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Short communication

# Synthesis and anti-metastatic effects of novel chiral ionone alkaloid derivatives

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ABSTRACT

Novel chiral ionone alkaloid derivatives were synthesized and evaluated their anti-metastatic effects in human MDA-MB-231 breast cancer cells. The chiral center C-6 of derivatives exerted an important role in response to the anti-metastatic activity. Comparing with a positive control of LY294002, compounds **17b** and **19a** exhibited potent inhibitory effects on the EGF-induced invasion of MDA-MB-231 cells with $IC_{50}$ values of 0.026 ± 0.003 and 0.016 ± 0.002 μM, respectively. Moreover, compounds **17b** and **19a** showed inhibitory effects on the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells in a dose-dependent manner. Thus, compounds **17b** and **19a** offer potential to be developed as novel anti-metastasis agents.



## 1. Introduction

Breast cancer (BC) is the most common malignancy in female patients and is the second leading cause of cancer-related mortality in worldwide [1]. Metastasis is the major driver in breast cancer deaths [2]. Cancer metastasis involves an extremely complex cascade of events that include detachment of cells within a primary tumor, intravasation, entering the circulation, extravasation, and establishment of new foci of malignancy at a secondary site [3,4]. Hence, the development and evaluation of biologically active agents will give new insight into this process, and would therefore allow potential chemotherapeutics that can effectively inhibit or even prevent the metastatic process and consequently be beneficial for cancer treatment [5].

In our previous study, a valuable racemic ionone alkaloid, (E)-4-(3-[(4- fluorobenzyl) (methyl)amino]but-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one(Ion-FBn), was found to have significant inhibition effect on the invasion of MDA-MB-231 cells induced by the chemokine epidermal growth factor (EGF) [6]. But this compound is a racemic mixture with two chiral centers. In this study, C-9 chiral center was eliminated as it might not be an important binding site for anti-metastatic effect.

Now it has been established that the desired biological response is possessed by one enantiomer only and that the other enantiomer generally either decreases biological activities or creates side effects (or toxic effects) [7]. Therefore, the synthesis of chiral derivatives will exert an important role in the study of structure–activity relationship and discovery of lead compound. This paper deals with the synthesis and anti-metastatic effects of novel C-6 chiral Ion-FBn derivatives, as well as their structure–activity relationship.

## 2. Results and discussion

### 2.1. Chemistry

As shown in Scheme 1, the targeted compounds **11a**,**b**–**23a**,**b** were synthesized. α-Ionone was used as the starting material, and it

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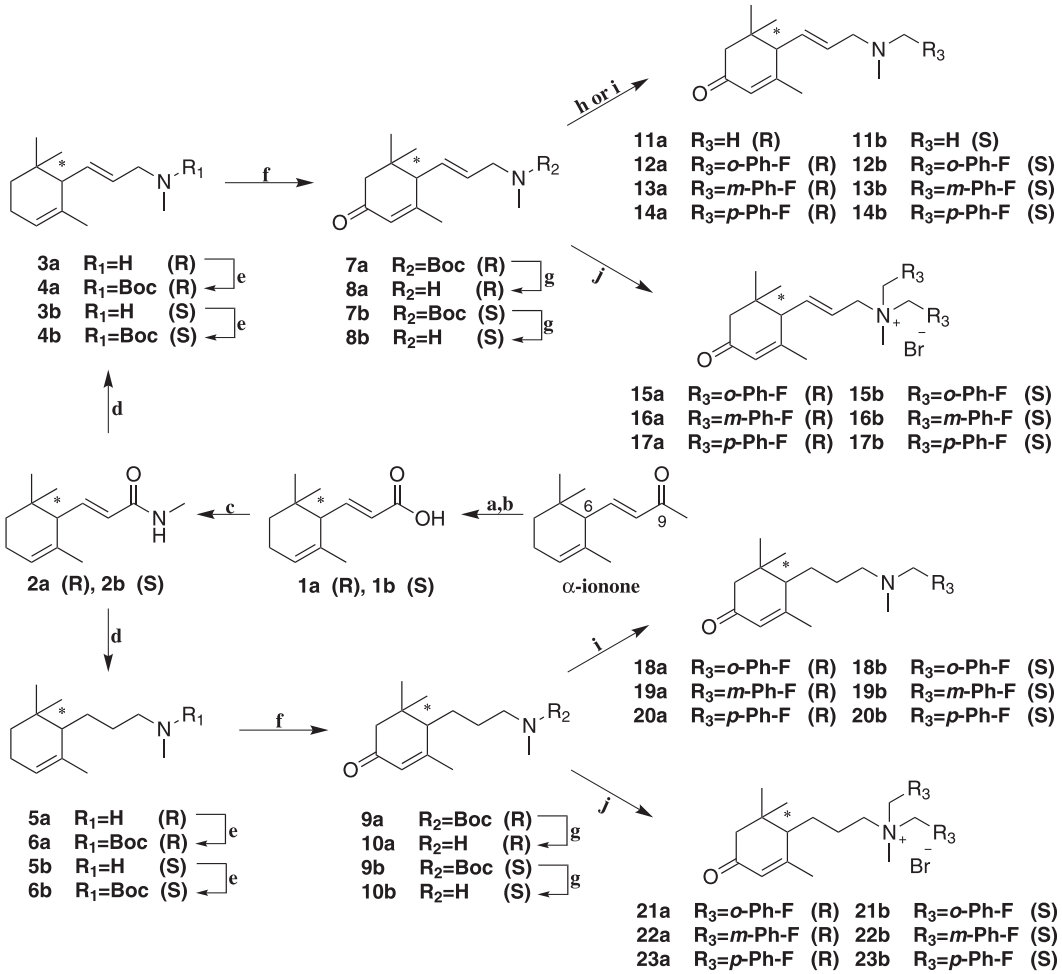


**Scheme 1.** The Synthetic route of chiral ionone alkaloid derivatives. Reagents and conditions: (a) NaOCl, EtOH, 0 °C to rt; (b) (R)-(+)-1-Phenylethylamine or (S)-(−)-1-Phenylethylamine, EtOAc, reflux to 4 °C; (c) EDCI, HOBt, DIEA, $CH_3NH_2 \cdot HCl$, $CH_2Cl_2$, rt; (d) $LiAlH_4$, THF, 40 °C; (e) $Boc_2O$, $NaHCO_3$, THF, rt; (f) $CrO_3$, 3, 5-dimethylprazole, $CH_2Cl_2$, −20 °C; (g) TFA, $CH_2Cl_2$, 0 °C to rt; (h) paraformaldehyde, $NaBH_3CN$, $CH_2Cl_2$, 40 °C; (i) substituted fluorobenzyl bromide, $K_2CO_3$, DMF, rt; (j) substituted fluorobenzyl bromide, $K_2CO_3$, $CH_3CN$, rt.

was used in haloform reaction with sodium hypochlorite to yield (±)-**1**. The chiral resolution of racemate is the main route for the separation of chiral compounds. Enantiomerically pure α-methylbenzylamine has been employed as a resolving agent to obtain the enantiomer of **1** [8]. The chiral acid was coupled to corresponding chiral amide **2** in the presence of HOBt, EDCI and DIEA. Reduction of chiral amide **2** by $LiAlH_4$ led to the amine mixture. The next step was followed by *N*-Boc protection of the forming amine mixture. Allylic oxidation of chiral compound **4** or **6** using chromium trioxide and 3,5-dimethylprazole produced the chiral compounds **7** and **9**. Reaction of chiral compound **7** or **9** with TFA occurred easily to obtain the required chiral amines **8** and **10**. Finally, the targeted compounds **11a,b**–**23a,b** were synthesized by methylation reaction and nucleophilic substitution reaction. All compounds were characterized using $^1H$ NMR, $^{13}C$ NMR and mass spectrometry methods.

### 2.2. Biological assessment

#### 2.2.1. Anti-invasive activity

The cytotoxic effects of chiral ionone alkaloid derivatives against MDA-MB-231 cells were evaluated by MTT assay. The results in Table 1 showed that the tested compounds do not display positive effects on the inhibition of cancer cell proliferation under the 100 μM concentration. The non-cytotoxic concentrations of derivatives were used for anti-invasive assay.

A remarkable characteristic of cancer metastasis is the invasive and migratory ability of cancer cells. The *in vitro* anti-invasive activities of all synthesized derivatives were evaluated against MDA-MB-231 cells in the non-cytotoxic concentrations by transwell invasion assay. The $IC_{50}$ values for Ion-FBn derivatives were summarized in Table 2. Compared with the positive control (LY294002, $IC_{50} = 1.18 \pm 0.137$ μM), compounds **17b** and **19a** exhibited significant inhibitory effects with $IC_{50}$ values of 0.026 ± 0.003 and 0.016 ± 0.002 μM, respectively.

Based on the above results, it is suggested that conformations of Ion-FBn derivatives are very critical to their anti-metastatic activities. In most of 7,8-dihydro derivatives, R-enantiomers show more potencies while S-enantiomers lose their activities. The *m*-fluoro substituted compound **19a** ($IC_{50} = 0.016 \pm 0.002$ μM) had much 10-fold higher than *o*- and *p*-fluoro substituted compounds **18a** ($IC_{50} = 0.232 \pm 0.033$ μM) and **20a** ($IC_{50} = 0.501 \pm 0.071$ μM). It is speculated that flexible R-enantiomers easily bind to receptors with one benzyl group.

