Discovery of Antimetastatic Chiral Ionone Alkaloid Derivatives Targeting HIF-1α/VEGF/VEGFR2 Pathway

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Novel chiral ionone alkaloid derivatives were synthesized and their antimetastatic effects were evaluated in human breast cancer cells using chemotaxis assay. Compared with positive control LY294002, a PI3 K inhibitor, derivatives **10a, 11a, 11c, 11g, 11j, 11k** and **11w** exhibited significant inhibitory effects against cancer cell migration. Especially, the IC_{50} for compound **11g** was as low as 0.035 ± 0.004 μM. Further investigations on compound **11g** revealed that it could exert inhibitory effects on the adhesion, migration and invasion of MDA-MB-231 cells. The mechanisms for the antitumor metastatic effects of **11g** might be through the inhibition of HIF-1α/VEGF/VEGFR2/Akt pathway, which suppressed the downstream signaling molecules, including Akt1/mTOR/p70S6K and Akt2/PKCζ/integrin β1 pathways. Taken together, chiral ionone alkaloid derivative **11g** has the potential to be developed into an antitumor metastatic agent for breast cancer.

1. Introduction

Breast cancer is the leading cause of deaths from malignancies in women. The major obstacle in the management of breast cancer, especially at advanced stages, is metastasis.[1] Metastasis is a complex, multistep biological process, involving a multitude of genes and biomolecules.[2] Hypoxia has been shown to be a hallmark of cancer progression and is associated with the metastatic potential of multiple solid tumors, where tumor cells proliferate rapidly and form large solid tumor masses, leading to obstruction and compression of the blood vessels surrounding these masses.[3] Activation of hypoxia-inducible factor-1α (HIF-1α) transcription factor is the most recognized pathway adopted by hypoxic cells.[4] Accumulating evidences have shown a strong correlation between elevated HIF-1α expression and tumor metastasis, angiogenesis as well as poor patient prognosis. Therefore, HIF-1α has been recognized as a potential target for cancer therapy.

We previously reported that, a novel chiral ionone alkaloid derivative (**1**) was able to inhibit EGF-induced invasion of MDA-MB-231 cells.[5] In this study, the structure optimization of **1** was performed and their antimetastatic activity was screened (Figure 1). Moreover, the molecular mechanisms of active derivatives were investigated.

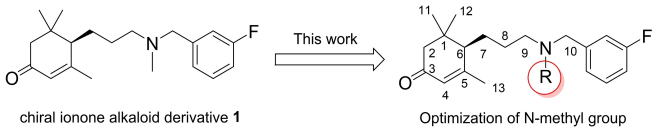

Figure 1. The structure of chiral ionone alkaloid derivative

2. Results and Discussion

2.1. Chemistry

As shown in Scheme 1, the 6R-α-ionone was an appropriate starting material. Several attempts were made to get 6R-α-ionone from α-ionone. Although 6R-α-ionone could be total synthesized through multiple steps,[6] it was not performed due to expensive and toxic metal reagents. The multiple resolving crystallization method used in our previous study was also discarded because of low yields. It was reported that 6S- and 6R-α-ionone could be obtained by stereoselective lipase-mediated esterification of α-ionol.[7] According to this, we obtained a large amount of 6R-α-ionone (**2**) from α-ionone. The [α]20 D (c = 0.8 $CHCl_3$) value of **2** was +441, which was as similar as the total synthesis 6R-α-ionone. To get acid **3**, 6R-α-ionone reacted with NaClO in EtOH for 8 h at 0 °C. Compound **3** was reacted with 3-fluorobenzylamine in the presence of HOBt, EDCI and triethylamine in CH_2Cl_2 for 12 h at room temperature. The resulted amide **4** was transformed into the corresponding amines **5** using the catalytic hydrogenation with Raney nickel. Then **5** was reduced to **6** with $LiAlH_4$ at room temperature for 8 h. After the amine was protected by the $(Boc)_2O$, the allyl position was oxidized to get **8**. Before reductive amination with the corresponding aldehyde, the protecting group was removed. Target compounds **10a–10e** were synthesized by nucleophilic substitution reaction. Compounds **11a–11x** were

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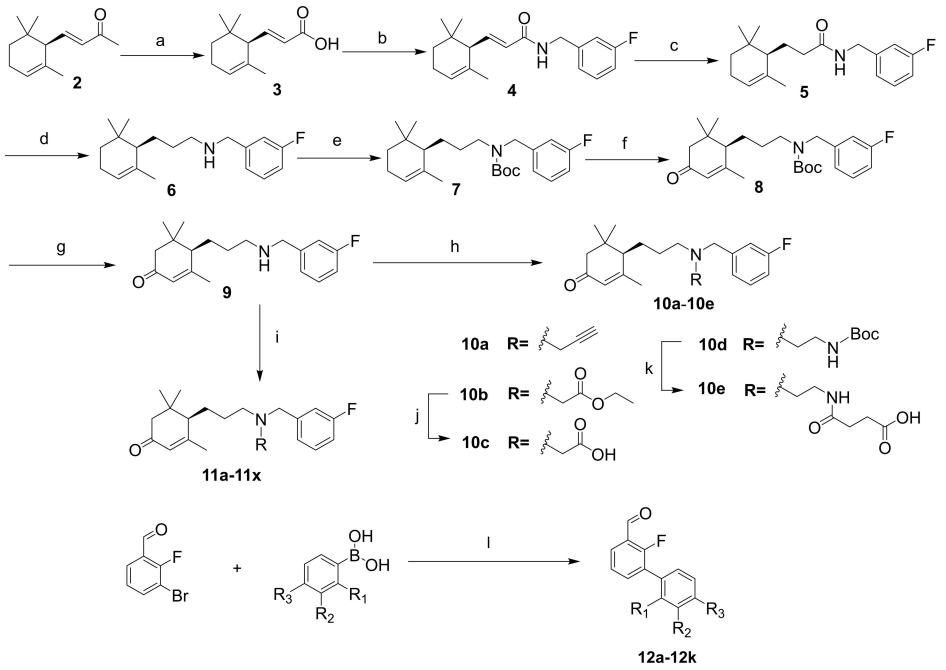
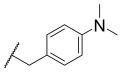
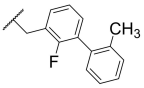
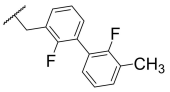
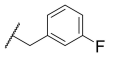
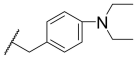
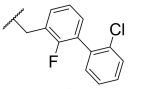
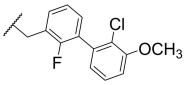
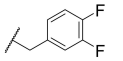
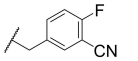
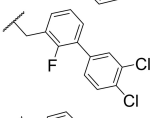
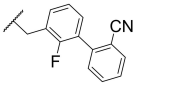
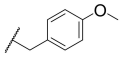
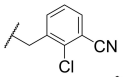
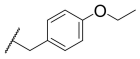
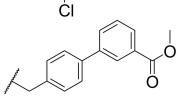
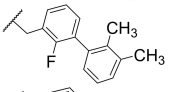
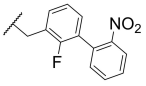
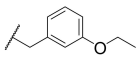
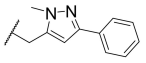
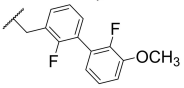
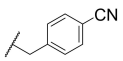
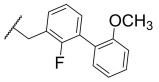
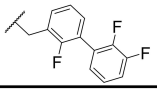


Scheme 1. Reagents and condictions: (a) NaOCl, EtOH, 0 °C to rt; (b) 3-fluorobenzylamine, triethylamine, HOBt, EDCI, CH_2Cl_2, rt; (c) Raney nickel, H_2, THF, rt; (d) $LiAlH_4$, THF, 0 °C to rt; (e) $(Boc)_2O$, $KHCO_3$, CH_2Cl_2, rt; (f) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, -20 °C; (g) TFA, CH_2Cl_2, 0 °C to rt; (h) R-Br, K_2CO_3, acetone, reflux; (i) commercial aldehyde or **12a-12k**, $ZnCl_2$, $Na(CN)BH_3$, MeOH, rt; (j) KOH,MeOH,rt; (k) 1)TFA, CH_2Cl_2,rt;2) pyridine,succinic anhydride, 0 °C to rt; (l) substituted phenylboronic acid, $(PPh_3)_4Pd$,1,4-dioxane:H_2O=5:1.

NO.	R	NO.	R	NO.	R	NO.	R
11a		11h		11o		11u	
11b		11i		11p		11v	
11c		11j		11q		11w	
11d		11k					
11e		11l		11r		11x	
11f		11m		11s			
11g		11n		11t			

Scheme 1. The synthesis of compound **10a-10e** and **11a-11x**

prepared using reductive amination. To get **11n–11x**, intermediate **12a–12k** were synthesized by Suzuki coupling reaction.

3. Biological assessment

3.1. Derivatives screening and structure-activity relationship

Tumor cell migration is one of the prominent features of cancer metastasis. Therefore the antimigratory effect of all derivatives was evaluated using in vitro transwell chemotaxis with MDA-MB-231 cells. The purities of compounds were determined by

Table 1. Inhibitory effects of derivatives on the migration of MB-MDA-231 cells.

No.	IC_{50}[a] [μM]	No.	IC_{50}[a] [μM]
10a	0.618 ± 0.012	11 l	1.179 ± 0.018
10b	18.57 ± 0.219	11 m	12.850 ± 0.155
10c	14.28 ± 0.203	11n	1.399 ± 0.028
10d	> 20	11o	8.880 ± 0.079
10e	> 20	11p	9.891 ± 0.046
11a	0.686 ± 0.027	11q	> 20
11b	5.103 ± 0.072	11r	7.488 ± 0.025
11c	0.534 ± 0.017	11 s	3.395 ± 0.026
11d	1.181 ± 0.033	11 t	3.435 ± 0.028
11e	7.921 ± 0.102	11u	5.835 ± 0.042
11f	2.713 ± 0.063	11v	1.096 ± 0.029
11 g	0.035 ± 0.004	11w	0.840 ± 0.015
11 h	1.300 ± 0.038	11x	2.150 ± 0.037
11i	7.416 ± 0.143	LY294002	1.010 ± 0.035
11j	0.943 ± 0.009	1	0.127 ± 0.006
11k	0.670 ± 0.011		

[a] The data are represented as mean ± S.D. of three independent experiments.

HPLC method, and the non-cytotoxic concentration of each compound, which was determined by MTT assay (see Supporting Information, Table S1 and S2), was used for the chemotaxis assay. The IC_{50} of the tested derivatives were listed in Table 1. In comparison with LY294002 (a PI3K inhibitor served as positive control[8]), derivatives **10a**, **11a**, **11c**, **11g**, **11j**, **11k** and **11w** showed obvious inhibitory effects on the migration of MDA-MB-231 cells. Among them, derivative **11g** was of highest potential with IC_{50} value of 0.035 μM.

Our previous study showed that fluorobenzene fragment in compound **1** had excellent antimetastatic effects. In this work, N-methyl group was optimized to obtain the compounds with better activity. At the first round of this structure optimization, **10a**–**10e** were designed and synthesized. The results showed that amide, ester and acid derivatives had lower activities. The alkynyl derivative **10a** exhibited good anti-invasion effect with IC_{50} of 0.618 μM. In some instances, aromatic ring and ethyln group were considered as bioisosteres.[9] Their replacement was widely used in structure optimization of lead compound. Based on this, N-benzylamine derivatives **11a**–**11k** were synthesized in the second-round optimization. The biological evaluation of these compounds showed that electron-deficient aromatic ring derivatives **11a, 11c, 11g** exhibited better anti-invasion activity. The IC_{50} value of benzonitrile derivative **11g** was four-fold less than the lead compound **1**. However, the substituted benzonitrile derivatives **11j** and **11k** did not exhibited better activities than **11g**. At the third-round of optimization, we constructed the biaromatic rings derivatives, because biaromatic rings structures often existed in small molecule tumor immunity inhibitors.[10] Unfortunately, **11l–11x** exhibited no better activity than that of **11g**. It was suggested that the nitrile group played a crucial role in the anti-invasive effects. Nitrile-containing drugs with diverse structures are widely used in a variety of medical treatments. Surveying the interactions of the nitrile group within these pharmaceuticals and drug candidates revealed that the biological function of the nitrile group varies considerably. Under some circumstances, the nitrile merely polarizes the adjacent electron density, whereas in other cases the nitrile is a key component for molecular recognition.[11] Therefore, **11g** was chosen for the further investigation on the antimetastatic activity and molecular mechanism.

