2.61–2.44 (m, 2H), 2.34 (s, 3H), 2.28–2.25 (m, 1H), 2.21 (s, 3H), 2.06–1.81 (m, 3H), 1.59–1.52 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{30}H_{35}NO_2$ (M^+): 441.2688, found: 441.2635.

*2-(E)-(4-Methyl)benzylidene-6-(N-methyl-N-phenyl)aminomethyl-1-(4-methoxy)phenylcyclohexanonol (**7j**)* Yield: 13.2 %. m.p.: 149–151 °C; MS (ESI, *m/z*): 427 (M +); IR (KBr) cm^{-1}: 3,565, 2,834, 1,609, 1,435, 1,155, 755; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.83–7.80 (d, $J = 09.0$ Hz, 2H), 7.25–7.21 (m, 3H), 7.10 (m, 6H), 6.93 (s, 1H), 6.87–6.84 (d, $J = 8.7$ Hz, 2H), 3.80 (s, 3H), 2.86–2.81 (tt, $J = 15.3$ Hz, 1H), 2.59–2.81 (m, 2H), 2.34–2.33 (m, 6H), 2.13–2.08 (m, 2H), 1.73–1.72 (m, 2H), 1.52 (m, 2H); HR-ESI-MS: *m/z*, calcd, $C_{29}H_{33}NO_2$ (M^+): 427.2511, found: 427.2583.

*2-(E)-(4-Methyl)benzylidene-6-(N-methy-N-(p-tolyl))aminomethyl-1-(4-methoxy)phenylcyclohexanonol (**7k**)* Yield: 13.5 %. m.p.: 153–155 °C; MS (ESI, *m/z*): 441 (M^+); IR (KBr) cm^{-1}: 3,565, 2,835, 1,609, 1,435, 1,155, 755; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.84–7.82 (d, $J = 8.9$ Hz, 2H), 7.26–7.24 (m, 2H), 7.19–7.08 (m, 6H), 6.87 (m, 1H), 6.85–6.83 (d, $J = 9.0$ Hz, 2H), 3.79 (s, 3H), 2.86–2.80 (tt, $J = 15.2$ Hz, 1H), 2.54 (m, 3H), 2.35 (s, 3H), 2.00–1.94 (m, 5H), 1.68–1.50 (m, 6H); HR-ESI-MS: *m/z*, calcd, $C_{30}H_{35}NO_2$ (M^+): 441.2668, found: 441.2681.

*2-(E)-(4-Methyl)benzylidene-6-(N-methy-N-(m-tolyl))aminomethyl-1-(4-methoxy)phenylcyclohexanonol (**7l**)* Yield: 12.5 %. m.p.: 144–147 °C; [α]D 25 = 0° ($c = 1$ % MeOH); MS (ESI, *m/z*): 441 (M^+); IR (KBr) cm^{-1}: 3,565, 2,835, 1,609, 1,434, 1,155, 755; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.84–7.81 (d, $J = 8.9$ Hz, 2H), 7.21–7.18 (m, 2H), 7.18–7.10 (m, 6H), 6.93 (m, 1H), 6.88–6.85 (d, $J = 9.0$ Hz, 2H), 3.80 (s, 3H), 2.87–2.82 (tt, $J = 15.1$ Hz, 1H), 2.55 (m, 3H), 2.34 (s, 3H), 2.08–1.99 (m, 5H), 1.82–1.79 (m, 4H), 1.55 (m, 2H); HR-ESI-MS: *m/z*, calcd, $C_{30}H_{35}NO_2$ (M^+): 441.2668, found: 441.2656.

Pharmacology

Male Kunming mice (18–22 g) and Wistar rats (160 ± 20 g) for the analgesic and anti-inflammatory assay were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. Each group consisted of ten mice, which were fasted for 12 h before performance. The vehicle (0.5 % sodium carboxymethyl cellulose, 0.5 % Na-CMC), positive control (ibuprofen), and test compounds were administered orally at a 200 mg/kg dose.

Analgesic evaluation by acetic acid-induced writhing test

One hour after the administration of the tested compounds, the mice were given an intraperitoneal injection of 0.7 % (v/v) acetic acid solution (10 mL/kg). A writhe was defined as stretching of the abdomen with swelling of the trunk and hind limbs, and rising of the rump. The number of writhes was counted for 15 min and the percent analgesic activity (PAA) was calculated by the following formula (Wang *et al.*, 2009),

$$\mathrm{PAA} = \frac{x - a}{x} \times 100\ \%$$

where, x is the mean writhing number of mice in the control group for administration of 0.5 % Na-CMC solution and a is the mean writhing number of mice administrated the tested compounds or ibuprofen.

Analgesic evaluation by hot plate test

Mice were placed on a hot plate maintained at 55 ± 0.5 °C and the time in seconds between the placement of mice on the platform and licking of the hind paw or jumping was recorded as the pain threshold. Pain threshold measures were recorded before drug and 1 h after the last drug administration. Mice exhibiting pain threshold time >30 s or <5 s were excluded. If the mice did not respond to the stimulus, the latency was recorded as 60 s.

The increasing percentage of pain threshold (PPT) was calculated by the following formula:

$$\mathrm{PPT} = \frac{t - t_0}{t_0} \times 100\ \%$$

where, t is the pain threshold time (s) of mice administrated the tested compounds or ibuprofen and t_0 is the pain threshold time (s) of mice before drug.

Anti-inflammatory assay by xylene-induced ear swelling

One hour later, after drug administration, inflammation was induced by the application of 0.03 mL xylene on the anterior and posterior surfaces of the right ear of mice. After 30 min, the animals were euthanized by cervical dislocation and both ears were removed. Disks of 8-mm diameter were removed from each ear using a cork borer and weighed. The swelling degree was estimated as the weight difference between the punches from right and left ears, and the percent swelling inhibition (PSI) was calculated by the following formula:

$$\mathrm{PSI} = \frac{\overline{S} - S}{\overline{S}} \times 100\ \%$$

where $\bar{S}$ is the mean swelling degree of mice in blank control group (0.5 % Na-CMC) and s is the mean swelling degree of mice administrated the tested compounds or ibuprofen.

Anti-inflammatory assay by carrageenan-induced paw edema

Carrageenan (1 % in saline) was injected into the plantar surface of the right hind paw of the rats 1 h after the last drug administration. Then, the hind paw volume was measured after 3 h and the rate of paw edema (RPE) was calculated according to the following equation (Kannur *et al.*, 2012),

$$\mathrm{RPE} = \left[1 - \left(\frac{a - x}{b - y}\right)\right] \times 100\ \%$$

where, a is the mean volume at time = 3 h of the test group, x is the mean volume at time = 0 of the test group, b is the mean volume at time = 3 h of the control group, and y is the mean volume at time = 0 of the control group.

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