On the other side, in most of 7-ene derivatives, S-enantiomers show more potencies than R-enantiomers, the S-enantiomers **12b** ($IC_{50} = 1.067 \pm 0.091$ μM) and **13b** ($IC_{50} = 0.134 \pm 0.019$ μM) showed positive activities, and their R-enantiomers revealed no activities on the tested concentrations. On the consideration of our previous reports [6,9–11] and this study, it is also seen that three tertiary amine substituent group had a positive impact on anti-

**Table 1**
Cytotoxicity data of chiral ionone alkaloid derivatives on MDA-MB-231 cells (n = 3).

| Compound | Inhibition (%) | | |
|---|---|---|---|
| | 10 μM | 50 μM | 100 μM |
| **8a** | 3.5 | 6.8 | 17.6 |
| **8b** | 4.1 | 3.2 | 9.1 |
| **11a** | 1.3 | 3.8 | 2.9 |
| **11b** | −2.9 | −1.1 | 5.9 |
| **12a** | 0.7 | 4.3 | 4.1 |
| **12b** | −5.8 | 1.1 | 4.6 |
| **13a** | −0.6 | 6.1 | 8.4 |
| **13b** | −6.7 | 17.8 | 14.1 |
| **14a** | 5.0 | 3.9 | 5.2 |
| **14b** | 7.1 | 5.4 | 11.1 |
| **15a** | 3.3 | 10.1 | 15.5 |
| **15b** | 0.9 | 2.7 | 12.1 |
| **16a** | 2.5 | 7.5 | 13.4 |
| **16b** | −1.7 | 2.9 | 10.1 |
| **17a** | 1.4 | 7.1 | 5.7 |
| **17b** | 0.9 | 3.6 | 6.0 |
| **18a** | −10.2 | 5.9 | 6.3 |
| **18b** | 1.9 | 9.4 | 8.1 |
| **19a** | 3.7 | 16.4 | 23.9 |
| **19b** | 4.1 | 20.6 | 26.3 |
| **20a** | −9.2 | −0.4 | −0.4 |
| **20b** | −4.4 | 0.7 | 2.2 |
| **21a** | 4.1 | 8.0 | 13.3 |
| **21b** | 1.3 | 5.5 | 16.1 |
| **22a** | −0.6 | 1.8 | 4.4 |
| **22b** | 1.5 | 1.1 | 2.7 |
| **23a** | 2.2 | 2.1 | −1.4 |
| **23b** | −5.0 | −3.9 | 6.1 |

metastatic activity.

Interestingly, only S-enantiomer **17b**, a quaternary ammonium salt, revealed potent anti-metastatic effect ($IC_{50}$ = 0.026 ± 0.003 μM), and compounds **15a**,**b**–**16a**,**b** and **17a** ($IC_{50}$ > 10 μM) showed no activities on the tested concentrations. It maybe matches some special sites of receptors with a stereoselectivity. These above phenomena are very useful to identify their targets. It is concluded that C-6 chirality, *N*-substitution condition and flexibility of these derivatives are co-factors to the anti-metastatic activities. A proper combine could generate a powerful potency.

### *2.2.2. The inhibition effects of compounds **17b** and **19a** on the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells*

PKCζ and integrin β1 are well reported to be related to cell metastasis [12,13]. To investigate the possible anti-metastatic mechanism of Ion-FBn derivatives, effects on the phosphorylations of PKCζ and integrin β1 proteins were examined by western blot. As a result, compounds **17b** and **19a** down regulated the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells in a dose-dependent manner (Fig. 1).

To date, many natural products and synthesized compounds have been reported to have anti-migration, invasion, and anti-angiogenesis effects against cancer cells, such as fisetin, myricetin, flavopiridol, mitoxantrone and staurosporine showed high or moderate inhibitory activity on PKCζ, with $IC_{50}$ values of 58 ± 9, 1.7 ± 0.4, 108 ± 17, 280 ± 47 and 0.019 ± 0.004 μM, respectively [14]. It is reported that 1,3,5-trisubstituted pyrazolines as potent and selective allosteric PKCζ inhibitors, and targeting of PKCζ in cells was confirmed by reporter gene assay, transfection assays, and western blotting [15]. A synthesized compound of PKCzI257.3 which inhibits EGF-induced breast cancer cell migration by suppressing PKCζ [16]. 3,4-methyenedioxy-β-nitrostyrene inhibits *in vitro* metastatic properties of triple negative breast cancer cells through suppression of β1 integrin activation and focal adhesion signaling [17]. In addition, Ginsenoside Rp1 down-regulates integrin β1 activation at concentrations between 10 to 40 μM and suppresses the *in vitro* tube formation of HUVEC [18].

Thus, to our knowledge, compounds **17b** and **19a** might exhibit the anti-metastatic activity via suppressing the expressions of p-PKCζ and p-integrin β1. The results in this present study suggest that chiral ionone alkaloid derivative might exhibited different anti-matastatic effect comparing with reported natural products and synthesized compounds, and may be promising candidate in the development of new anti-metastasis lead compound.

**Table 2**
Inhibitory effects of chiral ionone alkaloid derivatives on the invasion of MDA-MB-231 cells.

| No. | $IC_{50}$[a] (μM) | No. | $IC_{50}$[a] (μM) |
|---|---|---|---|
| **8a** | >10 | **8b** | >10 |
| **11a** | 8.76 ± 1.02 | **11b** | 4.59 ± 0.229 |
| **12a** | >10 | **12b** | 1.067 ± 0.091 |
| **13a** | >10 | **13b** | 0.134 ± 0.019 |
| **14a** | 5.974 ± 0.481 | **14b** | >10 |
| **15a** | >10 | **15b** | >10 |
| **16a** | >10 | **16b** | >10 |
| **17a** | >10 | **17b** | 0.026 ± 0.003 |
| **18a** | 0.232 ± 0.033 | **18b** | >10 |
| **19a** | 0.016 ± 0.002 | **19b** | >10 |
| **20a** | 0.501 ± 0.071 | **20b** | >10 |
| **21a** | 0.864 ± 0.107 | **21b** | >10 |
| **22a** | 4.135 ± 0.428 | **22b** | >10 |
| **23a** | >10 | **23b** | >10 |
| **LY294002**[b] | 1.18 ± 0.137 | | |

[a] $IC_{50}$ represents the concentration of the compound producing 50% inhibition against human MDA-MB-231 breast cancer cells.
[b] Positive control.

## 3. Conclusions

In summary, this work described the synthesis of novel chiral Ion-FBn derivatives, their chiral center of C-6 exert an important role in response to the anti-metastatic activity. Compounds **17b** and **19a** exhibited potent anti-metastasis effects compared with the positive control LY294002, and showed inhibitory effects on the expressions of p-PKCζ and p-integrin β1.

## 4. Experimental section

### *4.1. Chemistry*

#### *4.1.1. General instrumentation*

All reagents and solvents were obtained from commercial sources and used without purification unless mentioned. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel glass plates containing a fluorescence indicator. The column chromatography was carried out on silica gel ($SiO_2$; 300–400 mesh) and Toyopeal HW-40F (Tosoh Co., Ltd, Tokyo, Japan). The NMR spectra were taken using a Bruker AVANCE III 400 instrument ($^1H$ NMR, 400 MHz; $^{13}C$ NMR, 100 MHz). The high resolution mass spectra were recorded on an Agilent 1200 LC-MS in ESI mode (Agilent, Palo Alto, CA, USA). Optical rotation values were measured with a JASCO P-2000 digital polarimeter (Jasco Co., Ltd, Tokyo, Japan).

#### *4.1.2. General procedure for synthesis of **1a** and **1b***

To a solution of α-ionone (10 mL, 48.52 mmol) in EtOH (110 mL) was added NaClO solution (220 mL, 13%) in portions at 0 °C. The reaction mixture was stirred at room temperature for 12 h. When the reaction was completed, the mixture was acidified to pH = 1 with concentrated HCl. The reaction mixture was extracted with $CH_2Cl_2$ (150 mL), washed with water (50 mL), then washed with

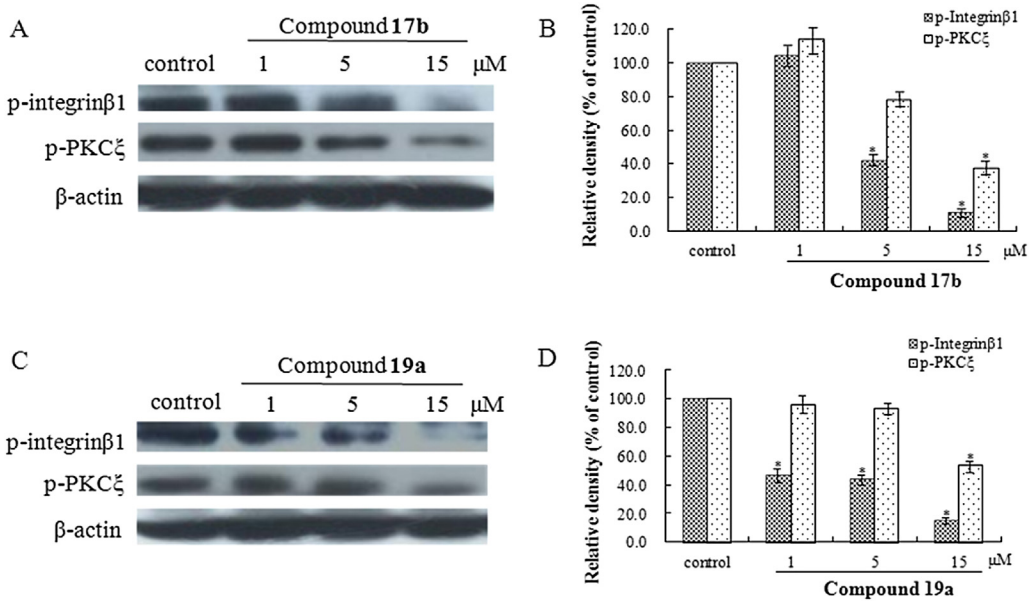


**Fig. 1.** The inhibition effects of compounds **17b** and **19a** on the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells. (A) Compound **17b** on the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells were determined by western blot; (B) Quantification of p-PKCζ and p-integrin β1 in MDA-MB-231 cells; (C) Compound **19a** on the expressions of p-PKCζ and p-integrin β1 in MDA-MB-231 cells were determined by western blot; (D) Quantification of p-PKCζ and p-integrin β1 in MDA-MB-231 cells. The relative band density was determined by densitometry with Image J software. The target protein expression was normalized to the β-actin expression level. Values are mean ± S.D (n = 3). $^{*}p < 0.05$ vs. control.

brine (50 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the crude product, which was purified through flash column chromatography (PE/EA = 10/1 + 0.3% HAc) to afford racemic **1** (8.68 g, 92.1%).