3.2. The antimetastatic effect of derivative 11g

Derivative **11g** supressed cell invasion at concentrations of 0.1–25 μM in transwell assay (Figure 2) and cell migration at concentrations of 0.1–25 μM in wound healing assay (Figure 3), both in a dose-dependent manner. Furthermore, **11g** (0.1–

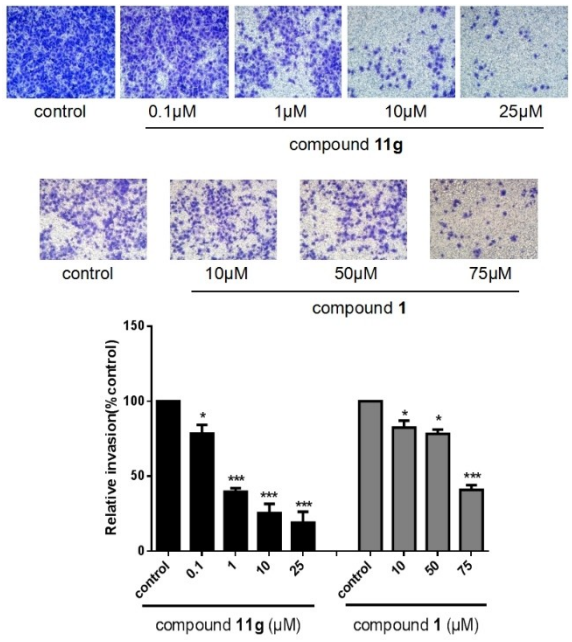


Figure 2. Anti-invasive effect of **11g** on MDA-MB-231 cells by transwell assay. MDA-MB-231 cells were cultured in the upper chamber with matrigel matrix. The cells that actively migrated to the lower surface of the filter were determined. The data are represented as mean ± S.D. of three independent experiments. (*$p < 0.05$, ***$p < 0.001$, compared with control).

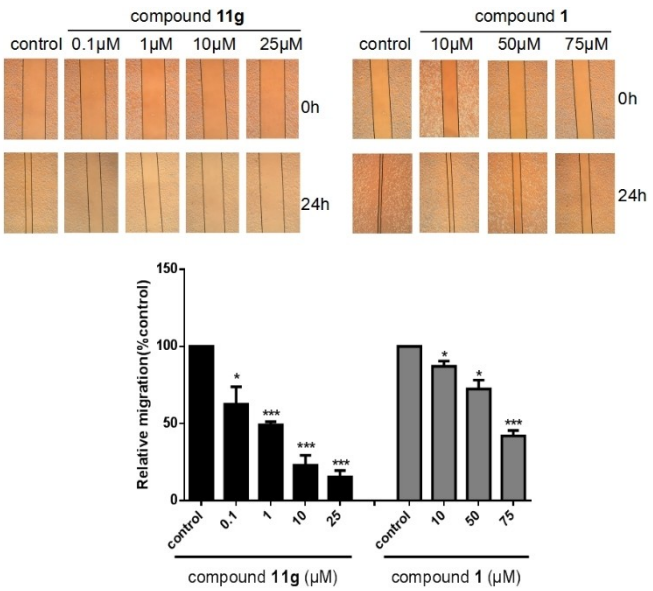


Figure 3. Antimigratory effect of **11 g** on MDA-MB-231 cells by wound healing assay. MDA-MB-231 cells were seeded in plates and generate a clean wound area. After 24 h, cells were monitored and photographed under microscope. The data are represented as mean ± S.D. of three independent experiments. (*$p < 0.05$, ***$p < 0.001$, compared with control).

25 μM) significantly decreased the adhesive capability of MDA-MB-231 cells in adhesion assay (Figure 4).

3.3. 11 g inhibited VEGF secretion and the phosphorylation of VEGFR2 in MDA-MB-231 cells

It is reported that β-Ionone exerts antitumor effects through the Akt pathway.[12] To further reveal the molecular mechanism of ionone alkaloid, we examined its effect on the expression and secretion of vascular endothelial growth factors (VEGFs). VEGFs are key mediators of angiogenesis, and are also reported to be important upstream signaling molecules of Akt[13] and crucial for the development and metastasis of tumors.[14] Tumor cells secrete VEGFs to promote the growth of blood vessels in the hypoxic area of tumor tissue to meet the demand for oxygen and nutrients. Studies have shown that the angiogenic function of VEGF was mediated by binding to its cognate receptor VEGF receptor 2 (VEGFR-2)[15], which further promotes the migration and invasion of tumor cells. The effect of **11 g** on VEGF secretion in MDA-MB-231 cells was determined using ELISA kit, and the expression and phosphorylation of VEGFR2 were determined by western blot. Results showed that **11 g** dose-dependently down regulated both secreted (extracellular) and intracellular VEGF (Figure 5). **11 g** also inhibited the phosphorylation of VEGFR-2 in a dose-dependent manner (Figure 6).

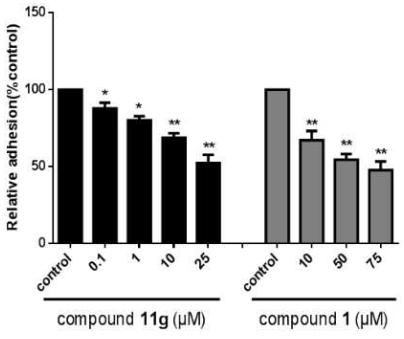


Figure 4. Antiadhesive effect of **11 g** on MDA-MB-231 cells. The data are represented as mean ± S.D. of three independent experiments. (*$p < 0.05$, **$p < 0.01$, compared with control).

3.4. 11 g suppressed hypoxia-induced HIF-1α expression

VEGF expression is regulated by HIF-1α, which binds to hypoxia response elements within the gene promoter region of VEGF.[16] HIF-1α is the most critical nuclear transcriptional regulator in the regulation of hypoxic response. Hypoxia is closely related to tumor invasion, metastasis, angiogenesis, and poor prognosis.[17] Under non-hypoxic conditions, HIF-1α is rapidly degraded through proteasomal degradation to the hydroxylation of pro402 and/or 564 by prolyl hydroxylase enzyme (PHD), which utilizes O_2 as substrate. However, under hypoxic conditions, decreased PHD activity results in the stabilization of HIF-1α protein, which binds to the hypoxia response elements (HREs) of the target gene promoter and activates downstream gene transcription involved in angiogenesis and metastasis.[18] Therefore identifying novel HIF-1α inhibitor has become a popular trend in antitumor drugs research and development. The effect of **11 g** on HIF-1α expression was determined by western blot.

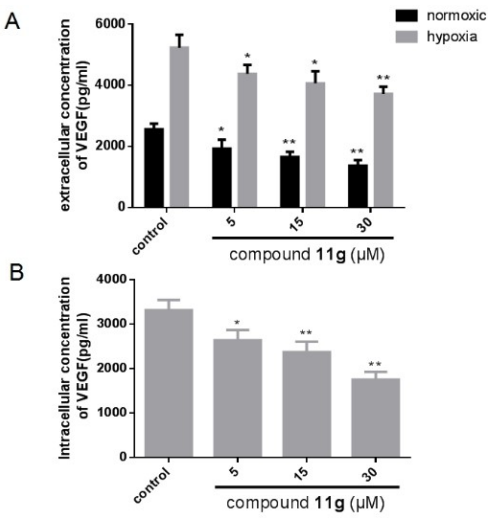


Figure 5. The protein level of VEGF was detected by ELISA. (A) The supernatant of breast cancer cells cultured with the compound in normoxia or hypoxia was collected, then the content of VEGF was measured. (B) The cell lysate of breast cancer cells cultured with the compound in hypoxia was collected, then the content of VEGF was measured. The data are represented as mean ± S.D. of three independent experiments. (*$p < 0.05$, **$p < 0.01$, compared with control).

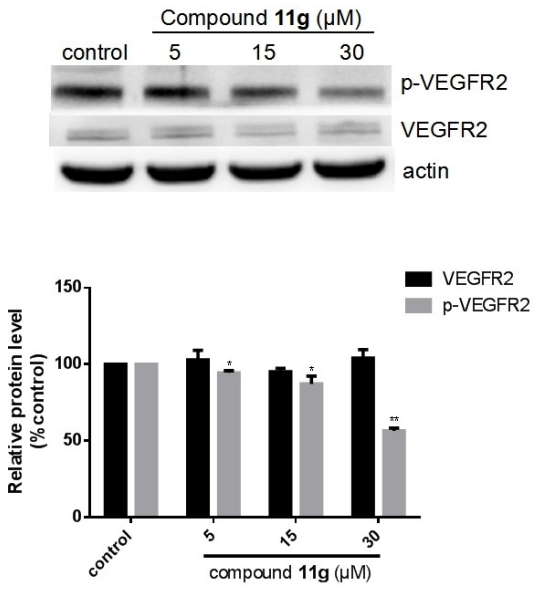


Figure 6. Effects of **11 g** on the protein expression and phosphorylation of VEGFR2 in MDA-MB-231 cells. The relative band density was determined by densitometry with Image J software. (*$p < 0.05$, **$p < 0.01$, compared with control).

The hypoxic cell model was established by pre-starvation of cells for 12 hours using starvation medium (containing only 1% FBS). Then, it was replaced with fresh starvation media with different concentrations of **11 g**, and the cells were cultured in anoxic culture boxes filled with nitrogen of high purity. The expression of HIF-1α protein was time-dependent under hypoxia and higher than that in normal oxygen (Figure 7A). The results showed that **11 g** significantly inhibited HIF-1α protein expression with dose-dependence (Figure 7B). In addition, we measured the expression of HIF-1α mRNA under hypoxia as well (Figure 8A). After cells were starved and incubated with compound **11 g** for 12 h, they were transferred to hypoxic condition for 1 hour before mRNA extraction. The results showed that **11 g** inhibited HIF-1α mRNA expression with dose-dependence (Figure 8B).

In order to determine whether the inhibition of HIF-1α was critical for the antimetastatic role of **11 g**, an agonist of HIF-1α, dimethyloxallyl glycine (DMOG), was adopted. Results showed that, upon administration of DMOG, the antimigratory activity of **11 g** was abolished (Figure 9). Further investigation showed that the inhibitory effect of **11 g** on pAkt was also eliminated by DMOG (Figure 10). These rusults revealed that **11 g** is a HIF-1α inhibitor that suppressed metastatic behavior of MDA-MB-231 cells through HIF-1α/VEGF/VEGFR2/Akt pathway.

3.5. 11 g suppressed the downstream molecules of AKT pathway

As key signaling molecules during tumor development, Akt promotes tumor metastasis by activating downstream mole-

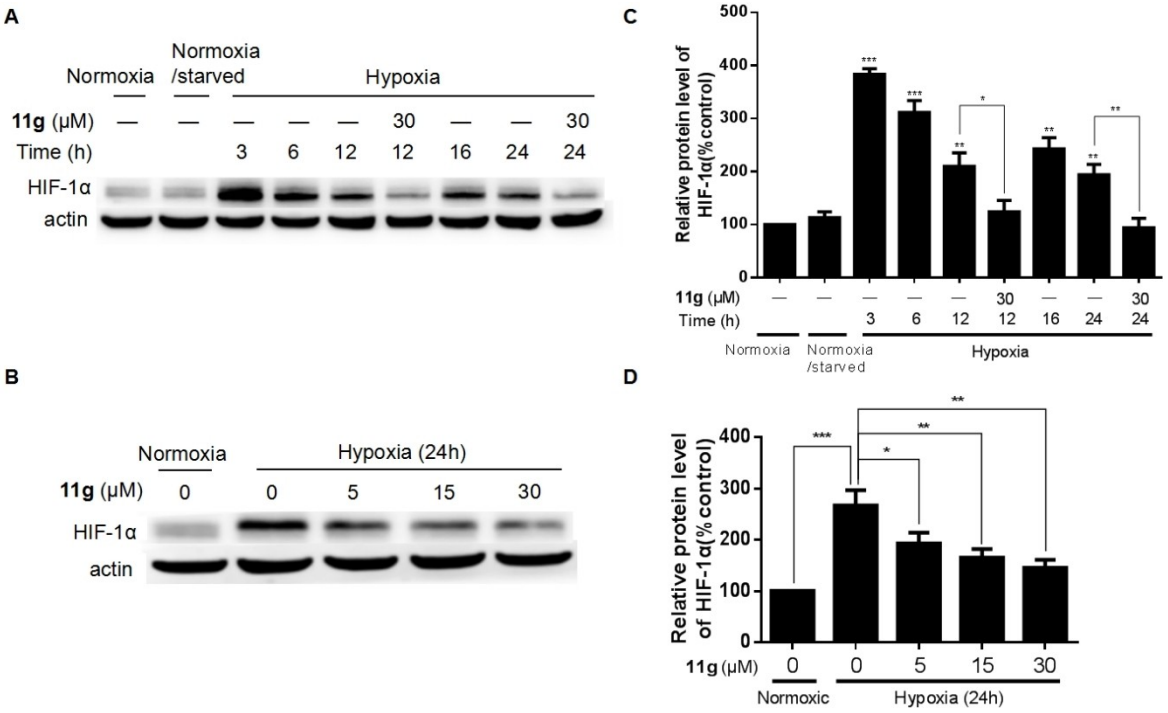


Figure 7. Influences of hypoxia and **11 g** on the protein expression level of HIF-1α in MDA-MB-231 cells. (A) the protein expression of HIF-1α in hypoxia-induced MDA-MB-231 cells were determined by western blot at different times ; (B) MDA-MB-231 cells was induced by hypoxia for 24 h in the presence of different concentrations of **11 g**; thereafter, the protein levels of HIF-1α was examined by western blot. (C, D) was HIF-1α relative protein level. The relative band density was determined by densitometry with Image J software. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control).

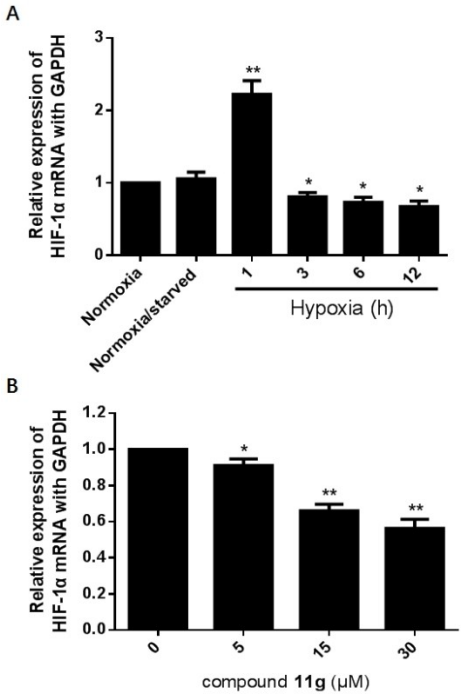


Figure 8. Influences of hypoxia and **11 g** on the HIF-1α mRNA level in MDA-MB-231 cells. (A) the mRNA expression of HIF-1α in hypoxia-induced MDA-MB-231 cells was determined by qRT-PCR at different times ; (B) MDA-MB-231 cells was induced by hypoxia for 1 h in the presence of different concentrations of **11 g**; thereafter, the mRNA expression of HIF-1α was determined by qRT-PCR. The data are represented as mean ± S.D. of three independent experiments. (*$p < 0.05$, **$p < 0.01$, compared with control).

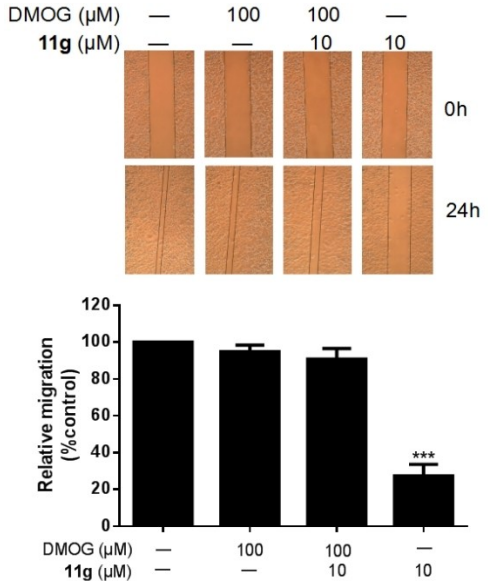


Figure 9. Antimigratory effect of DMOG and **11 g** combined on MDA-MB-231 cell in hypoxia by wound healing assay. The data are represented as mean ± S.D. of three independent experiments. (***$p < 0.001$, compared with control).

cules including mTOR, p70S6K and 4EBP1.[19] Studies have shown that Akt1 is critical for the growth of breast cancer, while

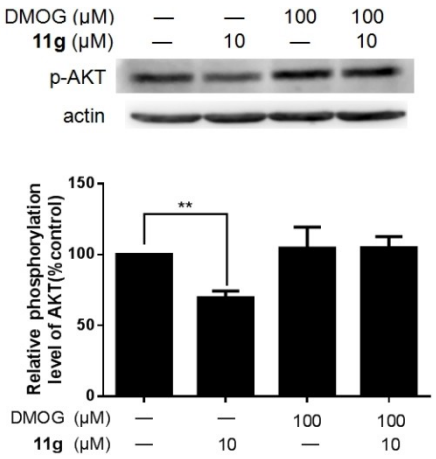


Figure 10. Effect of DMOG combined with **11 g** on the phosphorylation of Akt in hypoxia. The relative band density was determined by densitometry with Image J software. (**$p < 0.01$, compared with control).

Akt2 is more involved in metastatic transmission.[20] Akt2 has been found to be able to promote tumor metastasis through regulating downstream PKCζ, integrinβ1 and FAK.[21] Our results showed that **11 g** reduced the phosphorylations of Akt2 and Akt1. **11 g** also reduced the phosphorylations of mTOR, p70S6K, 4EBP1, PKCζ, integrin β1 and FAK, respectively. The above data were consistent with the previous phenotypic results (Figure 11).

Recent studies have shown that β-ionone has properties of antiproliferation, antimetastasis and apoptosis in vitro and in vivo.[22] However, the antitumor activity of α-ionone alkaloid is rarely reported. In this paper, a series of α-ionone alkaloid derivatives were synthesized and screened, and a compound (**11 g**) with significant antitumor metastatic activity was investigated.

The development of HIF-1α inhibitors has attracted increasing attention in cancer research: These inhibitors have different mechanisms. (1) Inhibit HIF-1α mRNA expression. (2) Inhibit HIF-1α translation. (3) Inhibit HIF-1α stabilization (including Hsp90 inhibitors and Histone deacetylase (HDAC) inhibitors). (4) Inhibit HIF-1 dimerization. After nuclear translocation of HIF-1α, it forms a dimer with HIF-1β to bind to downstream hypoxia response elements. Inhibition of dimerization can inhibit the activity of HIF-1α. (5) Inhibit HIF-1/DNA binding. (6) Inhibit HIF-1 transcriptional activity. Interruption of HIF-1α and HIF-1β binding to the co-factor p300 to form an active transcription complex is the final step towards HIF-1 transactivation.[23] In this study, compound **11 g** dose-dependently down regulated the mRNA expression of HIF-1α. That may be one of the mechanisms of **11 g**.

These inhibitors suppress tumor growth and metastasis through different signaling pathways. For example, SYP-5 inhibited the tumor angiogenesis and cell invasion by decreasing HIF-1α and HIF-1β expression through down regulated EGFR/PI3K/Akt and MAPK/ERK pathway;[24] benzophenone-1B inhibits the lung and breast cancer cells migration and invasion by down regulated HIF-1α nuclear translocation and inhibited

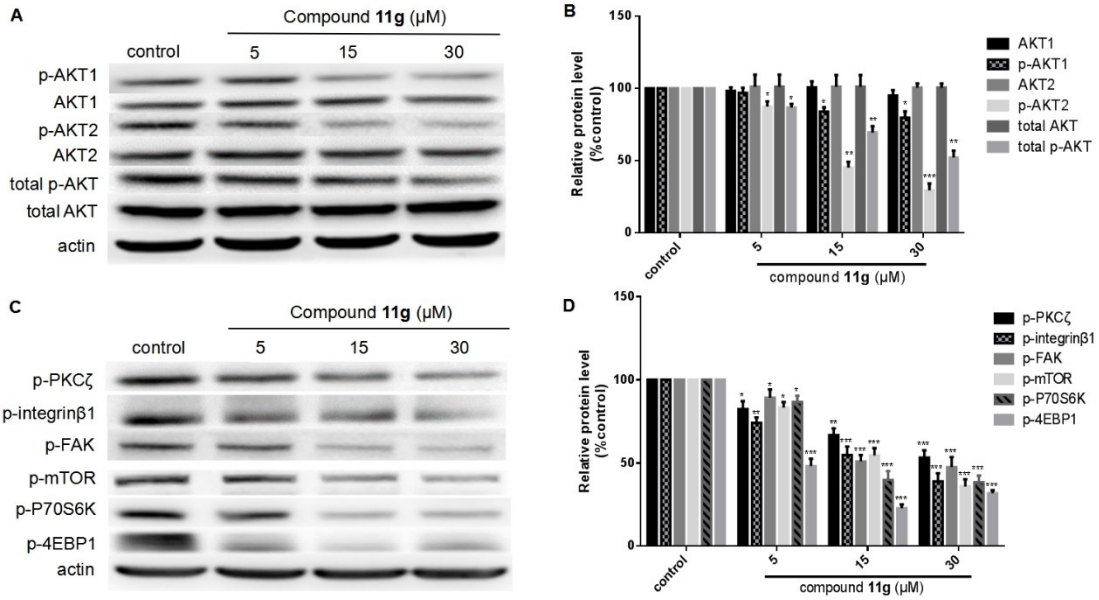


Figure 11. Effects of **11 g** on the protein expressions and phosphorylations of Akt, mTOR, p70S6K, FAK, 4EBP1, PKCζ and integrin β1 in MDA-MB-231 cells. (A,C) The protein expressions and phosphorylations in MDA-MB-231 cells were determined by western blot analysis; (B,D) were relative protein level. The relative bands density was determined by densitometry with Image J software. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control).

the expressions of MMP-2 and MMP-9;[25] ursolic acid derivative inhibited Hep3B cancer cell growth by suppressing the HIF-1 and VEGF expression;[26] bishonokiol A inhibited the breast cancer cell migration and invasion through suppressing the accumulation of HIF-1α, which was regulated by PI3K/Akt pathway;[27] the HIF-1α inhibitor echinomycin suppressed leukaemia cell growth through NOTCH signalling pathway.[28] In this article,we reported a novel chiral ionone alkaloid derivative **11 g**, which inhibited breast cancer cell adhesion, migration and invasion through the downregulation of the HIF-1α/VEGF/VEGFR2/Akt pathway, and suppression of the downstream molecules, Akt1/mTOR/p70S6K and Akt2/PKCζ/integrin β1/FAK. The potential mechanism of **11 g** was showed as Figure 12.

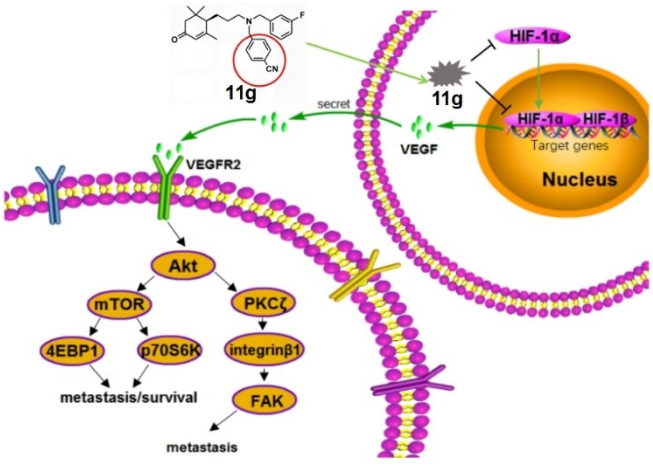


Figure 12. Potential antitumor metastasis mechanism of **11 g**

4. Conclusion

To summarize, in this study, a series of novel chiral ionone alkaloid derivatives were synthesized and their antitumor activity were screened. Compound **11 g** exhibited significant inhibitory effect on breast cancer cell adhesion, migration, and invasion through the inhibition of HIF-1α and VEGF/VEGFR2/Akt pathway, which suppressed the downstream molecules, Akt1/mTOR/p70S6K and Akt2/PKCζ/integrin β1/FAK. Therefore, chiral ionone alkaloid derivative **11 g** has the potential to be developed into an antitumor metastatic agent for breast cancer.