To a solution of (±)-**1** (47.30 g, 243.47 mmol) in EtOAc (254 mL) was added (R)-(+)-1-phenethylamine (29.51 g, 243.47 mmol) in portions at 50 °C. The reaction mixture was refluxed for 10 min. The solution was allowed to cool and crystallization occurred within 36 h at 4 °C. The precipitated was collected using suction filtration and washed three times with cold EtOAc (10 mL). This salt (36.99 g) was dissolved in hot EtOAc and recrystallized three times at 4 °C. The chiral resolution salt was then dissolved in 200 mL of $CH_2Cl_2$, and adjusted to pH = 2 with conc. HCl. The organic layers were collected and dried by $MgSO_4$, and the solvent was removed *in vacuo* to afford the desired product **1a**. The procedure described above (using the same quantities of (S)-(−)-1-phenethylamine and solvent) was also followed to yield the enantiopure **1b**.

*4.1.3. (R,E)-3-(2,6,6-trimethylcyclohex-2-en-1-yl)acrylic acid (**1a**)*

Yield 32.1%, orange oil, $[\alpha]_D^{20}$ +362.5 (*c* 1.042, ethanol), consistent with that reported in the literature $[\alpha]_D^{22} = +367$ in the ethanol [19], *ee* 92.7%. $^1$H NMR (400 MHz, $CDCl_3$): δ 6.92 (dd, *J* = 15.4 Hz, 9.9 Hz, 1H, 7-H), 5.81 (d, *J* = 15.4 Hz, 1H, 8-H), 5.50 (br s, 1H, 4-H), 2.31 (d, *J* = 9.9 Hz, 1H, 6-H), 2.04 (br s, 2H, 3-H), 1.57 (d, *J* = 1.5 Hz, 3H, 13-H), 1.47 (m, 1H, 2-H), 1.20 (m, 1H, 2-H), 0.93 (s, 3H, 11-H), 0.86 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 172.2 (9-C), 152.9 (7-C), 131.6 (5-C), 122.8 (8-C), 121.7 (4-C), 54.1 (6-C), 32.5 (1-C), 31.1 (2-C), 27.7 (11-C), 26.7 (12-C), 23.0 (3-C), 22.7 (13-C). ESI-MS *m/z* 217.1 $[M+Na]^+$.

*4.1.4. (S,E)-3-(2,6,6-trimethylcyclohex-2-en-1-yl)acrylic acid (**1b**)*

Yield 34.2%, orange oil, $[\alpha]_D^{20}$ −363.7 (*c* 1.081, ethanol), *ee* 92.9%. $^1$H NMR (400 MHz, $CDCl_3$): δ 6.92 (dd, *J* = 15.2 Hz, 9.8 Hz, 1H, 7-H), 5.81 (d, *J* = 15.2 Hz, 1H, 8-H), 5.50 (br s, 1H, 4-H), 2.31 (d, *J* = 9.8 Hz, 1H, 6-H), 2.04 (br s, 2H, 3-H), 1.57 (s, 3H, 13-H), 1.47 (m, 1H, 2-H), 1.21 (m, 1H, 2-H), 0.93 (s, 3H, 11-H), 0.86 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 171.4 (9-C), 152.9 (7-C), 131.6 (5-C), 122.8 (8-C), 121.5 (4-C), 54.1 (6-C), 32.5 (1-C), 31.1 (2-C), 27.7 (11-C), 26.8 (12-C), 23.0 (3-C), 22.8 (13-C). ESI-MS *m/z* 217.1 $[M+Na]^+$.

*4.1.5. General procedure for synthesis of **2a** and **2b***

To a solution of **1a** or **1b** (9.20 g, 47.35 mmol) in anhydrous $CH_2Cl_2$ (110 mL) were added methylamine hydrochloride (4.47 g, 66.20 mmol), DIEA (23.48 mL, 134.79 mmol), HOBt (9.60 g, 71.05 mmol), and EDCI (13.62 g, 71.05 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h and then concentrated under reduced pressure. The residue was diluted with $CH_2Cl_2$ (70 mL) and the solution was washed with aqueous HCl (0.06 M, 20 mL), saturated aqueous $NaHCO_3$ (40 mL), and brine (20 mL), the organic layer was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (PE/EA = 2/1) to give **2a** or **2b**.

*4.1.6. (R,E)-N-methyl-3-(2,6,6-trimethylcyclohex-2-en-1-yl) acrylamide (**2a**)*

Yield 89.9%, colorless oil, $[\alpha]_D^{20}$ +278.2 (*c* 1.054, ethanol).$^1$H NMR (400 MHz, $CDCl_3$): δ 6.65 (dd, *J* = 15.2 Hz, 9.7 Hz, 1H, 7-H), 6.13 (br s, 1H, NH), 5.78 (d, *J* = 15.2 Hz, 1H, 8-H), 5.45 (br s, 1H, 4-H), 2.87 (d, *J* = 4.9 Hz, 3H, *N*–$CH_3$), 2.23 (d, *J* = 9.7 Hz, 1H, 6-H), 2.01 (br s, 2H, 3-H), 1.55 (d, *J* = 1.4 Hz, 3H, 13-H), 1.46 (m, 1H, 2-H), 1.17 (m, 1H, 2-H), 0.90 (s, 3H, 11-H), 0.84 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 166.7 (9-C), 144.8 (7-C), 132.3 (5-C), 124.7 (8-C), 122.2 (4-C), 53.8 (6-C), 32.4 (1-C), 31.1 (2-C), 27.7 (11-C), 26.8 (12-C), 26.2 (*N*–$CH_3$), 23.0 (3-C), 22.8 (13-C). ESI-MS *m/z* 208.2 $[M+H]^+$.

*4.1.7. (S,E)-N-methyl-3-(2,6,6-trimethylcyclohex-2-en-1-yl) acrylamide (**2b**)*

Yield 87.8%, colorless oil, $[\alpha]_D^{20}$ −281.5 (*c* 1.072, ethanol).$^1$H NMR (400 MHz, $CDCl_3$): δ 6.64 (dd, *J* = 15.2 Hz, 9.7 Hz, 1H, 7-H), 6.61 (br s, 1H, NH), 5.83 (d, *J* = 15.2 Hz, 1H, 8-H), 5.45 (br s, 1H, 4-H), 2.85 (d, *J* = 4.8 Hz, 3H, *N*–$CH_3$), 2.23 (d, *J* = 9.7 Hz, 1H, 6-H), 2.01 (br s, 2H, 3-H), 1.55 (s, 3H, 13-H), 1.46 (m, 1H, 2-H), 1.18 (m, 1H, 2-H), 0.90 (s, 3H,

11-H), 0.84 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 166.8 (9-C), 144.6 (7-C), 132.3 (5-C), 124.8 (8-C), 122.1 (4-C), 53.8 (6-C), 32.3 (1-C), 31.2 (2-C), 27.6 (11-C), 26.8 (12-C), 26.2 (*N*–$CH_3$), 23.0 (3-C), 22.8 (13-C). ESI-MS *m/z* 208.2 $[M+H]^+$.

*4.1.8. General procedure for synthesis of **3a**, **3b**, **5a** and **5b***

To the solution of amide **2a** or **2b** (3.87 g, 18.67 mmol) in anhydrous THF (25 mL), $LiAlH_4$ (1.42 g, 37.29 mmol) was added in portions at 0 °C under nitrogen. The reaction was stirred at room temperature for 24 h, and then quenched by careful addition of 4 N aqueous NaOH (5 mL) and saturated potassium sodium tartrate (25 mL) at 0 °C. The mixture was filtered *in vacuo* and the filtrate was evaporated. The mixture of **3a** and **5a** or the mixture of **3b** and **5b** thus obtained was immediately used in the next reaction without purification.

*4.1.9. General procedure for synthesis of **4a**, **4b**, **6a** and **6b***

To the mixture of **3a** and **5a** (1.67 g, 8.64 mmol) and $NaHCO_3$ (1.44 g, 17.14 mmol) in THF (15 mL) was added a solution of $Boc_2O$ (2.82 g, 12.92 mmol) in THF (5 mL) when the mixture was stirred in an ice bath. The mixture was stirred at room temperature for 12 h, evaporated just to dryness with a vacuum evaporator. The reaction mixture was extracted with $CH_2Cl_2$ (20 mL), washed with water (15 mL), then washed with brine (15 mL), and the organic layer was dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the crude product, which was separated by preparative HPLC (YMC-Pack SIL-06, S-5 μm, 250 × 20 mm I.D., silica gel, normal phase) column chromatography (PE/EA = 20/1) to obtain pure **4a** and **6a**.

The procedure described above (using the same quantities of reagents) was followed to yield pure **4b** and **6b**.

*4.1.10. General procedure for synthesis of **7a**, **7b**, **9a** and **9b***

To a suspension of $CrO_3$ (5.62 g, 56.21 mmol) in $CH_2Cl_2$ (40 mL) at −20 °C, 3,5-dimethylpyrazole (5.40 g, 56.17 mmol) was added and the resulting mixture was stirred at −20 °C for 10 min. A solution of **4a** (1.65 g, 5.62 mmol) in $CH_2Cl_2$ (8 mL) was then added dropwise and the mixture was stirred continuously for 1 h. The reaction mixture was then filtered through a pad of Celite and evaporated to dryness. The residue was purified by column chromatography over silica gel (PE/EA = 8/1) to afford the crude product **7a** which could be used in the next reaction.