Experimental Section

Chemistry

General procedure for synthesis of 3

To a solution of (6R)-α-ionone (10 g) in EtOH (110 mL) was added NaClO solution (230 mL, 13%) in portions at 0 °C. The reaction mixture was stirred at room temperature for 8 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 brine (50 mL) and dried over $MgSO_4$. After removal of the solvent, the residue **3** was immediately used in the next reaction without purification.

General procedure for synthesis of 4

To a solution of **3** (4.1 g, 21.1 mmol, 1 eq.) and 3-fluorobenzylamine (2.65 mL, 23.2 mmol, 1.1 eq.)in CH_2Cl_2 (40 ml) at 0 °C were added HOBt (3.14 g, 23.2 mmol, 1.1 eq.), EDCI (3.14 g, 23.2 mmol, 1.1 eq.)

and triethylamine(7.3 mL, 52.8 mmol, 2.5 eq.). 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 **4**.

(R, E)-N-(3-fluorobenzyl)-3-(1,1,5-trimethylcyclohex-4-en-6-yl) acrylamide (4)

Yield 73.5%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (m, 1H, Ar–H), 7.00 (m, 1H, Ar–H), 6.90 (m, 2H, Ar–H), 6.68 (dd, *J* = 15.2 Hz, 9.6 Hz, 1H, 7-H), 5.86 (d, *J* = 15.2 Hz, 1H, 8-H), 5.43 (s, 1H, 4-H), 4.38 (d, *J* = 5.6 Hz, 2H, Ar-CH_2), 2.20 (d, *J* = 9.6 Hz, 1H, 6-H), 1.99 (br s, 2H, 3-H), 1.54 (s, 3H, 13-H), 1.43 (m, 1H, 2-H), 1.16 (m, 1H, 2-H), 0.89 (s, 3H, 12-H), 0.82 (s, 3H, 11-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2 (9-C), 162.9 (d, *J* = 244.7 Hz, Ar–C), 145.7 (7-C), 141.2 (d, *J* = 6.9 Hz, Ar–C), 132.2 (5-C), 130.0 (d, *J* = 8.2 Hz, Ar–C), 124.5 (8-C), 123.1 (d, *J* = 2.8 Hz, Ar–C), 122.3 (4-C), 114.4 (d, *J* = 21.6 Hz, Ar–C), 114.1 (d, *J* = 20.9 Hz, Ar–C), 53.8 (6-C), 42.9 (Ar-$\underline{C}H_2$), 32.4 (2-C), 31.2 (1-C), 27.7 (12-C), 26.8 (11-C), 23.0 (3-C), 22.9 (13-C).

General procedure for synthesis of 5

Weighed 3 g of compound **4** and dissolved it in THF(40 mL), added Raney nickel mixture(15 mL), stirred at room temperature under hydrogen for 8 h. The reaction mixture was filtered through a pad of celite, collected the filtrate and concentrated to obtain crude **5**, which can be used for the next step without purification.

General procedure for synthesis of 6

To the solution of crude amide **5** (3.16 g) in anhydrous THF (40 mL), $LiAlH_4$ (593 mg, 15.62 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 (40 mL) at 0 °C. The mixture was filtered in vacuo and the filtrate was evaporated. The mixture of **6** or the mixture of **6** thus obtained was immediately used in the next reaction without purification.

General procedure for synthesis of 7

To the mixture of crude **6** (5.6 g) and $KHCO_3$ (4.84 g, 29.03 mmol) in CH_2Cl_2 (100 mL) was added a solution of Boc_2O (10.5 g, 21.29 mmol) in CH_2Cl_2 (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 (50 mL), washed with water (50 mL), then washed with brine (50 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 column chromatography (PE/EA = 20/1) to obtain pure **7**.

General procedure for synthesis of 8

To a suspension of CrO_3 (11.7 g, 115.5 mmol, 10.0 eq.) in CH_2Cl_2 (120 mL) at −20 °C, 3,5-dimethylpyrazole (11.25 g, 115.5 mmol, 10.0 eq.) was added and the resulting mixture was stirred at −20 °C for 10 min. A solution of **7** (4.5 g, 11.55 mmol, 1.0 eq.) in CH_2Cl_2 (8 mL) was then added dropwise and the mixture was stirred continuously for 5 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 = 10/1) to afford the crude product **8** which could be used in the next reaction.

Tert-butyl (R)-(3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6-yl)propyl)carbamate (8)

Yield 57.3%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 1H, Ar–H), 7.07 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.79 (s, 2H, Ar-CH_2), 2.63 (t, *J* = 6.8 Hz, 2H, 9-H), 2.36 (d, *J* = 17.2 Hz, 1H, 2-H), 2.01 (d, *J* = 17.2 Hz, 1H, 2-H), 1.97 (s, 3H, 13-H), 1.86 (t, *J* = 5.2 Hz, 1H, 6-H), 1.74 (m, 1H, 7-H), 1.60 (m, 2H, 8-H), 1.47 (s, 9H, $(CH_3)_3$), 1.43 (m, 1H, 7-H), 1.05 (s, 3H, 11-H), 1.02 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 165.5 (5-C), 163.0 (d, *J* = 244.4 Hz, Ar–C), 156.5 (CO), 138.5 (d, *J* = 6.9 Hz, Ar–C), 129.9 (d, *J* = 8.1 Hz, Ar–C), 125.2 (4-C), 123.7 (d, *J* = 2.2 Hz, Ar–C), 114.9 (d, *J* = 21.1 Hz, Ar–C), 113.9 (d, *J* = 21.0 Hz, Ar–C), 79.8 (C), 65.3 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.2 (2-C), 47.1 (9-C), 36.3 (1-C), 30.6 (12-C), 28.7 (7-C), 28.4 ($(CH_3)_3$), 27.4 (8-C), 27.1 (11-C), 24.6 (13-C).

General procedure for synthesis of 9

Compound **8** (2.3 g, 5.7 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (40 mL), TFA (1.27 mL, 17.1 mmol, 3.0 eq.) was added. Then the reaction was stirred at room temperature for 5 h. The reaction mixture was treated with saturated Na_2CO_3 solution until a pH of 8–9 and extracted by CH_2Cl_2 (50 mL), washed with brine (50 mL). The organic layers were dried by $MgSO_4$, and the solvent was removed in vacuo to afford the residue to yield the desired product **9**.

(R)-6-(3-((3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (9)

Yield 92.3%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 1H, Ar–H), 7.07 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.79 (s, 2H, Ar-CH_2), 2.63 (t, *J* = 7.2 Hz, 2H, 9-H), 2.36 (d, *J* = 17.2 Hz, 1H, 2-H), 2.01 (d, *J* = 17.2 Hz, 1H, 2-H), 1.97 (s, 3H, 13-H), 1.86 (t, *J* = 5.2 Hz, 2H, 9-H), 1.74 (m, 2H, 7-H), 1.60 (m, 2H, 8-H), 1.44 (m, 2H, 7-H), 1.05 (s, 3H, 12-H), 1.01 (s, 3H, 11-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.6 (5-C), 163.0 (d, *J* = 244.4 Hz, Ar–C), 142.5 (d, *J* = 6.9 Hz, Ar–C), 129.9 (d, *J* = 8.2 Hz, Ar–C), 125.1 (4-C), 123.7 (d, *J* = 2.2 Hz, Ar–C), 114.9 (d, *J* = 21.1 Hz, Ar–C), 113.9 (d, *J* = 21.2 Hz, Ar–C), 53.3 (d, *J* = 1.4 Hz, Ar-$\underline{C}H_2$), 51.1 (6-C), 49.5 (9-C), 47.1 (2-C), 36.3 (1-C), 29.7 (12-C), 28.7 (7-C), 27.8 (8-C), 27.1 (11-C), 24.7 (13-C).

General procedure for synthesis of 12a–12k

3-Bromo-2-fluorobenzaldehyde (1.0 eq.) was dissolved in solvent (1,4-dioxane/H_2O = 5/1), Substituted phenylboronic acid (1.8 eq.), K_2CO_3 (5.0 eq.), $Pd[P(C_6H_5)_3]_4$ (0.07 eq.) were added. Then the reaction was stirred at reflux temperature. The reaction mixture was filtered through a pad of celite. The filtration was finally purified by column chromatography in PE/EA as eluent to afford the target compounds **12a–12k**.

2-fluoro-2′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (12a)

Yield 94.2%, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.42 (s, 1H, CHO), 7.86 (m, 1H, Ar–H), 7.60 (m, 1H, Ar–H), 7.40 (m, 1H, Ar–H), 7.28 (m, 2H, Ar–H), 6.99 (m, 2H, Ar–H), 3.81 (s, 3H, OCH_3).^{13}C NMR (100 MHz, $CDCl_3$): δ 187.7 (d, *J* = 7.9 Hz, CHO), 162.2 (d, *J* = 258.6 Hz, Ar–C), 156.9 (Ar–C), 138.3 (d, *J* = 5.1 Hz, C), 131.5 (Ar–C), 131.2 (d, *J* = 1.0 Hz, Ar–C), 130.1 (Ar–C), 127.8 (d, *J* = 9.7 Hz, C), 127.6 (d, *J* =

1.8 Hz, C), 124.2 (d, J = 4.1 Hz, Ar–C), 123.5 (Ar–C), 120.7 (Ar–C), 111.1 (Ar–C), 55.7 (d, J = 2.3 Hz, OCH_3).

2-fluoro-2′-methyl-[1,1′-biphenyl]-3-carbaldehyde (12 b)

Yield 95.1 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.42 (s, 1H, CHO), 7.89 (m, 1H, Ar–H), 7.52 (m, 1H, Ar–H), 7.31 (m, 4H, Ar–H), 7.21 (m, 1H, Ar–H), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.5 (d, J = 7.7 Hz, CHO), 161.7 (d, J = 256.6 Hz, Ar–C), 137.9 (d, J = 5.1 Hz, Ar–C), 136.7 (Ar–C), 134.2 (Ar–C), 130.6 (d, J = 16.6 Hz, Ar–C), 130.2 (Ar–C), 130.0 (Ar–C), 128.6 (Ar–C), 127.8 (d, J = 1.6 Hz, Ar–C), 125.9 (Ar–C), 124.5 (d, J = 4.3 Hz, Ar–C), 124.3 (d, J = 8.9 Hz, Ar–C), 20.0 (d, J = 2.7 Hz, CH_3).

2′-chloro-2-fluoro- [1,1′-biphenyl] −3-carbaldehyde (12 c)

Yield 91.5 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.42 (s, 1H, CHO), 7.93 (m, 1H, Ar–H), 7.58 (m, 1H, Ar–H), 7.52 (m, 1H, Ar–H), 7.36 (m, 4H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.2 (d, J = 7.4 Hz, CHO), 161.7 (d, J = 258.2 Hz, Ar–C), 137.8 (d, J = 4.3 Hz, Ar–C), 133.5 (d, J = 19.4 Hz, Ar–C), 131.5 (Ar–C), 129.9 (Ar–C), 129.8 (Ar–C), 128.5 (d, J = 1.5 Hz, Ar–C), 128.2 (d, J = 15.6 Hz, Ar–C), 126.9 (Ar–C), 124.4 (d, J = 4.5 Hz, Ar–C), 124.2 (Ar–C).

3′,4′-dichloro-2-fluoro-[1,1′-biphenyl]-3-carbaldehyde (12 d)

Yield 89.4 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.44 (s, 1H, CHO), 7.90 (m, 1H, Ar–H), 7.66 (m, 2H, Ar–H), 7.56 (d, J = 8.0 Hz, 1H, Ar–H), 7.37 (m, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.1 (d, J = 8.1 Hz, CHO), 161.6 (d, J = 259.1 Hz, Ar–C), 136.5 (d, J = 4.2 Hz, Ar–C), 134.2 (Ar–C), 132.8 (d, J = 17.1 Hz, Ar–C), 130.9 (d, J = 2.9 Hz, Ar–C), 130.7 (Ar–C), 128.6 (d, J = 1.7 Hz, Ar–C), 128.3 (d, J = 3.2 Hz, Ar–C), 128.0 (d, J = 13.0 Hz, Ar–C), 125.0 (d, J = 4.4 Hz, Ar–C), 124.8 (d, J = 8.8 Hz, Ar–C).

2-fluoro-2′,3′-dimethyl-[1,1′-biphenyl]-3-carbaldehyde (12 e)

Yield 92.3 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.41 (s, 1H, CHO), 7.88 (m, 1H, Ar–H), 7.51 (m, 1H, Ar–H), 7.31 (m, 1H, Ar–H), 7.20 (m, 2H, Ar–H), 7.06 (m, 1H, Ar–H), 3.25 (s, 3H, CH_3), 2.11 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.5 (d, J = 7.4 Hz, CHO), 161.6 (d, J = 256.5 Hz, Ar–C), 138.1 (d, J = 4.8 Hz, Ar–C), 137.3 (Ar–C), 135.1 (Ar–C), 134.3 (Ar–C), 131.2 (d, J = 16.5 Hz, Ar–C), 130.1 (Ar–C), 127.8 (Ar–C), 127.6 (d, J = 1.8 Hz, Ar–C), 125.6 (Ar–C), 124.5 (d, J = 4.3 Hz, Ar–C), 124.2 (d, J = 9.1 Hz, Ar–C), 20.6 (CH_3), 16.8 (d, J = 2.1 Hz, CH_3).

2,2′-difluoro-3′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (12 f)

Yield 90.3 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.42 (s, 1H, CHO), 7.91 (m, 1H, Ar–H), 7.64 (m, 1H, Ar–H), 7.35 (m, 1H, Ar–H), 7.18 (m, 1H, Ar–H), 7.06 (m, 1H, Ar–H), 6.94 (m, 1H, Ar–H), 3.93 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.3 (d, J = 7.9 Hz, CHO), 161.8 (d, J = 259.6 Hz, Ar–C), 149.6 (d, J = 247.4 Hz, Ar–C), 148.1 (d, J = 10.8 Hz, Ar–C), 137.8 (d, J = 2.5 Hz, Ar–C), 137.7 (d, J = 2.3 Hz, Ar–C), 128.4 (d, J = 1.9 Hz, Ar–C), 124.7 (d, J = 15.5 Hz, Ar–C), 124.4 (d, J = 4.4 Hz, Ar–C), 124.1 (d, J = 4.8 Hz, Ar–C), 122.8 (d, J = 13.0 Hz, Ar–C), 122.4 (d, J = 2.7 Hz, Ar–C), 113.5 (d, J = 1.7 Hz, Ar–C), 56.3 (OCH_3).

2,2′,3′-trifluoro-[1,1′-biphenyl]-3-carbaldehyde (12 g)

Yield 88.7 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.44 (s, 1H, CHO), 7.95 (m, 1H, Ar–H), 7.66 (m, 1H, Ar–H), 7.38 (m, 1H, Ar–H), 7.22 (m, 3H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.0 (d, J = 7.8 Hz, CHO), 161.7 (d, J = 259.8 Hz, Ar–C), 150.9 (dd, J = 247.5 Hz, 12.9 Hz, Ar–C), 148.1 (dd, J = 249.6 Hz, 13.2 Hz, Ar–C), 137.6 (dd, J = 4.3 Hz, 1.2 Hz, Ar–C), 128.9 (d, J = 2.0 Hz, Ar–C), 126.1 (dd, J = 3.1 Hz, 1.1 Hz, Ar–C), 124.7 (d, J = 4.4 Hz, Ar–C), 124.5 (d, J = 8.7 Hz, Ar–C), 124.3 (dd, J = 4.6 Hz, 2.3 Hz, Ar–C), 124.2 (d, J = 2.8 Hz, Ar–C), 123.7 (dd, J = 15.2 Hz, 2.4 Hz, Ar–C), 117.6 (d, J = 17.1 Hz, Ar–C).

2,2′-difluoro-3′-methyl-[1,1′-biphenyl]-3-carbaldehyde (12 h)

Yield 93.6 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.42 (s, 1H, CHO), 7.90 (m, 1H, Ar–H), 7.63 (m, 1H, Ar–H), 7.33 (m, 1H, Ar–H), 7.26 (m, 1H, Ar–H), 7.19 (m, 1H, Ar–H), 7.13 (m, 1H, Ar–H), 2.34 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.3 (d, J = 7.7 Hz, CHO), 161.9 (d, J = 259.1 Hz, Ar–C), 158.2 (d, J = 245.4 Hz, Ar–C), 137.9 (d, J = 4.3 Hz, Ar–C), 137.8 (d, J = 4.4 Hz, Ar–C), 132.0 (d, J = 5.3 Hz, Ar–C), 128.8 (d, J = 1.9 Hz, Ar–C), 128.1 (d, J = 1.7 Hz, Ar–C), 125.5 (d, J = 17.2 Hz, Ar–C), 125.2 (d, J = 15.4 Hz, Ar–C), 124.5 (d, J = 4.2 Hz, Ar–C), 123.8 (d, J = 4.3 Hz, Ar–C), 121.7 (d, J = 16.2 Hz, Ar–C), 14.7 (d, J = 4.4 Hz, Ar–C).

2′-chloro-2-fluoro-3′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (12 i)

Yield 87.6 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.43 (s, 1H, CHO), 7.93 (m, 1H, Ar–H), 7.57 (m, 1H, Ar–H), 7.34 (m, 1H, Ar–H), 7.03 (m, 1H, Ar–H), 6.95 (m, 1H, Ar–H), 3.97 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.3 (d, J = 7.4 Hz, CHO), 161.7 (d, J = 258.5 Hz, Ar–C), 155.4 (Ar–C), 137.8 (d, J = 3.9 Hz, Ar–C), 135.0 (Ar–C), 128.4 (d, J = 1.3 Hz, Ar–C), 127.7 (Ar–C), 127.3 (Ar–C), 124.3 (d, J = 6.8 Hz, Ar–C), 124.1 (d, J = 6.9 Hz, Ar–C), 123.6 (Ar–C), 122.2 (d, J = 15.4 Hz, Ar–C), 111.9 (Ar–C), 56.4 (CH_3).

2′-fluoro-3′-formyl-[1,1′-biphenyl]-2-carbonitrile (12 j)

Yield 84.6 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.45 (s, 1H, CHO), 8.00 (m, 1H, Ar–H), 7.83 (m, 1H, Ar–H), 7.71 (m, 2H, Ar–H), 7.56 (m, 2H, Ar–H), 7.42 (m, 1H, Ar–H).^{13}C NMR (100 MHz, $CDCl_3$): δ 186.8 (d, J = 7.8 Hz, CHO), 161.4 (d, J = 258.9 Hz, Ar–C), 138.0 (Ar–C), 137.4 (d, J = 3.7 Hz, Ar–C), 133.4 (Ar–C), 132.8 (Ar–C), 130.9 (d, J = 1.3 Hz, Ar–C), 129.5 (d, J = 2.0 Hz, Ar–C), 128.9 (Ar–C), 127.1 (d, J = 14.6 Hz, Ar–C), 124.9 (d, J = 4.5 Hz, Ar–C), 124.7 (d, J = 8.7 Hz, Ar–C), 117.7 (CN), 112.9 (Ar–C).

2-fluoro-2′-nitro-[1,1′-biphenyl]-3-carbaldehyde (12 k)

Yield 81.2 %, white solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.37 (s, 1H, CHO), 7.94 (m, 1H, Ar–H), 7.71 (m, 2H, Ar–H), 7.61 (m, 2H, Ar–H), 7.45 (m, 1H, Ar–H), 7.38 (m, 1H, Ar–H).^{13}C NMR (100 MHz, $CDCl_3$): δ 186.3 (d, J = 7.5 Hz, CHO), 161.6 (d, J = 258.5 Hz, Ar–C), 138.2 (Ar–C), 137.3 (d, J = 3.7 Hz, Ar–C), 133.4 (Ar–C), 132.8 (Ar–C), 131.0 (d, J = 1.3 Hz, Ar–C), 129.4 (d, J = 2.0 Hz, Ar–C), 128.9 (Ar–C), 127.0 (d, J = 14.6 Hz, Ar–C), 124.8 (d, J = 4.5 Hz, Ar–C), 124.8 (d, J = 8.7 Hz, Ar–C), 112.7 (Ar–C).

General procedure for synthesis of 10 a-10 b

To the solution of compound **9** (1.0 eq.) in acetone, K_2CO_3 (3.0 eq.) and corresponding bromides were added in portions.The reaction was stirred at 50 °C for 6 h, and then quenched by careful addition of 1 N aqueous NaOH. The residue was diluted with EA and the solution was washed with brine, the organic layer was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (PE/EA = 10/1).

(R)-6-(3-((3-fluorobenzyl) (prop-2-yn-1-yl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (10a)

Yield 82.7%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 1H, Ar–H), 7.08 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.81 (s, 1H, 4-H), 3.61 (s, 2H, Ar-CH_2), 3.31 (d, J = 2.0 Hz, 2H, N-CH_2), 2.54 (t, J = 6.8 Hz, 2H, 9-H), 2.37 (d, J = 17.2 Hz, 1H, 2-H), 2.26 (t, J = 2.0 Hz, 1H, CH), 2.02 (d, J = 17.2 Hz, 1H, 2-H), 1.97 (s, 3H, 13-H), 1.86 (t, J = 5.4 Hz, 1H, 6-H), 1.75 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.44 (m, 1H, 7-H), 1.05 (s, 3H, 11-H), 1.01 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 165.4 (5-C), 162.9 (d, J = 244.2 Hz, Ar–C), 141.4 (d, J = 3.9 Hz, Ar–C), 129.8 (d, J = 8.2 Hz, Ar–C), 125.0 (4-C), 124.5 (d, J = 2.6 Hz, Ar–C), 115.6 (d, J = 21.1 Hz, Ar–C), 114.1 (d, J = 21.0 Hz, Ar–C), 78.0 (C), 73.5 (CH), 57.4 (Ar-$\underline{C}H_2$), 53.5 (9-C), 51.1 (6-C), 47.1 (2-C), 41.5 (N-$\underline{C}H_2$), 36.3 (1-C), 28.7 (12-C), 27.8 (7-C), 27.2 (11-C and 8-C), 24.7 (13-C). HR-ESIMS m/z 342.2219 $[M+H]^+$, calcd for $C_{22}H_{29}FNO$, 342.2233.

Ethyl(R)-N-(3-fluorobenzyl)-N-(3-(1,1,5-trimethyl-3-oxocyclohex −4-en-6-yl) propyl) glycinate (10b)

Yield 84.3%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 1H, Ar–H), 7.09 (m, 2H, Ar–H), 6.93 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 4.16 (q, J = 7.0 Hz, 2H, OCH_2), 3.77 (s, 2H, Ar-CH_2), 3.31 (s, 2H, Ar-CH_2), 2.63 (t, J = 6.8 Hz, 2H, 9-H), 2.35 (d, J = 17.4 Hz, 1H, 2-H), 2.01 (d, J = 17.4 Hz, 1H, 2-H), 1.95 (d, J = 1.0 Hz, 3H, 13-H), 1.84 (t, J = 5.2 Hz, 1H, 6-H), 1.72 (m, 1H, 7-H), 1.55 (m, 2H, 8-H), 1.43 (m, 1H, 7-H), 1.27 (t, J = 7.0 Hz, 3H, CH_3), 1.04 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.1 (3-C), 177.1 (CO), 165.4 (5-C), 163.0 (d, J = 244.2 Hz, Ar–C), 142.0 (d, J = 6.8 Hz, Ar–C), 129.8 (d, J = 8.1 Hz, Ar–C), 125.1 (4-C), 124.3 (d, J = 2.6 Hz, Ar–C), 115.5 (d, J = 21.2 Hz, Ar–C), 114.0 (d, J = 21.1 Hz, Ar–C), 60.3 (OCH_2), 58.0 (Ar-$\underline{C}H_2$), 54.5 (N-$\underline{C}H_2$), 54.2 (9-C), 51.1 (6-C), 47.2 (2-C), 36.3 (1-C), 28.8 (12-C), 27.7 (7-C), 27.5 (8-C), 27.1 (11-C), 24.7 (13-C), 14.3 (CH_3). HR-ESIMS m/z 390.2428 $[M+H]^+$, calcd for $C_{23}H_{33}FNO_3$, 390.2444.