The procedure described above (using the same quantities of reagents) was followed to yield **7b**, **9a** and **9b**.

*4.1.11. General procedure for synthesis of **8a**, **8b**, **10a** and **10b***

Compound **7a** or **7b** (212 mg) was dissolved in $CH_2Cl_2$ (5 mL), TFA (420 μL, 5.49 mmol) was added. Then the reaction was stirred in an ice bath for 1 h. The reaction mixture was treated with saturated $NaHCO_3$ solution until a pH of 8–9 and extracted by $CH_2Cl_2$ (10 mL), washed with brine (25 mL). The organic layers were dried by $MgSO_4$, and the solvent was removed *in vacuo* to afford the residue which was purified by flash column chromatography over silica gel (PE/diethylamine = 10/1) to yield the desired product **8a** or **8b**.

The procedure described above (using the same quantities of reagents) was followed to yield **10a** and **10b**, which were immediately used in the next reaction.

*4.1.12. (R,E)-3,5,5-trimethyl-4-(3-(methylamino)prop-1-en-1-yl) cyclohex-2-en-1-one (**8a**)*

Yield 71.4%, orange oil, $[\alpha]_D^{20}$ +128.2 (*c* 1.014, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.86 (br s, 1H, 4-H), 5.64 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.47 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.21 (d, *J* = 6.4 Hz, 2H, 9-H), 2.51 (d, *J* = 9.2 Hz, 1H, 6-H), 2.39 (s, 3H, 10-H), 2.32 (d, *J* = 16.8 Hz, 1H, 2-H), 2.05 (d, *J* = 16.8 Hz, 1H, 2-H), 1.88 (s, 3H, 13-H), 1.00 (s, 3H, 11-H), 0.94 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 162.2 (5-C), 132.9 (8-C), 128.9 (7-C), 125.7 (4-C), 55.9 (6-C), 53.3 (9-C), 47.5 (2-C), 36.1 (1-C), 35.9 (10-C), 27.9 (11-C), 27.2 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 208.1707 $[M+H]^+$, calcd for $C_{13}H_{22}NO$, 208.1701.

*4.1.13. (S,E)-3,5,5-trimethyl-4-(3-(methylamino)prop-1-en-1-yl) cyclohex-2-en-1-one (**8b**)*

Yield 60.2%, orange oil, $[\alpha]_D^{20}$ −125.2 (*c* 1.031, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.86 (br s, 1H, 4-H), 5.64 (dt, *J* = 15.2 Hz, 6.0 Hz, 1H, 8-H), 5.47 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.20 (d, *J* = 6.0 Hz, 2H, 9-H), 2.51 (d, *J* = 9.2 Hz, 1H, 6-H), 2.39 (s, 3H, 10-H), 2.32 (d, *J* = 16.8 Hz, 1H, 2-H), 2.05 (d, *J* = 16.8 Hz, 1H, 2-H), 1.88 (s, 3H, 13-H), 1.00 (s, 3H, 11-H), 0.94 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 162.1 (5-C), 133.0 (8-C), 128.8 (7-C), 125.7 (4-C), 55.9 (6-C), 53.4 (9-C), 47.5 (2-C), 36.1 (1-C), 35.9 (10-C), 27.9 (11-C), 27.2 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 208.1708 $[M+H]^+$, calcd for $C_{13}H_{22}NO$, 208.1701.

*4.1.14. General procedure for synthesis of **11a** and **11b***

To a mixture of compound **8a** or **8b** (1.0 eq) and paraformaldehyde (3.0 eq) in $CH_2Cl_2$ (4 mL), which was stirred for 1 h while maintaining gentle reflux, was added $NaBH_3CN$ (3.0 eq). The reaction mixture was refluxed until the starting material disappeared, as indicated by TLC. The solvent was removed from the reaction mixture under reduced pressure. Saturated $NaHCO_3$ solution (10 mL) was added, and the aqueous phase was extracted with $CH_2Cl_2$ (15 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified using flash column chromatography over silica gel (PE/diethylamine = 25/1) to obtain the product **11a** or **11b**.

*4.1.15. (R,E)-4-(3-(dimethylamino)prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**11a**)*

Yield 78.1%, orange oil, $[\alpha]_D^{20}$ +148.5 (*c* 1.089, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.88 (br s, 1H, 4-H), 5.65 (dt, *J* = 15.2 Hz, 6.8 Hz, 1H, 8-H), 5.48 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 2.93 (d, *J* = 6.8 Hz, 2H, 9-H), 2.54 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.23 (s, 6H, 10-H), 2.08 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (d, *J* = 1.2 Hz, 3H, 13-H), 1.02 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.1 (3-C), 161.9 (5-C), 132.0 (8-C), 130.3 (7-C), 125.8 (4-C), 61.6 (9-C), 55.9 (6-C), 47.6 (2-C), 45.2 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 222.1859 $[M+H]^+$, calcd for $C_{14}H_{24}NO$, 222.1858.

*4.1.16. (S,E)-4-(3-(dimethylamino)prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**11b**)*

Yield 68.2%, orange oil, $[\alpha]_D^{20}$ −152.1 (*c* 1.064, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.88 (br s, 1H, 4-H), 5.64 (dt, *J* = 15.2 Hz, 6.8 Hz, 1H, 8-H), 5.48 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 2.93 (d, *J* = 6.8 Hz, 2H, 9-H), 2.54 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.22 (s, 6H, 10-H), 2.08 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (d, *J* = 1.2 Hz, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.1 (3-C), 161.9 (5-C), 132.0 (8-C), 130.2 (7-C), 125.8 (4-C), 61.6 (9-C), 55.9 (6-C), 47.6 (2-C), 45.2 (10-C), 36.1 (1-C), 27.9 (11-C), 27.2 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 222.1856 $[M+H]^+$, calcd for $C_{14}H_{24}NO$, 222.1858.

*4.1.17. General procedure for synthesis of **12a,b**–**14a,b** and **18a,b**–**20a,b***

To a mixture of chiral amine (1.0 eq) and $K_2CO_3$ (1.5 eq) in 0.6 mL of DMF, various substituted fluorobenzyl bromide (1.1 eq) was added in drops. Then, the reaction was stirred for 6–10 min. The reaction mixture was filtered through a pad of Celite. The filtration was finally purified by column chromatography (Toyopearl HW-

40F) in $CH_2Cl_2$:MeOH (2:1) as eluent to afford the target compounds **12a**,**b**–**14a**,**b** and **18a,b**–**20a**,**b**.

*4.1.18. (R,E)-4-(3-[(2-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**12a**)*

Yield 58.3%, orange oil, $[\alpha]_D^{20}$ +116.6 (*c* 1.134, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 1H, Ar–H), 7.23 (m, 1H, Ar–H), 7.11 (m, 1H, Ar–H), 7.03 (m, 1H, Ar–H), 5.89 (br s, 1H, 4-H), 5.70 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.53 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.57 (br s, 2H, N–$CH_2$), 3.07 (d, *J* = 6.4 Hz, 2H, 9-H), 2.56 (d, *J* = 9.2 Hz, 1H, 6-H), 2.34 (d, *J* = 16.7 Hz, 1H, 2-H), 2.22 (s, 3H, 10-H), 2.08 (d, *J* = 16.7 Hz, 1H, 2-H), 1.90 (d, *J* = 1.0 Hz, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.1 (3-C), 161.9 (5-C), 161.4 (d, *J* = 244.9 Hz, Ar–C), 132.0 (8-C), 131.5 (d, *J* = 4.4 Hz, Ar–C), 130.4 (7-C), 128.8 (d, *J* = 8.0 Hz, Ar–C), 125.8 (4-C), 125.4 (d, *J* = 14.6 Hz, Ar–C), 123.9 (d, *J* = 3.5 Hz, Ar–C), 115.3 (d, *J* = 22.0 Hz, Ar–C), 59.4 (9-C), 56.0 (6-C), 54.1 (N–$\underline{C}H_2$-Ar), 47.6 (2-C), 42.0 (10-C), 36.2 (1-C), 27.9 (11-C), 27.3 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 316.2074 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.19. (S,E)-4-(3-[(2-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**12b**)*

Yield 51.1%, orange oil, $[\alpha]_D^{20}$ –115.1 (*c* 1.062, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 1H, Ar–H), 7.23 (m, 1H, Ar–H), 7.10 (m, 1H, Ar–H), 7.02 (m, 1H, Ar–H), 5.88 (br s, 1H, 4-H), 5.69 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.52 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.55 (br s, 2H, N–$CH_2$), 3.07 (d, *J* = 6.4 Hz, 2H, 9-H), 2.55 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.4 Hz, 1H, 2-H), 2.21 (s, 3H, 10-H), 2.07 (d, *J* = 16.4 Hz, 1H, 2-H), 1.90 (d, *J* = 1.2 Hz, 3H, 13-H), 1.02 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 162.0 (5-C), 161.4 (d, *J* = 244.8 Hz, Ar–C), 132.0 (8-C), 131.5 (d, *J* = 4.5 Hz, Ar–C), 130.4 (7-C), 128.8 (d, *J* = 8.3 Hz, Ar–C), 125.7 (4-C), 125.3 (d, *J* = 14.5 Hz, Ar–C), 123.9 (d, *J* = 3.6 Hz, Ar–C), 115.3 (d, *J* = 22.1 Hz, Ar–C), 59.4 (9-C), 55.9 (6-C), 54.1 (N–$\underline{C}H_2$-Ar), 47.6 (2-C), 42.0 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.6 (13-C). HR-ESIMS *m/z* 316.2076 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.20. (R,E)-4-(3-[(3-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**13a**)*