General procedure for synthesis of 10c

To the solution of compound **10b** (350 mg, 0.9 mmol, 1.0 eq.) in MeOH, KOH (66 mg, 1.17 mmol, 1.3 eq.) was added in portions. The reaction was stirred at room temperature for 5 h, and then quenched by addition of saturated citric acid solution until a pH of 5–6, extracted by EA (50 mL), washed with brine (50 mL), the organic layer was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 30/1) to give **10c**.

(R)-N-(3-fluorobenzyl)-N-(3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6-yl) propyl) glycine (10c)

Yield 86.2%, yellow oil, ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, OH), 7.37 (m, 1H, Ar–H), 7.18 (m, 2H, Ar–H), 7.09 (m, 1H, Ar–H), 5.71 (s, 1H, 4-H), 3.81 (s, 2H, Ar-CH_2), 3.32 (s, 2H, Ar-CH_2), 2.62 (t, J = 6.8 Hz, 2H, 9-H), 2.30 (d, J = 17.2 Hz, 1H, 2-H), 1.90 (m, 4H, 13-H and 2-H), 1.82 (t, J = 5.4 Hz, 1H, 6-H), 1.64 (m, 1H, 7-H), 1.49 (m, 2H, 8-H), 1.30 (m, 1H, 7-H), 0.96 (s, 3H, 11-H), 0.92 (s, 3H, 12-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8 (3-C), 171.9 (CO), 165.6 (5-C), 162.2 (d, J = 241.7 Hz, Ar–C), 141.7 (d, J = 5.9 Hz, Ar–C), 130.1 (d, J = 8.2 Hz, Ar–C), 124.7 (d, J = 2.6 Hz, Ar–C), 124.2 (4-C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.9 (d, J = 20.9 Hz, Ar–C), 57.2 (Ar-$\underline{C}H_2$), 54.2 (N-CH_2), 53.6 (9-C), 50.0 (6-C), 46.8 (2-C), 35.9 (1-C), 28.3 (12-C), 26.9 (7-C), 26.5 (11-C), 24.0 (8-C), 21.0 (13-C). HR-ESIMS m/z 362.2114 $[M+H]^+$, calcd for $C_{21}H_{29}FNO_3$, 362.2131.

General procedure for synthesis of 10d

To the solution of compound **9** (490 mg, 1.61 mmol, 1.0 eq.) in DMF, Cs_2CO_3 (2.63 g, 8.07 mmol, 5.0 eq.), NaI (245 mg, 1.61 mmol, 1.0 eq.) and tert-butyl (2-bromoethyl) carbamate (1.81 g, 8.07 mmol, 5.0 eq.) were added in portions. The reaction was stirred at 100 °C for 48 h under nitrogen, and then quenched by careful addition of 1 N aqueous NaOH. The residue was diluted with EA and the solution was washed with brine, the organic layer was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (PE/EA = 6/1) to give **10d**.

tert-Butyl (R)-(2-((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6-yl) propyl) amino) ethyl) carbamate (10d)

Yield 54.8%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 1H, Ar–H), 7.04 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.55 (s, 2H, Ar-CH_2), 3.19 (m, 2H, CH_2), 2.54 (t, J = 6.0 Hz, 2H, 9-H), 2.44 (t, J = 6.6 Hz, 2H, N-CH_2), 2.35 (d, J = 17.2 Hz, 1H, 2-H), 2.01 (d, J = 17.2 Hz, 1H, 2-H), 1.95 (s, 3H, 13-H), 1.82 (t, J = 5.2 Hz, 1H, 6-H), 1.68 (m, 1H, 7-H), 1.53 (m, 2H, 8-H), 1.44 (s, 9H, $(CH_3)_3$), 1.35 (m, 1H, 7-H), 1.04 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.2 (3-C), 165.5 (5-C), 162.9 (d, J = 244.3 Hz, Ar–C), 155.9 (CO), 141.2 (d, J = 5.7 Hz, Ar–C), 129.8 (d, J = 8.1 Hz, Ar–C), 125.0 (4-C), 124.2 (d, J = 2.1 Hz, Ar–C), 115.4 (d, J = 21.2 Hz, Ar–C), 114.0 (d, J = 21.0 Hz, Ar–C), 79.1 (C), 58.2 (Ar-$\underline{C}H_2$), 54.3 (N-CH_2), 53.5 (9-C), 51.0 (6-C), 47.1 (2-C), 38.0 (CH_2), 36.3 (1-C), 28.7 (12-C), 28.4 ($(CH_3)_3$), 28.0 (7-C), 27.2 (11-C), 26.9 (8-C), 24.8 (13-C). HR-ESIMS m/z 447.3002 $[M+H]^+$, calcd for $C_{26}H_{40}FN_2O_3$, 447.3023.

General procedure for synthesis of 10e

Compound **10d** (1.5 g, 3.36 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (40 mL), TFA (0.75 mL, 10.1 mmol, 3.0 eq.) was added. Then the reaction was stirred at room temperature for 5 h. The reaction mixture was treated with 1 N aqueous NaOH and stirred for 1 h, then extracted by CH_2Cl_2 (50 mL), washed with brine (50 mL). The organic layers were dried by $MgSO_4$, and the solvent was removed in vacuo to afford the residue to yield the desired product. The crude product in CH_2Cl_2 was added pyridine (1.4 mL, 17.35 mmol, 5.0 eq.) and succinic anhydride (1.74 g, 17.35 mmol, 5.0 eq.) 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 solvent was evaporated under reduced pressure to give the crude product, which was separated by column chromatography (CH_2Cl_2/MeOH = 20/1) to obtain pure **10e**.

(R)-4-((2-((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6-yl) propyl) amino) ethyl) amino)-4-oxobutanoic acid (10e)

Yield 73.5%, yellow oil, ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, OH), 7.79 (s, 1H, NH), 7.34 (m, 1H, Ar–H), 7.15 (m, 2H, Ar–H), 7.05 (m, 1H, Ar–H), 5.70 (s, 1H, 4-H), 3.59 (s, 2H, Ar-CH_2), 3.15 (q, J = 6.1 Hz, 2H, CH_2), 2.42 (m, 6H, CH_2), 2.31 (m, 3H, 2-H and CH_2), 1.91 (s, 3H, 13-H), 1.86 (m, 2H, 2-H and 6-H), 1.64 (m, 1H, 7-H), 1.48 (m, 2H, 8-H), 1.29 (m, 1H, 7-H), 0.97 (s, 3H, 11-H), 0.92 (s, 3H, 12-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8 (3-C), 173.7 (CO), 170.8 (CO), 165.6 (5-C), 162.2 (d, J = 241.5 Hz, Ar–C), 143.0 (d, J = 6.3 Hz, Ar–C), 129.9 (d, J = 8.2 Hz, Ar–C), 124.4 (d, J = 2.6 Hz, Ar–C), 124.1 (4-C), 115.0 (d, J = 21.1 Hz, Ar–C), 113.5 (d, J = 20.7 Hz, Ar–C), 57.5 (Ar-$\underline{C}H_2$), 53.8 (N-CH_2), 53.0 (9-C), 50.2 (6-C), 46.9 (2-C), 39.7 (CH_2), 36.6 (CH_2), 35.9 (1-C), 30.0 (CH_2), 29.2 (CH_2), 28.3 (12-C), 27.2 (7-C), 26.5 (11-C), 26.4 (8-

C), 24.0 (13-C). HR-ESIMS *m/z* 447.2638 $[M+H]^+$, calcd for $C_{25}H_{36}FN_2O_4$, 447.2659.

General procedure for synthesis of 11 a–11 x

To a solution of amine **9** (1.0 eq.) in MeOH was added at 0 °C $NaBH_3CN$ (1.5 eq.) and $ZnCl_2$ (0.5 eq.) which had been dissolved by MeOH followed by substituted aldehyde (3.0 eq.) and 0.5 mL of H_2O. The reaction mixture was warmed to room temperature, stirred 12 h, evaporated just to dryness with a vacuum evaporator. The viscous suspension was transferred to a separatory funnel and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil that was purified by column chromatography (PE/EA) to afford **11 a–11 x**.

(R)-6-(3-((3-fluorobenzyl) (pyridin-4-ylmethyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 a)

Yield 77.3 %, yellow oil, ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.55 (d, *J* = 5.4 Hz, 2H, Ar–H), 7.29 (d, *J* = 5.4 Hz, 2H, Ar–H), 7.27 (m, 1H, Ar–H), 7.09 (m, 2H, Ar–H), 6.95 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.55 (s, 4H, Ar-CH_2), 2.42 (t, *J* = 6.9 Hz, 2H, 9-H), 2.33 (d, *J* = 17.4 Hz, 1H, 2-H), 2.02 (d, *J* = 17.4 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.80 (t, *J* = 5.4 Hz, 1H, 6-H), 1.66 (m, 1H, 7-H), 1.55 (m, 2H, 8-H), 1.35 (m, 1H, 7-H), 1.01 (s, 3H, 11-H), 0.99 (s, 3H, 12-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 199.3 (3-C), 165.4 (5-C), 163.0 (d, *J* = 244.4 Hz, Ar–C), 149.8 (C), 148.9 (C), 141.8 (d, *J* = 7.1 Hz, Ar–C), 129.9 (d, *J* = 8.1 Hz, Ar–C), 125.2 (4-C), 124.1 (d, *J* = 2.6 Hz, Ar–C), 123.6 (C), 115.3 (d, *J* = 21.2 Hz, Ar–C), 114.1 (d, *J* = 21.0 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 57.7 (9-C), 54.4 (*N*-$\underline{C}H_2$-Ar), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (8-C), 26.9 (11-C), 24.8 (13-C). HR-ESIMS *m/z* 395.2479 $[M+H]^+$, calcd for $C_{25}H_{32}FN_2O$, 395.2499.

(R)-6-(3-(bis(3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex- 4-en-3-one (11 b)

Yield 81.6 %, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 2H, Ar–H), 7.09 (m, 4H, Ar–H), 6.93 (m, 2H, Ar–H), 5.79 (s, 1H, 4-H), 3.53 (s, 4H, Ar-CH_2), 2.41 (t, *J* = 6.8 Hz, 2H, 9-H), 2.33 (d, *J* = 10.8 Hz, 3H, 13-H), 2.03 (d, *J* = 10.8 Hz, 3H, 13-H), 1.90 (s, 3H, 13-H), 1.78 (t, *J* = 5.4 Hz, 1H, 6-H), 1.66 (m, 1H, 7-H), 1.54 (m, 2H, 8-H), 1.33 (m, 1H, 7-H), 1.01 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.6 (5-C), 163.0 (d, *J* = 244.2 Hz, Ar–C), 142.3 (d, *J* = 6.9 Hz, Ar–C), 129.8 (d, *J* = 8.2 Hz, Ar–C), 125.1 (4-C), 124.2 (d, *J* = 2.4 Hz, Ar–C), 115.4 (d, *J* = 21.2 Hz, Ar–C), 113.9 (d, *J* = 21.0 Hz, Ar–C), 58.2 (Ar-$\underline{C}H_2$), 54.1 (9-C), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 28.0 (7-C), 27.2 (11-C), 26.9 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 412.2437 $[M+H]^+$, calcd for $C_{26}H_{32}F_2NO$, 412.2452.