Yield 48.1%, orange oil, $[\alpha]_D^{20}$ +113.6 (*c* 1.035, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 1H, Ar–H), 7.05 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.90 (br s, 1H, 4-H), 5.68 (dt, *J* = 15.2 Hz, 6.8 Hz, 1H, 8-H), 5.51 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.48 (br s, 2H, N–$CH_2$), 3.03 (d, *J* = 6.8 Hz, 2H, 9-H), 2.56 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.19 (s, 3H, 10-H), 2.08 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.1 (3-C), 163.0 (d, *J* = 243.8 Hz, Ar–C), 161.9 (5-C), 141.7 (d, *J* = 6.9 Hz, Ar–C), 132.0 (8-C), 130.3 (7-C), 129.7 (d, *J* = 8.2 Hz, Ar–C), 125.8 (4-C), 124.3 (d, *J* = 2.5 Hz, Ar–C), 115.6 (d, *J* = 21.1 Hz, Ar–C), 113.9 (d, *J* = 20.9 Hz, Ar–C), 61.3 (N–$\underline{C}H_2$-Ar), 59.3 (9-C), 55.9 (6-C), 47.6 (2-C), 42.2 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.6 (13-C). HR-ESIMS *m/z* 316.2080 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.21. (S,E)-4-(3-[(3-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**13b**)*

Yield 50.1%, orange oil, $[\alpha]_D^{20}$ –118.0 (*c* 1.110, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 1H, Ar–H), 7.05 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.89 (br s, 1H, 4-H), 5.68 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.51 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.48 (br s, 2H, N–$CH_2$), 3.03 (d, *J* = 6.4 Hz, 2H, 9-H), 2.56 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.19 (s, 3H, 10-H), 2.09 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (d, *J* = 1.2 Hz, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 163.0 (d, *J* = 243.8 Hz, Ar–C), 161.9 (5-C), 141.7 (d, *J* = 7.0 Hz, Ar–C), 132.0 (8-C), 130.3 (7-C), 129.7 (d, *J* = 8.3 Hz, Ar–C), 125.8 (4-C), 124.3 (d, *J* = 2.8 Hz, Ar–C), 115.6 (d, *J* = 21.1 Hz, Ar–C), 113.9 (d, *J* = 21.0 Hz, Ar–C), 61.3 (N–$\underline{C}H_2$-Ar), 59.3 (9-C), 55.9 (6-C), 47.6 (2-C), 42.2 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.6 (13-C). HR-ESIMS *m/z* 316.2074 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.22. (R,E)-4-(3-[(4-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**14a**)*

Yield 47.2%, orange oil, $[\alpha]_D^{20}$ +115.8 (*c* 1.054, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 2H, Ar–H), 7.00 (m, 2H, Ar–H), 5.89 (br s, 1H, 4-H), 5.67 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.51 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.45 (br s, 2H, N–$CH_2$), 3.02 (d, *J* = 6.4 Hz, 2H, 9-H), 2.55 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.17 (s, 3H, 10-H), 2.08 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (d, *J* = 1.2 Hz, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 162.0 (d, *J* = 243.4 Hz, Ar–C), 161.9 (5-C), 134.4 (Ar–C), 131.9 (8-C), 130.5 (d, *J* = 7.9 Hz, Ar–C), 130.3 (7-C), 125.8 (4-C), 115.1 (d, *J* = 21.1 Hz, Ar–C), 61.0 (N–$\underline{C}H_2$-Ar), 59.2 (9-C), 55.9 (6-C), 47.6 (2-C), 42.0 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.6 (13-C). HR-ESIMS *m/z* 316.2074 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.23. (S,E)-4-(3-[(4-fluorobenzyl) (methyl)amino]prop-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one (**14b**)*

Yield 53.7%, orange oil, $[\alpha]_D^{20}$ –113.9 (*c* 1.036, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 2H, Ar–H), 7.00 (m, 2H, Ar–H), 5.89 (br s, 1H, 4-H), 5.67 (dt, *J* = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.51 (dd, *J* = 15.2 Hz, 9.2 Hz, 1H, 7-H), 3.45 (br s, 2H, N–$CH_2$), 3.02 (d, *J* = 6.4 Hz, 2H, 9-H), 2.55 (d, *J* = 9.2 Hz, 1H, 6-H), 2.33 (d, *J* = 16.8 Hz, 1H, 2-H), 2.17 (s, 3H, 10-H), 2.08 (d, *J* = 16.8 Hz, 1H, 2-H), 1.90 (d, *J* = 0.8 Hz, 3H, 13-H), 1.03 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 162.0 (d, *J* = 243.5 Hz, Ar–C), 161.9 (5-C), 134.5 (Ar–C), 132.0 (8-C), 130.5 (d, *J* = 7.9 Hz, Ar–C), 130.3 (7-C), 125.8 (4-C), 115.1 (d, *J* = 21.1 Hz, Ar–C)), 61.0 (N–$\underline{C}H_2$-Ar), 59.2 (9-C), 55.9 (6-C), 47.6 (2-C), 42.0 (10-C), 36.1 (1-C), 27.9 (11-C), 27.3 (12-C), 23.6 (13-C). HR-ESIMS *m/z* 316.2080 $[M+H]^+$, calcd for $C_{20}H_{27}FNO$, 316.2077.

*4.1.24. (R)-4-(3-[(2-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**18a**)*

Yield 49.7%, orange oil, $[\alpha]_D^{20}$ +121.8 (*c* 1.079, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 1H, Ar–H), 7.23 (m, 1H, Ar–H), 7.10 (m, 1H, Ar–H), 7.02 (m, 1H, Ar–H), 5.80 (br s, 1H, 4-H), 3.55 (br s, 2H, N–$CH_2$), 2.39 (d, *J* = 17.2 Hz, 1H, 2-H), 2.35 (m, 2H, 9-H), 2.21 (s, 3H, 10-H), 2.01 (d, *J* = 17.2 Hz, 1H, 2-H), 1.97 (d, *J* = 1.2 Hz, 3H, 13-H), 1.84 (m, 1H, 6-H), 1.72 (m, 1H, 7-H), 1.60 (m, 2H, 8-H), 1.40 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.8 (5-C), 161.4 (d, *J* = 244.7 Hz, Ar–C), 131.5 (d, *J* = 4.6 Hz, Ar–C), 128.8 (d, *J* = 8.1 Hz, Ar–C), 125.3 (d, *J* = 13.8 Hz, Ar–C), 125.0 (4-C), 123.8 (d, *J* = 3.6 Hz, Ar–C), 115.3 (d, *J* = 22.2 Hz, Ar–C), 57.6 (9-C), 54.6 (N–$\underline{C}H_2$-Ar), 51.2 (6-C), 47.1 (2-C), 42.2 (10-C), 36.4 (1-C), 28.8 (12-C), 27.9 (7-C), 27.3 (8-C), 27.2 (11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2236 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.25. (S)-4-(3-[(2-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**18b**)*

Yield 54.7%, orange oil, $[\alpha]_D^{20}$ –124.9 (*c* 1.009, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.33 (m, 1H, Ar–H), 7.22 (m, 1H, Ar–H), 7.09 (m, 1H, Ar–H), 7.01 (m, 1H, Ar–H), 5.80 (br s, 1H, 4-H), 3.54 (br s, 2H, N–$CH_2$), 2.38 (d, *J* = 17.2 Hz, 1H, 2-H), 2.35 (m, 2H, 9-H), 2.20 (s, 3H, 10-H), 2.00 (d, *J* = 17.2 Hz, 1H, 2-H), 1.96 (s, 3H, 13-H), 1.83 (m, 1H, 6-H), 1.71 (m, 1H, 7-H), 1.59 (m, 2H, 8-H), 1.40 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.7 (5-C), 161.4 (d, *J* = 244.4 Hz, Ar–C), 131.5 (d, *J* = 4.6 Hz, Ar–C),

128.7 (d, $J$ = 8.2 Hz, Ar–C), 125.4 (d, $J$ = 14.3 Hz, Ar–C), 125.0 (4-C), 123.8 (d, $J$ = 3.5 Hz, Ar–C), 115.2 (d, $J$ = 22.1 Hz, Ar–C), 57.6 (9-C), 54.6 (N–$\underline{C}H_2$-Ar), 51.2 (6-C), 47.2 (2-C), 42.2 (10-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.3 (8-C), 27.2 (11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2234 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.26. (R)-4-(3-[(3-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**19a**)*

Yield 47.5%, orange oil, $[\alpha]_D^{20}$ +127.3 (*c* 1.021, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 1H, Ar–H), 7.04 (m, 2H, Ar–H), 6.93 (m, 1H, Ar–H), 5.81 (br s, 1H, 4-H), 3.45 (br s, 2H, N–$CH_2$), 2.37 (d, $J$ = 17.6 Hz, 1H, 2-H), 2.34 (m, 2H, 9-H), 2.17 (s, 3H, 10-H), 2.02 (d, $J$ = 17.6 Hz, 1H, 2-H), 1.97 (d, $J$ = 1.2 Hz, 3H, 13-H), 1.85 (m, 1H, 6-H), 1.72 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.40 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.7 (5-C), 162.9 (d, $J$ = 244.1 Hz, Ar–C), 141.9 (d, $J$ = 6.8 Hz, Ar–C), 129.6 (d, $J$ = 8.2 Hz, Ar–C), 125.1 (4-C), 124.4 (d, $J$ = 2.8 Hz, Ar–C), 115.6 (d, $J$ = 21.1 Hz, Ar–C), 113.9 (d, $J$ = 21.1 Hz, Ar–C), 62.0 (N–$\underline{C}H_2$-Ar), 57.7 (9-C), 51.2 (6-C), 47.2 (2-C), 42.3 (10-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.3 (8-C), 27.2 (11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2236 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.27. (S)-4-(3-[(3-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**19b**)*