(R)-6-(3-((3,4-difluorobenzyl) (3-fluorobenzyl) amino) propyl)-1,1,5- trimethylcyclohex-4-en-3-one (11 c)

Yield 83.5 %, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 1H, Ar–H), 7.19 (m, 1H, Ar–H), 7.11 (m, 4H, Ar–H), 6.96 (m, 1H, Ar–H), 5.82 (s, 1H, 4-H), 3.54 (s, 2H, Ar-CH_2), 3.51 (s, 2H, Ar-CH_2), 2.41 (t, *J* = 6.8 Hz, 2H, 9-H), 2.34 (d, *J* = 17.4 Hz, 1H, 2-H), 2.03 (d, *J* = 17.4 Hz, 1H, 2-H), 1.93 (s, 3H, 13-H), 1.81 (t, *J* = 5.4 Hz, 1H, 6-H), 1.66 (m, 1H, 7-H), 1.56 (m, 2H, 8-H), 1.36 (m, 1H, 7-H), 1.03 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.6 (5-C), 163.0 (d, *J* = 244.2 Hz, Ar–C), 150.3 (dd, J_1 = 246.3 Hz, J_2 = 12.7 Hz, Ar–C), 149.4 (dd, J_1 = 245.4 Hz, J_2 = 12.8 Hz, Ar–C), 142.1 (d, *J* = 4.0 Hz, Ar–C), 136.6 (d, *J* = 2.4 Hz, Ar–C), 129.8 (d, *J* = 8.1 Hz, Ar–C), 125.1 (4-C), 124.3 (), 124.1 (d, *J* = 2.6 Hz, Ar–C), 117.3 (d, *J* = 17.2 Hz, Ar–C), 117.0 (d, *J* = 16.9 Hz, Ar–C), 115.3 (d, *J* = 21.2 Hz, Ar–C), 114.2 (d, *J* = 21.1 Hz, Ar–C), 58.1 (Ar-$\underline{C}H_2$), 57.7 (9-C), 54.0 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (8-C), 26.8 (11-C), 24.8 (13-C). HR-ESIMS *m/z* 430.2341 $[M+H]^+$, calcd for $C_{26}H_{31}F_3NO$, 430.2358.

(R)-6-(3-((3-fluorobenzyl) (4-methoxybenzyl) amino) propyl)-1,1,5- trimethylcyclohex-4-en-3-one (11 d)

Yield 77.2 %, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 3H, Ar–H), 7.08 (m, 2H, Ar–H), 6.91 (m, 2H, Ar–H), 6.85 (d, *J* = 8.4 Hz, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.79 (s, 3H, OCH_3), 3.51 (s, 2H, Ar-CH_2), 3.49 (s, 2H, Ar-CH_2), 2.39 (t, *J* = 6.8 Hz, 2H, 9-H), 2.32 (d, *J* = 17.2 Hz, 1H, 2-H), 2.00 (d, *J* = 17.2 Hz, 1H, 2-H), 1.89 (s, 3H, 13-H), 1.77 (t, *J* = 5.6 Hz, 1H, 6-H), 1.64 (m, 2H, 7-H), 1.54 (m, 2H, 8-H), 1.00 (s, 3H, 12-H), 0.98 (s, 3H, 11-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.6 (5-C), 163.0 (d, *J* = 243.9 Hz, Ar–C), 158.7 (Ar–C), 142.8 (d, *J* = 6.4 Hz, Ar–C), 129.9 (Ar–C), 129.6 (d, *J* = 8.9 Hz, Ar–C), 125.1 (4-C), 124.2 (d, *J* = 2.6 Hz, Ar–C), 115.3 (d, *J* = 21.3 Hz, Ar–C), 113.7 (d, *J* = 20.8 Hz, Ar–C), 113.7 (Ar–C), 58.1 (Ar-CH_2), 55.2 (OCH_3), 53.9 (Ar-$\underline{C}H_2$), 51.1 (6-C), 47.16 (2-C), 36.3 (1-C), 28.7 (12-C), 28.0 (7-C), 27.2 (8-C), 26.9 (11-C), 24.8 (13-C). HR-ESIMS *m/z* 424.2630 $[M+H]^+$, calcd for $C_{27}H_{35}FNO_2$, 424.2652.

(R)-6-(3-((4-ethoxybenzyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 e)

Yield 76.4 %, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19 (m, 3H, Ar–H), 7.01 (m, 2H, Ar–H), 6.84 (m, 1H, Ar–H), 6.77 (d, *J* = 8.4 Hz, 1H, Ar–H), 5.71 (s, 1H, 4-H), 3.95 (q, *J* = 6.8 Hz, 2H, OCH_2), 3.43 (s, 2H, Ar-CH_2), 3.41 (s, 2H, Ar-CH_2), 2.31 (t, *J* = 6.8 Hz, 2H, 9-H), 2.25 (d, *J* = 17.6 Hz, 1H, 2-H), 1.92 (d, *J* = 17.6 Hz, 1H, 2-H), 1.82 (s, 3H, 13-H), 1.69 (t, *J* = 5.4 Hz, 1H, 6-H), 1.56 (m, 2H, 7-H), 1.46 (m, 2H, 8-H), 1.33 (t, *J* = 6.8 Hz, 3H, CH_3), 0.93 (s, 3H, 12-H), 0.90 (s, 3H, 11-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.6 (5-C), 163.0 (d, *J* = 243.7 Hz, Ar–C),157.2 (Ar–C), 143.2 (d, *J* = 6.8 Hz, Ar–C), 130.1 (Ar–C), 129.5 (d, *J* = 8.1 Hz, Ar–C), 127.8 (Ar–C), 127.6 (Ar–C), 125.0 (4-C), 124.1 (d, *J* = 2.0 Hz, Ar–C), 120.3 (Ar–C), 115.4 (d, *J* = 21.2 Hz, Ar–C), 113.6 (d, *J* = 21.1 Hz, Ar–C), 111.4 (Ar–C), 64.1 (OCH_2), 62.4 (Ar-CH_2), 57.0 (Ar-$\underline{C}H_2$), 52.8 (Ar-$\underline{C}H_2$), 50.1 (6-C), 46.1 (2-C), 35.3 (1-C), 27.7 (12-C), 27.0 (7-C), 26.12 (8-C), 25.9 (11-C), 23.7 (13-C), 13.9 (CH_3). HR-ESIMS *m/z* 438.2775 $[M+H]^+$, calcd for $C_{28}H_{37}FNO_2$, 438.2808.

(R)-6-(3-((3-ethoxybenzyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 f)

Yield 78.3 %, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23 (m, 2H, Ar–H), 7.09 (m, 2H, Ar–H), 6.91 (m, 3H, Ar–H), 6.85 (d, *J* = 7.2 Hz, 1H, Ar–H), 5.79 (s, 1H, 4-H), 4.02 (q, *J* = 7.2 Hz, 2H, OCH_2), 3.53 (s, 2H, Ar-CH_2), 3.52 (s, 2H, Ar-CH_2), 2.41 (t, *J* = 6.8 Hz, 2H, 9-H), 2.32 (d, *J* = 17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.77 (t, *J* = 5.2 Hz, 1H, 6-H), 1.66 (m, 2H, 7-H), 1.53 (m, 2H, 8-H), 1.41 (t, *J* = 7.2 Hz, 3H, CH_3), 1.01 (s, 3H, 12-H), 0.98 (s, 3H, 11-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ199.2 (3-C), 165.5 (5-C), 163.0 (d, *J* = 244.0 Hz, Ar–C),159.1 (Ar–C), 142.7 (d, *J* = 6.9 Hz, Ar–C), 141.1 (Ar–C), 129.6 (d, *J* = 8.1 Hz, Ar–C), 129.0 (Ar–C), 125.1 (4-C), 124.2 (d, *J* = 2.4 Hz, Ar–C), 120.9 (Ar–C), 115.4 (d, *J* = 21.3 Hz, Ar–C), 115.0 (Ar–C), 113.8 (d, *J* = 21.1 Hz, Ar–C), 112.9 (Ar–C), 63.3 (OCH_2), 58.8 (Ar-CH_2), 58.3 (Ar-$\underline{C}H_2$), 54.1 (Ar-$\underline{C}H_2$), 51.2 (6-C), 47.2 (2-C), 36.3 (1-C), 28.7 (12-C), 28.0 (7-C), 27.1 (8-C), 27.1 (11-C), 24.7 (13-C), 14.9 (CH_3). HR-ESIMS *m/z* 438.2787 $[M+H]^+$, calcd for $C_{28}H_{37}FNO_2$, 438.2808.

(R)-4-(((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6- yl) propyl) amino) methyl) benzonitrile (11 g)

Yield 76.2%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, J= 7.6 Hz, 2H, Ar–H), 7.45 (d, J=7.6 Hz, 2H, Ar–H), 7.29 (m, 1H, Ar–H), 7.08 (m, 2H, Ar–H), 6.96 (m, 1H, Ar–H), 5.82 (s, 1H, 4-H), 3.60 (s, 2H, Ar-CH_2), 3.55 (s, 2H, Ar-CH_2), 2.42 (t, J=6.4 Hz, 2H, 9-H), 2.34 (d, J= 17.4 Hz, 1H, 2-H), 2.04 (d, J=17.4 Hz, 1H, 2-H), 1.92 (s, 3H, 13-H), 1.81 (t, J=5.2 Hz, 1H, 6-H), 1.64 (m, 1H, 7-H), 1.55 (m, 2H, 8-H), 1.36 (m, 1H, 7-H), 1.02 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.5 (5-C), 163.0 (d, J=244.5 Hz, Ar–C), 145.4 (Ar–C), 141.8 (d, J=6.6 Hz, Ar–C), 132.2 (Ar–C), 129.9 (d, J=8.0 Hz, Ar–C), 129.4 (Ar–C), 125.2 (4-C), 124.1 (Ar–C), 118.9 (CN), 115.3 (d, J=21.0 Hz, Ar–C), 114.1 (d, J=21.1 Hz, Ar–C), 110.9 (Ar–C), 58.4 (Ar-$\underline{C}H_2$), 58.3 (9-C), 54.3 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.8 (12-C), 27.9 (7-C), 27.1 (8-C), 26.8 (11-C), 24.8 (13-C). HR-ESIMS *m/z* 419.2477 $[M+H]^+$, calcd for $C_{27}H_{32}FN_2O$, 419.2499.

(R)-6-(3-((4-(dimethylamino) benzyl) (3-fluorobenzyl) amino) propyl)- 1,1,5-trimethylcyclohex-4-en-3-one (11 h)

Yield 73.2%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 1H, Ar–H), 7.20 (d, J=7.6 Hz, 2H, Ar–H), 7.11 (d, J=7.6 Hz, 2H, Ar–H), 6.93 (m, 1H, Ar–H), 6.73 (d, J=7.4 Hz, 2H, Ar–H), 5.81 (s, 1H, 4-H), 3.52 (s, 2H, Ar-CH_2), 3.49 (s, 2H, Ar-CH_2), 2.96 (s, 6H, $N(CH_3)_2$), 2.41 (t, J=6.4 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 2.02 (d, J=17.2 Hz, 1H, 2-H), 1.93 (s, 3H, 13-H), 1.80 (t, J=5.2 Hz, 1H, 6-H), 1.68 (m, 1H, 7-H), 1.55 (m, 2H, 8-H), 1.36 (m, 1H, 7-H), 1.03 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6 (3-C), 166.0 (5-C), 162.9 (d, J=244.0 Hz, Ar–C), 149.8 (Ar–C), 143.0 (d, J=7.3 Hz, Ar–C), 129.7 (Ar–C), 129.5 (d, J=8.3 Hz, Ar–C), 126.9 (Ar–C), 125.0 (4-C), 124.1 (d, J=2.1 Hz, Ar–C), 115.3 (d, J=21.1 Hz, Ar–C), 113.6 (d, J= 21.0 Hz, Ar–C), 112.5 (Ar–C), 58.0 (Ar-$\underline{C}H_2$), 57.9 (9-C), 53.7 (Ar-$\underline{C}H_2$), 51.1 (6-C), 47.1 (2-C), 40.7 ($N(\underline{C}H_3)_2$), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (8-C), 26.9 (11-C), 24.8 (13-C). HR-ESIMS *m/z* 437.2948 $[M+H]^+$, calcd for $C_{28}H_{38}FN_2O$, 437.2968.