Yield 52.1%, orange oil, $[\alpha]_D^{20}$ −128.4 (*c* 0.997, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 1H, Ar–H), 7.04 (m, 2H, Ar–H), 6.92 (m, 1H, Ar–H), 5.81 (br s, 1H, 4-H), 3.45 (br s, 2H, N–$CH_2$), 2.36 (d, $J$ = 17.2 Hz, 1H, 2-H), 2.33 (m, 2H, 9-H), 2.17 (s, 3H, 10-H), 2.02 (d, $J$ = 17.2 Hz, 1H, 2-H), 1.97 (s, 3H, 13-H), 1.84 (m, 1H, 6-H), 1.71 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.41 (m, 1H,7-H), 1.04 (m, 3H, 11-H), 1.00 (m, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.6 (5-C), 163.0 (d, $J$ = 243.7 Hz, Ar–C), 142.0 (d, $J$ = 6.9 Hz, Ar–C), 129.6 (d, $J$ = 8.1 Hz, Ar–C), 125.1(4-C), 124.3 (d, $J$ = 2.7 Hz, Ar–C), 115.6 (d, $J$ = 21.1 Hz, Ar–C), 113.8 (d, $J$ = 21.1 Hz, Ar–C), 62.0 (N–$\underline{C}H_2$-Ar), 57.7 (9-C), 51.2 (6-C), 47.2 (2-C), 42.3 (10-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.3 (8-C), 27.2 (11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2233 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.28. (R)-4-(3-[(4-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**20a**)*

Yield 44.9%, orange oil, $[\alpha]_D^{20}$ +121.2 (*c* 1.064, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 2H, Ar–H), 7.00 (m, 2H, Ar–H), 5.81 (br s, 1H, 4-H), 3.44 (br s, 2H, N–$CH_2$), 2.37 (d, $J$ = 17.2 Hz, 1H, 2-H), 2.33 (m, 2H, 9-H), 2.16 (s, 3H, 10-H), 2.02 (d, $J$ = 17.2 Hz, 1H, 2-H), 1.97 (d, $J$ = 1.2 Hz, 3H, 13-H), 1.84 (m, 1H, 6-H), 1.70 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.40 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (m, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.6 (5-C), 162.0 (d, $J$ = 243.3 Hz, Ar–C), 134.5 (Ar–C), 130.4 (d, $J$ = 7.8 Hz, Ar–C), 125.1 (4-C), 115.1 (d, $J$ = 21.1 Hz, Ar–C), 61.6 (N–$\underline{C}H_2$-Ar), 57.5 (9-C), 51.1 (6-C), 47.2 (2-C), 42.1 (10-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.2 (8-C and 11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2233 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.29. (S)-4-(3-[(4-fluorobenzyl) (methyl)amino]propyl)-3,5,5-trimethylcyclohex-2-en-1-one (**20b**)*

Yield 56.2%, orange oil, $[\alpha]_D^{20}$ −118.9 (*c* 1.061, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 2H, Ar–H), 7.00 (m, 2H, Ar–H), 5.81 (br s, 1H, 4-H), 3.44 (br s, 2H, N–$CH_2$), 2.37 (d, $J$ = 17.2 Hz, 1H, 2-H), 2.33 (m, 2H, 9-H), 2.17 (s, 3H, 10-H), 2.02 (d, $J$ = 17.2 Hz, 1H, 2-H), 1.97 (s, 3H, 13-H), 1.85 (m, 1H, 6-H), 1.71 (m, 1H, 7-H), 1.59 (m, 2H, 8-H), 1.41 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (m, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.6 (5-C), 161.9 (d, $J$ = 243.0 Hz, Ar–C), 134.7 (Ar–C), 130.4 (d, $J$ = 7.8 Hz, Ar–C), 125.0 (4-C), 115.0 (d, $J$ = 21.0 Hz, Ar–C), 61.7 (N–$\underline{C}H_2$-Ar), 57.6 (9-C), 51.2 (6-C), 47.2 (2-C), 42.1 (10-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.2 (8-C and 11-C), 24.7 (13-C). HR-ESIMS *m/z* 318.2234 $[M+H]^+$, calcd for $C_{20}H_{29}FNO$, 318.2233.

*4.1.30. General procedure for synthesis of **15a**,**b**–**17a**,**b** and **21a**,**b**-**23a**,**b***

A mixture of chiral amine (1.0 eq, **8a**, **8b** or **10a**, **10b**) and $K_2CO_3$ (3.0 eq) in $CH_3CN$ (2.5 mL) was stirred at room temperature. After 10 min, various substituted fluorobenzyl bromide (2.5 eq) was added in drops. The reaction was stirred for 8–24 h. After completion of the reaction, as indicated by TLC, the reaction mixture was filtered by a pad of Celite and evaporated to dryness. The residue was subsequently purified through a gel permeation column chromatography (Toyopearl HW-40F) with the mobile phase ($CH_2Cl_2$:MeOH = 2:1, V/V) to obtain the target compounds **15a**,**b**-**17a**,**b** and **21a**,**b**-**23a**,**b**.

*4.1.31. (R,E)-N,N-bis(2-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**15a**)*

Yield 70.2%, orange oil, $[\alpha]_D^{20}$ +67.1 (*c* 1.054, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.03 (m, 2H, Ar–H), 7.50 (m, 2H, Ar–H), 7.29 (m, 2H, Ar–H), 7.14 (m, 2H, Ar–H), 6.21 (dd, $J$ = 15.2 Hz, 9.4 Hz, 1H, 7-H), 5.86 (dt, $J$ = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.85 (br s, 1H, 4-H), 5.29 (d, $J$ = 12.9 Hz, 1H, 14-H), 5.23 (d, $J$ = 12.9 Hz, 1H, 14-H), 5.04 (d, $J$ = 12.7 Hz, 1H, 15-H), 4.98 (d, $J$ = 12.9 Hz, 1H, 15-H), 4.43 (m, 2H, 9-H), 2.89 (s, 3H, 10-H), 2.73 (d, $J$ = 9.4 Hz, 1H, 6-H), 2.27 (d, $J$ = 16.8 Hz, 1H, 2-H), 2.06 (d, $J$ = 16.8 Hz, 1H, 2-H), 1.86 (d, $J$ = 0.9 Hz, 3H, 13-H), 1.01 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.1 (3-C), 161.8 (d, $J$ = 248.0 Hz, Ar–C), 159.1 (5-C), 143.7 (7-C), 136.4 (Ar–C), 133.6 (d, $J$ = 8.7 Hz, Ar–C), 126.8 (4-C), 125.6 (d, $J$ = 3.4 Hz, Ar–C), 120.1 (8-C), 116.3 (d, $J$ = 21.9 Hz, Ar–C), 114.6 (d, $J$ = 13.6 Hz, Ar–C), 63.0 (9-C), 58.8 (14-C and 15-C), 55.9 (6-C), 47.4 (2-C), 45.5 (10-C), 36.5 (1-C), 27.8 (11-C), 27.4 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 584.0807 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804.

*4.1.32. (S,E)-N,N-bis(2-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**15b**)*

Yield 63.2%, orange oil, $[\alpha]_D^{20}$ −65.8 (*c* 1.073, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.01 (m, 2H, Ar–H), 7.49 (m, 2H, Ar–H), 7.27 (m, 2H, Ar–H), 7.13 (m, 2H, Ar–H), 6.21 (dd, $J$ = 15.2 Hz, 9.4 Hz, 1H, 7-H), 5.87 (dt, $J$ = 15.2 Hz, 6.4 Hz, 1H, 8-H), 5.85 (br s, 1H, 4-H), 5.27 (d, $J$ = 12.9 Hz, 1H, 14-H), 5.22 (d, $J$ = 12.9 Hz, 1H, 14-H), 5.02 (d, $J$ = 12.9 Hz, 1H, 15-H), 4.97 (d, $J$ = 12.9 Hz, 1H, 15-H), 4.42 (m, 2H, 9-H), 2.89 (s, 3H, 10-H), 2.73 (d, $J$ = 9.4 Hz, 1H, 6-H), 2.27 (d, $J$ = 16.8 Hz, 1H, 2-H), 2.04 (d, $J$ = 16.8 Hz, 1H, 2-H), 1.86 (s, 3H, 13-H), 1.00 (s, 3H, 11-H), 0.95 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.1 (3-C), 161.8 (d, $J$ = 248.1 Hz, Ar–C), 159.2 (5-C), 143.7 (7-C), 136.3 (Ar–C), 133.6 (d, $J$ = 8.7 Hz, Ar–C), 126.8 (4-C), 125.6 (d, $J$ = 3.4 Hz, Ar–C), 120.1 (8-C), 116.3 (d, $J$ = 22.2 Hz, Ar–C), 114.6 (d, $J$ = 13.5 Hz, Ar–C), 63.0 (9-C), 58.8 (14-C and 15-C), 55.9 (6-C), 47.4 (2-C), 45.6 (10-C), 36.4 (1-C), 27.8 (11-C), 27.4 (12-C), 23.5 (13-C). HR-ESIMS *m/z* 584.0784 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804.

*4.1.33. (R,E)-N,N-bis(3-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**16a**)*

Yield 67.5%, orange oil, $[\alpha]_D^{20}$ +66.8 (*c* 1.154, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.51 (m, 2H, Ar–H), 7.42 (m, 4H, Ar–H), 7.17 (m, 2H, Ar–H), 6.09 (m, 1H, 7-H), 6.00 (m, 1H, 8-H), 5.84 (br s, 1H, 4-H), 5.28 (d, $J$ = 13.1 Hz, 1H, 14-H), 5.25 (d, $J$ = 13.1 Hz, 1H, 14-H), 5.08 (d, $J$ = 12.8 Hz, 1H, 15-H), 5.03 (d, $J$ = 12.8 Hz, 1H, 15-H), 4.27 (m, 2H, 9-H), 3.08 (s, 3H, 10-H), 2.83 (d, $J$ = 9.0 Hz, 1H, 6-H), 2.25 (d, $J$ = 16.9 Hz, 1H, 2-H), 2.06 (d, $J$ = 16.9 Hz, 1H, 2-H), 1.89 (d, $J$ = 0.8 Hz, 3H, 13-H), 1.01 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.2 (3-C), 162.7 (d, $J$ = 248.1 Hz, Ar–C), 159.8 (5-C), 142.6

(7-C), 131.2 (d, $J$ = 8.1 Hz, Ar–C), 129.4 (d, $J$ = 7.2 Hz, Ar–C), 129.3 (Ar–C), 126.6 (4-C), 120.7 (8-C), 120.1 (d, $J$ = 19.9 Hz, Ar–C), 118.2 (d, $J$ = 20.8 Hz, Ar–C), 64.7 (14-C and 15-C), 62.6 (9-C), 55.6 (6-C), 47.5 (2-C), 45.9 (10-C), 36.4 (1-C), 27.8 (11-C), 27.4 (12-C), 23.7 (13-C). HR-ESIMS $m/z$ 584.0810 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804.

*4.1.34. (S,E)-N,N-bis(3-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**16b**)*

Yield 64.9%, orange oil, $[\alpha]_D^{20}$ −67.1 ($c$ 1.098, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.52 (m, 2H, Ar–H), 7.42 (m, 4H, Ar–H), 7.19 (m, 2H, Ar–H), 6.10 (m, 1H, 7-H), 6.00 (m, 1H, 8-H), 5.89 (br s, 1H, 4-H), 5.27 (d, $J$ = 13.0 Hz, 1H, 14-H), 5.24 (d, $J$ = 13.0 Hz, 1H, 14-H), 5.13 (d, $J$ = 16.6 Hz, 1H, 15-H), 5.09 (d, $J$ = 16.6 Hz, 1H, 15-H), 4.28 (m, 2H, 9-H), 3.05 (s, 3H, 10-H), 2.83 (d, $J$ = 9.0 Hz, 1H, 6-H), 2.26 (d, $J$ = 16.9 Hz, 1H, 2-H), 2.10 (d, J = 16.9 Hz, 1H, 2-H), 1.91 (d, $J$ = 1.0 Hz, 3H, 13-H), 1.04 (s, 3H, 11-H), 0.99 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.1 (3-C), 162.7 (d, $J$ = 248.5 Hz, Ar–C), 159.4 (5-C), 142.9 (7-C), 131.3 (d, $J$ = 8.2 Hz, Ar–C), 129.4 (Ar–C), 129.2 (d, $J$ = 7.2 Hz, Ar–C), 126.8 (4-C), 120.4 (8-C), 120.0 (d, $J$ = 21.7 Hz, Ar–C), 118.2 (d, $J$ = 20.8 Hz, Ar–C), 64.7 (14-C and 15-C), 62.4 (9-C), 55.7 (6-C), 47.5 (2-C), 45.9 (10-C), 36.5 (1-C), 27.8 (11-C), 27.5 (12-C), 23.7 (13-C). HR-ESIMS $m/z$ 584.0801 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804.

*4.1.35. (R,E)-N,N-bis(4-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**17a**)*

Yield 68.2%, orange oil, $[\alpha]_D^{20}$ +69.3 ($c$ 1.089, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.67 (m, 4H, Ar–H), 7.11 (m, 4H, Ar–H), 6.07 (m, 1H, 7-H), 5.96 (m, 1H, 8-H), 5.86 (br s, 1H, 4-H), 5.20 (d, $J$ = 12.6 Hz, 1H, 14-H), 5.17 (d, $J$ = 12.6 Hz, 1H, 14-H), 5.05 (d, $J$ = 13.6 Hz, 1H, 15-H), 5.02 (d, $J$ = 13.6 Hz, 1H, 15-H), 4.20 (m, 2H, 9-H), 2.98 (s, 3H, 10-H), 2.80 (d, $J$ = 8.9 Hz, 1H, 6-H), 2.24 (d, $J$ = 16.9 Hz, 1H, 2-H), 2.06 (d, $J$ = 16.9 Hz, 1H, 2-H), 1.88 (d, $J$ = 1.0 Hz, 3H, 13-H), 1.02 (s, 3H, 11-H), 0.96 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.1 (3-C), 164.1 (d, $J$ = 251.4 Hz, Ar–C), 159.6 (5-C), 142.5 (7-C), 135.4 (d, $J$ = 8.6 Hz, Ar–C), 126.7 (4-C), 123.1 (d, $J$ = 3.5 Hz, Ar–C), 120.6 (8-C), 116.7 (d, $J$ = 21.6 Hz, Ar–C), 64.5 (14-C and 15-C), 62.0 (9-C), 55.6 (6-C), 47.4 (2-C), 45.3 (10-C), 36.4 (1-C), 27.8 (11-C), 27.5 (12-C), 23.7 (13-C). HR-ESIMS $m/z$ 584.0806 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804.

*4.1.36. (S,E)-N,N-bis(4-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)prop-2-en-1-aminium bromide (**17b**)*

Yield 68.2%, orange oil, $[\alpha]_D^{20}$ −66.3 ($c$ 1.105, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.68 (m, 4H, Ar–H), 7.13 (m, 4H, Ar–H), 6.08 (m, 1H, 7-H), 5.96 (m, 1H, 8-H), 5.89 (br s, 1H, 4-H), 5.21 (d, $J$ = 12.6 Hz, 1H, 14-H), 5.17 (d, $J$ = 12.6 Hz, 1H, 14-H), 5.10 (d, $J$ = 12.4 Hz, 1H, 15-H), 5.05 (d, $J$ = 12.4 Hz, 1H, 15-H), 4.22 (m, 2H, 9-H), 2.97 (s, 3H, 10-H), 2.81 (d, $J$ = 8.9 Hz, 1H, 6-H), 2.26 (d, $J$ = 16.8 Hz, 1H, 2-H), 2.09 (d, $J$ = 16.8 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.04 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.1 (3-C), 164.2 (d, $J$ = 251.6 Hz, Ar–C), 159.4 (5-C), 142.7 (7-C), 135.4 (d, $J$ = 8.4 Hz, Ar–C), 126.7 (4-C), 123.0 (d, $J$ = 3.3 Hz, Ar–C), 120.4 (8-C), 116.7 (d, $J$ = 21.6 Hz, Ar–C), 64.5 (14-C and 15-C), 61.9 (9-C), 55.6 (6-C), 47.4 (2-C), 45.3 (10-C), 36.4 (1-C), 27.8 (11-C), 27.5 (12-C), 23.7 (13-C). HR-ESIMS $m/z$ 514.2415 $[M-Br+2(HCOOH-H)]^-$, calcd for $C_{29}H_{34}F_2NO_5$, 514.2411.

*4.1.37. (R)-N,N-bis(2-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**21a**)*

Yield 64.8%, orange oil, $[\alpha]_D^{20}$ +74.3 ($c$ 1.134, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.03 (m, 2H, Ar–H), 7.49 (m, 2H, Ar–H), 7.26 (m, 2H, Ar–H), 7.13 (m, 2H, Ar–H), 5.76 (br s, 1H, 4-H), 5.11 (m, 4H, 14-H and 15-H), 3.45 (m, 2H, 9-H), 3.14 (s, 3H, 10-H), 2.67 (d, $J$ = 17.4 Hz, 1H, 2-H), 2.01 (s, 3H, 13-H), 1.96 (d, $J$ = 17.4 Hz, 1H, 2-H), 1.95 (m, 3H, 6-H and 8-H), 1.70 (m, 1H, 7-H), 1.33 (m, 1H, 7-H), 1.03 (s, 3H, 11-H), 0.99 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.7 (3-C), 164.4 (5-C), 161.8 (d, $J$ = 247.9 Hz, Ar–C), 136.3 (d, $J$ = 1.8 Hz, Ar–C), 133.5 (d, $J$ = 8.7 Hz, Ar–C), 125.6 (d, $J$ = 3.3 Hz, Ar–C), 125.5 (4-C), 116.2 (d, $J$ = 22.0 Hz, Ar–C), 114.8 (Ar–C), 114.7 (Ar–C), 61.2 (9-C), 59.2 (14-C and 15-C), 50.5 (6-C), 46.9 (2-C), 46.7 (10-C), 36.3 (1-C), 28.5 (12-C), 27.1 (11-C), 27.0 (8-C), 25.0 (13-C), 23.0 (7-C). HR-ESIMS $m/z$ 586.0942 $[M+Br]^-$, calcd for $C_{27}H_{34}Br_2F_2NO$, 586.0961.

*4.1.38. (S)–N,N-bis(2-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**21b**)*

Yield 67.8%, orange oil, $[\alpha]_D^{20}$ −73.1 ($c$ 1.112, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.01 (m, 2H, Ar–H), 7.48 (m, 2H, Ar–H), 7.25 (m, 2H, Ar–H), 7.13 (m, 2H, Ar–H), 5.75 (br s, 1H, 4-H), 5.10 (m, 4H, 14-H and 15-H), 3.43 (m, 2H, 9-H), 3.13 (s, 3H, 10-H), 2.26 (d, $J$ = 17.4 Hz, 1H, 2-H), 2.00 (s, 3H, 13-H), 1.95 (d, $J$ = 17.4 Hz, 1H, 2-H), 1.94 (m, 3H, 6-H and 8-H), 1.70 (m, 1H, 7-H), 1.32 (m, 1H, 7-H), 1.02 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.8 (3-C), 164.5 (5-C), 161.9 (d, $J$ = 247.8 Hz, Ar–C), 136.3 (d, $J$ = 1.8 Hz, Ar–C), 133.6 (d, $J$ = 8.7 Hz, Ar–C), 125.6 (d, $J$ = 3.3 Hz, Ar–C), 125.5 (4-C), 116.3 (d, $J$ = 22.1 Hz, Ar–C), 114.8 (d, $J$ = 1.3 Hz, Ar–C), 114.7 (d, $J$ = 1.4 Hz, Ar–C), 61.2 (9-C), 59.3 (14-C and 15-C), 50.5 (6-C), 46.9 (2-C), 46.8 (10-C), 36.4 (1-C), 28.6 (12-C), 27.2 (11-C), 27.1 (8-C), 25.0 (13-C), 23.0 (7-C). HR-ESIMS $m/z$ 586.0977 $[M+Br]^-$, calcd for $C_{27}H_{34}Br_2F_2NO$, 586.0961.

*4.1.39. (R)-N,N-bis(3-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**22a**)*

Yield 61.2%, orange oil, $[\alpha]_D^{20}$ +72.7 ($c$ 1.075, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.42 (m, 4H, Ar–H), 7.33 (m, 4H, Ar–H), 7.09 (m, 2H, Ar–H), 5.67 (br s, 1H, 4-H), 5.24 (m, 2H, 14-H), 4.94 (m, 2H, 15-H), 3.36 (m, 2H, 9-H), 3.14 (s, 3H, 10-H), 2.22 (d, $J$ = 17.4 Hz, 1H, 2-H), 1.96 (s, 3H, 13-H), 1.91 (d, $J$ = 17.4 Hz, 1H, 2-H), 1.90 (m, 3H, 6-H and 8-H), 1.59 (m, 1H, 7-H), 1.24 (m, 1H, 7-H), 0.96 (s, 3H, 11-H), 0.91 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.8 (3-C), 164.8 (5-C), 162.6 (d, $J$ = 247.5 Hz, Ar–C), 131.0 (d, $J$ = 8.0 Hz, Ar–C), 129.6 (d, $J$ = 7.4 Hz, Ar–C), 129.1 (Ar–C), 125.4 (4-C), 120.0 (d, $J$ = 22.8 Hz, Ar–C), 117.9 (d, $J$ = 20.6 Hz, Ar–C), 64.8 (14-C and 15-C), 61.0 (9-C), 50.3 (6-C), 47.0 (2-C), 46.5 (10-C), 36.3 (1-C), 28.4 (12-C), 27.0 (11-C), 26.9 (8-C), 25.1 (13-C), 23.0 (7-C). HR-ESIMS $m/z$ 586.0958 $[M+Br]^-$, calcd for $C_{27}H_{34}Br_2F_2NO$, 586.0961.

*4.1.40. (S)–N,N-bis(3-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**22b**)*

Yield 65.1%, orange oil, $[\alpha]_D^{20}$ −73.4 ($c$ 1.097, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.47 (m, 2H, Ar–H), 7.39 (m, 4H, Ar–H), 7.15 (m, 2H, Ar–H), 5.76 (br s, 1H, 4-H), 5.26 (m, 2H, 14-H), 5.14 (m, 2H, 15-H), 3.33 (m, 2H, 9-H), 3.18 (s, 3H, 10-H), 2.27 (d, $J$ = 17.6 Hz, 1H, 2-H), 2.04 (d, $J$ = 1.0 Hz, 3H, 13-H), 1.97 (d, $J$ = 17.6 Hz, 1H, 2-H), 1.96 (m, 3H, 6-H and 8-H), 1.64 (m, 1H, 7-H), 1.27 (m, 1H, 7-H), 1.03 (s, 3H, 11-H), 0.99 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.8 (3-C), 164.6 (5-C), 162.7 (d, $J$ = 248.1 Hz, Ar–C), 131.1 (d, $J$ = 8.1 Hz, Ar–C), 129.5 (d, $J$ = 7.3 Hz, Ar–C), 129.1 (d, $J$ = 2.7 Hz, Ar–C), 125.5 (4-C), 120.0 (d, $J$ = 22.2 Hz, Ar–C), 118.1 (d, $J$ = 20.6 Hz, Ar–C), 64.8 (14-C and 15-C), 60.5 (9-C), 50.4 (6-C), 47.0 (2-C), 46.6 (10-C), 36.3 (1-C), 28.5 (12-C), 27.1 (11-C), 27.0 (8-C), 25.2 (13-C), 23.1 (7-C). HR-ESIMS $m/z$ 516.2578 $[M-Br+2(HCOOH-H)]^-$, calcd for $C_{29}H_{36}F_2NO_5$, 516.2567.

*4.1.41. (R)-N,N-bis(4-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**23a**)*

Yield 70.3%, orange oil, $[\alpha]_D^{20}$ +73.2 ($c$ 1.102, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.61 (m, 4H, Ar–H), 7.00 (m, 4H, Ar–H), 5.66 (br s, 1H, 4-H), 5.12 (m, 2H, 14-H), 4.92 (m, 2H, 15-H), 3.28 (m, 2H, 9-

H), 3.04 (s, 3H, 10-H), 2.21 (d, $J$ = 17.5 Hz, 1H, 2-H), 1.95 (s, 3H, 13-H), 1.90 (d, $J$ = 17.5 Hz, 1H, 2-H), 1.89 (m, 3H, 6-H and 8-H), 1.57 (m, 1H, 7-H), 1.22 (m, 1H, 7-H), 0.95 (s, 3H, 11-H), 0.90 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.7 (3-C), 164.8 (5-C), 164.0 (d, $J$ = 251.0 Hz, Ar–C), 135.3 (d, $J$ = 3.1 Hz, Ar–C), 135.2 (d, $J$ = 3.0 Hz, Ar–C), 125.4 (4-C), 123.3 (Ar–C), 116.4 (d, $J$ = 21.6 Hz, Ar–C), 64.6 (14-C and 15-C), 60.3 (9-C), 50.3 (6-C), 46.9 (2-C), 46.0 (10-C), 36.3 (1-C), 28.4 (12-C), 27.0 (8-C and 11-C), 25.2 (13-C), 22.8 (7-C). HR-ESIMS $m/z$ 516.2573 $[M\text{-}Br+2(HCOOH-H)]^-$, calcd for $C_{29}H_{36}F_2NO_5$, 516.2567.

*4.1.42. (S)–N,N-bis(4-fluorobenzyl)-N-methyl-3-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)propan-1-aminium bromide (**23b**)*

Yield 60.6%, orange oil, $[\alpha]_D^{20}$ −71.5 ($c$ 1.081, ethanol). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.61 (m, 4H, Ar–H), 7.03 (m, 4H, Ar–H), 5.70 (br s, 1H, 4-H), 5.14 (m, 2H, 14-H), 4.98 (m, 2H, 15-H), 3.26 (m, 2H, 9-H), 3.06 (s, 3H, 10-H), 2.23 (d, $J$ = 17.6 Hz, 1H, 2-H), 1.98 (s, 3H, 13-H), 1.92 (d, $J$ = 17.6 Hz, 1H, 2-H), 1.91 (m, 3H, 6-H and 8-H), 1.59 (m, 1H, 7-H), 1.24 (m, 1H, 7-H), 0.97 (s, 3H, 11-H), 0.93 (s, 3H, 12-H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 198.7 (3-C), 164.7 (5-C), 164.0 (d, $J$ = 251.0 Hz, Ar–C), 135.3 (d, $J$ = 3.1 Hz, Ar–C), 135.2 (d, $J$ = 3.0 Hz, Ar–C), 125.4 (4-C), 123.4 (Ar–C), 116.5 (d, $J$ = 21.6 Hz, Ar–C), 64.7 (14-C and 15-C), 60.1 (9-C), 50.3 (6-C), 46.9 (2-C), 46.0 (10-C), 36.3 (1-C), 28.5 (12-C), 27.1 (8-C and 11-C), 25.2 (13-C), 22.8 (7-C). HR-ESIMS $m/z$ 584.0810 $[M+Br]^-$, calcd for $C_{27}H_{32}Br_2F_2NO$, 584.0804. HR-ESIMS $m/z$ 516.2558 $[M\text{-}Br+2(HCOOH-H)]^-$, calcd for $C_{29}H_{36}F_2NO_5$, 516.2567.

### 4.2. Biological evaluation

#### 4.2.1. Invasion assay

The chemotaxis invasion assay was performed as described by Zhang et al. [20] using nontoxic concentrations of each compound. MDA-MB-231 cells were pretreated with the compounds at the indicated concentrations for 24 h at 37 °C in six-well cell culture plates. A chemoattractant (EGF; 1 ng/mL, 30 μL/well) was loaded into the lower chemotaxis chamber. Control (cells only) and pretreated cells were resuspended in binding medium (RPMI 1640 containing 0.1% BSA and 25 mM HEPES) at a density of 0.5 × $10^6$ cells/mL and placed into the upper chamber (50 μL/well). The 8 μm filter membranes (Neuroprobe), which had previously been pretreated with 0.001% fibronectin in serum-free medium at 4 °C overnight and air-dried, were inserted between the upper and lower chambers. The cells were incubated at 37 °C in 5% $CO_2$ for 3.5 h; then the filter membrane was rinsed, fixed, and stained. The number of migrating cells in three separate fields was counted using light microscopy at 400×. The inhibitory ratio (IR) was calculated as follows: IR% = (1 − number of migrated cells in sample/number of migrated cells in control) × 100%. The potencies of the products were expressed as the median inhibitory concentration ($IC_{50}$) values. LY294002 (Camarillo, CA, USA) was used as a positive control substance for this assay [21,22].

#### 4.2.2. Western blotting

MDA-MB-231 cells were cultured in 12-well plates and lysed on ice in 200 μL of RIPA buffer [100 mM NaCl, 0.25% w/v sodium deoxycholate, 1.0% w/v NP40, 0.1% w/v sodium dodecyl sulfate (SDS), 2 mM ethylenediaminetetraacetic acid, 50 mM NaF, 10 nM okadaic acid, 1 mM sodium orthovanadate, protease inhibitor cocktail, and 50 mM Tris–HCl, pH 7.2]. The samples were electrophoresed on 7.5% SDS–polyacrylamide gels, transferred to polyvinylidene fluoride membranes, blocked for 1 h in 5% (w/v) bovine serum albumin, and then incubated with primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies for 1 h at room temperature. The bands were detected using chemiluminescent reagent and autoradiographic film.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.037.

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