(R)-6-(3-((4-(diethylamino) benzyl) (3-fluorobenzyl) amino) propyl)- 1,1,5-trimethylcyclohex-4-en-3-one (11 i)

Yield 72.6%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (m, 1H, Ar–H), 7.17 (d, J=7.6 Hz, 2H, Ar–H), 7.11 (d, J=7.6 Hz, 2H, Ar–H), 6.93 (m, 1H, Ar–H), 6.66 (d, J=7.4 Hz, 2H, Ar–H), 5.82 (s, 1H, 4-H), 3.53 (s, 2H, Ar-CH_2), 3.47 (s, 2H, Ar-CH_2), 3.36 (q, J=6.8 Hz, 4H, $(CH_2)_2$), 2.39 (m, 3H, 9-H and 2-H), 2.02 (d, J=17.2 Hz, 1H, 2-H), 1.93 (s, 3H, 13-H), 1.80 (t, J=5.2 Hz, 1H, 6-H), 1.69 (m, 1H, 7-H), 1.56 (m, 2H, 8-H), 1.36 (m, 1H, 7-H), 1.17(t, J=6.8 Hz, 6H, $(CH_3)_2$), 1.03 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6 (3-C), 166.1 (5-C), 162.9 (d, J=243.6 Hz, Ar–C), 146.9 (Ar–C), 143.1 (d, J= 6.7 Hz, Ar–C), 129.9 (Ar–C), 129.5 (d, J=8.2 Hz, Ar–C), 125.5 (Ar–C), 124.9 (4-C), 124.2 (d, J=2.1 Hz, Ar–C), 115.3 (d, J=21.1 Hz, Ar–C), 113.6 (d, J=21.1 Hz, Ar–C), 111.6 (Ar–C), 58.0 (Ar-$\underline{C}H_2$), 57.7 (9-C), 53.7 (Ar-$\underline{C}H_2$), 51.1 (6-C), 47.1 (2-C), 44.4 ($(\underline{C}H_2)_2$), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (8-C), 26.9 (11-C), 24.8 (13-C), 12.6 ($(\underline{C}H_3)_2$). HR-ESIMS *m/z* 465.3265 $[M+H]^+$, calcd for $C_{30}H_{42}FN_2O$, 465.3281.

(R)-2-fluoro-5-(((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex- 4-en-6-yl) propyl) amino) methyl) benzonitrile (11 j)

Yield 76.5%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (m, 2H, Ar–H), 7.29 (m, 1H, Ar–H), 7.18 (m, 1H, Ar–H), 7.06 (m, 2H, Ar–H), 6.95 (m, 1H, Ar–H), 5.81 (s, 1H, 4-H), 3.54 (s, 4H, Ar-CH_2), 2.41 (t, J= 6.8 Hz, 2H, 9-H), 2.32 (t, J=17.2 Hz, 1H, 2-H), 2.02 (d, J=17.2 Hz, 1H, 2-H), 1.93 (s, 3H, 13-H), 1.81 (t, J=5.2 Hz, 1H, 6-H), 1.64 (m, 1H, 7-H), 1.56 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.02 (s, 3H, 11-H), 1.00 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.4 (5-C), 163.0 (d, J=244.7 Hz, Ar–C), 162.2 (d, J=256.8 Hz, Ar–C), 141.7 (d, J=6.6 Hz, Ar–C), 136.8 (d, J=14.0 Hz, Ar–C), 135.1 (d, J=7.7 Hz, Ar–C), 133.1 (Ar–C), 129.9 (d, J=8.1 Hz, Ar–C), 125.2 (4-C), 124.1 (d, J=4.3 Hz, Ar–C), 116.4 (d, J=19.4 Hz, Ar–C), 115.3 (d, J=21.3 Hz, Ar–C), 114.3 (d, J=21.1 Hz, Ar–C), 114.1(CN), 101.2 (d, J=15.5 Hz, Ar–C), 58.2 (Ar-$\underline{C}H_2$), 57.2 (Ar-$\underline{C}H_2$), 54.5 (9-C), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (11-C), 26.8 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 437.2387 $[M+H]^+$, calcd for $C_{27}H_{31}F_2N_2O$, 437.2404.

(R)-2-chloro-3-(((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex- 4-en-6-yl) propyl) amino) methyl) benzonitrile (11 k)

Yield 77.4%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J= 7.6 Hz, 1H, Ar–H), 7.58 (d, J=7.6 Hz, 1H, Ar–H), 7.37 (m, 1H, Ar–H), 7.27 (m, 1H, Ar–H), 7.07 (m, 2H, Ar–H), 6.94 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.71 (s, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.45 (t, J=6.8 Hz, 2H, 9-H), 2.31 (t, J=17.2 Hz, 1H, 2-H), 2.01 (d, J=17.2 Hz, 1H, 2-H), 1.91 (s, 3H, 13-H), 1.79 (t, J=5.2 Hz, 1H, 6-H), 1.64 (m, 1H, 7-H), 1.55 (m, 2H, 8-H), 1.32 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.99 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3 (3-C), 165.4 (5-C), 162.9 (d, J= 244.5 Hz, Ar–C), 141.7 (d, J=6.8 Hz, Ar–C), 139.2 (Ar–C), 136.0 (Ar–C), 134.4 (Ar–C), 132.6 (Ar–C), 129.9 (d, J=8.1 Hz, Ar–C), 127.1 (Ar–C), 125.2 (4-C), 124.1 (d, J=4.3 Hz, Ar–C), 116.2 (Ar–C), 115.3 (d, J=21.4 Hz, Ar–C), 114.2 (d, J=21.0 Hz, Ar–C), 114.0 (CN), 58.6 (Ar-$\underline{C}H_2$), 55.4 (Ar-$\underline{C}H_2$), 54.7 (9-C), 51.0 (6-C), 47.0 (2-C), 36.3 (1-C), 28.7 (12-C), 28.0 (7-C), 27.2 (11-C), 26.9 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 453.1994 $[M+H]^+$, calcd for $C_{27}H_{31}ClFN_2O$, 453.2019.

methyl(R)-4′-(((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex- 4-en-6-yl) propyl) amino) methyl)-[1,1′-biphenyl]-3-carboxylate (11 l)

Yield 76.5%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (m, 1H, Ar–H), 8.00 (m, 1H, Ar–H), 7.78 (m, 1H, Ar–H), 7.59 (d, J=8.0 Hz, 2H, Ar–H), 7.50 (m, 1H, Ar–H), 7.43 (d, J=8.0 Hz, 2H, Ar–H), 7.27 (m, 1H, Ar–H), 7.11 (m, 2H, Ar–H), 6.93 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.94 (s, 3H, OCH_3), 3.60 (s, 2H, Ar-CH_2), 3.56 (s, 2H, Ar-CH_2), 2.44 (t, J= 6.8 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 2.01 (d, J=17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.79 (t, J=5.4 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.56 (m, 2H, 8-H), 1.36 (m, 1H, 7-H), 1.02 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 167.0 (CO), 165.7 (5-C), 162.9 (d, J=244.1 Hz, Ar–C), 142.5 (d, J=6.8 Hz, Ar–C), 141.1 (Ar–C), 139.1 (Ar–C), 138.8 (Ar–C), 131.4 (Ar–C), 130.6 (Ar–C), 129.6 (d, J=8.2 Hz, Ar–C), 129.2 (Ar–C), 128.9 (Ar–C), 128.3 (Ar–C), 128.1 (Ar–C), 127.0 (Ar–C), 125.0 (4-C), 124.1 (d, J=2.4 Hz, Ar–C), 115.3 (d, J=21.1 Hz, Ar–C), 113.8 (d, J=21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 58.2 (Ar-$\underline{C}H_2$), 54.0 (9-C), 52.2 ($O\underline{C}H_3$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 26.9 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 528.2883 $[M+H]^+$, calcd for $C_{34}H_{39}FNO_3$, 528.2914.

(R)-6-(3-((3-fluorobenzyl) ((1-methyl-3-phenyl-1H-pyrazol-5- yl) methyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 m)

Yield 76.8%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J= 7.2 Hz, 2H, Ar–H), 7.37 (m, 2H, Ar–H), 7.28 (m, 2H, Ar–H), 7.04 (m, 2H, Ar–H), 6.95 (m, 1H, Ar–H), 6.45 (s, 1H), 5.79 (s, 1H, 4-H), 3.84 (s, 3H, *N*-CH_3), 3.56 (s, 4H, Ar-CH_2), 2.44 (t, J=6.8 Hz, 2H, 9-H), 2.31 (d, J=17.2 Hz, 1H, 2-H), 2.00 (d, J=17.2 Hz, 1H, 2-H), 1.89 (s, 3H, 13-H), 1.77 (t, J=5.2 Hz, 1H, 6-H), 1.60 (m, 1H, 7-H), 1.53 (m, 2H, 8-H), 1.31 (m, 1H, 7-H), 0.99 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 199.3 (3-C), 165.5 (5-C), 162.8 (d, J = 244.6 Hz, Ar–C), 149.8 (C), 141.6 (d, J = 6.7 Hz, Ar–C), 140.6 (Ar–C), 133.3 (C), 129.8 (d, J = 8.3 Hz, Ar–C), 128.6 (Ar–C), 127.5 (Ar–C), 125.3 (Ar–C), 125.1 (4-C), 124.4 (d, J = 2.5 Hz, Ar–C), 115.5 (d, J = 21.1 Hz, Ar–C), 114.1 (Ar–C), 104.3 (CH), 58.4 (Ar-$\underline{C}H_2$), 54.2 (9-C), 50.9 (Ar-$\underline{C}H_2$), 49.5 (6-C), 47.0 (2-C), 36.8 (*N*-CH_3), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 26.6 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 474.2895 $[M+H]^+$, calcd for $C_{30}H_{37}FN_3O$, 474.2921.

(R)-6-(3-(((2-fluoro-2′-methoxy-[1,1′-biphenyl]-3-yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 n)

Yield 90.2%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (m, 2H, Ar–H), 7.25 (m, 3H, Ar–H), 7.14 (m, 3H, Ar–H), 7.01 (m, 2H, Ar–H), 6.91 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.78 (s, 3H, OCH_3), 3.69 (s, 2H, Ar-CH_2), 3.59 (s, 2H, Ar-CH_2), 2.45 (t, J = 6.8 Hz, 2H, 9-H), 2.33 (d, J = 17.2 Hz, 1H, 2-H), 2.00 (d, J = 17.2 Hz, 1H, 2-H), 1.89 (s, 3H, 13-H), 1.78 (t, J = 5.2 Hz, 1H, 9-H), 1.67 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.35 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.9 (5-C), 162.9 (d, J = 243.9 Hz, Ar–C), 158.5 (d, J = 246.6 Hz, Ar–C), 156.8 (Ar–C), 142.7 (d, J = 6.8 Hz, Ar–C), 131.2 (Ar–C), 130.6 (d, J = 3.5 Hz, Ar–C), 130.1 (d, J = 4.5 Hz, Ar–C), 129.6 (d, J = 8.2 Hz, Ar–C), 129.3 (Ar–C), 126.2 (d, J = 17.1 Hz, Ar–C), 125.7 (d, J = 15.5 Hz, Ar–C), 125.1 (4-C), 124.9 (Ar–C), 124.1 (d, J = 2.1 Hz, Ar–C), 123.4 (d, J = 3.9 Hz, Ar–C), 120.5 (Ar–C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.8 (d, J = 21.1 Hz, Ar–C), 111.0 (Ar–C), 58.2 (Ar-$\underline{C}H_2$), 55.6 (OCH_3), 54.0 (9-C), 51.4 (d, J = 1.8 Hz, Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.7 (13-C). HR-ESIMS *m/z* 518.2841 $[M+H]^+$, calcd for $C_{33}H_{38}F_2NO_2$, 518.2871.

(R)-6-(3-(((2-fluoro-2′-methyl-[1,1′-biphenyl]-3-yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 o)

Yield 89.2%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (m, 1H, Ar–H), 7.26 (m, 4H, Ar–H), 7.16 (m, 5H, Ar–H), 6.92 (m, 1H, Ar–H), 6.91 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.68 (s, 2H, Ar-CH_2), 3.59 (s, 2H, Ar-CH_2), 2.45 (t, J = 6.8 Hz, 2H, 9-H), 2.33 (d, J = 17.6 Hz, 1H, 2-H), 2.19 (s, 3H, CH_3), 2.00 (d, J = 17.6 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.78 (t, J = 5.2 Hz, 1H, 9-H), 1.66 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.8 (5-C), 162.9 (d, J = 243.8 Hz, Ar–C), 158.1 (d, J = 246.6 Hz, Ar–C), 142.6 (d, J = 7.0 Hz, Ar–C), 136.5 (Ar–C), 135.8 (Ar–C), 130.3 (d, J = 8.9 Hz, Ar–C), 130.3 (Ar–C), 129.9 (d, J = 9.6 Hz, Ar–C), 129.6 (d, J = 8.2 Hz, Ar–C), 129.2 (d, J = 7.8 Hz, Ar–C),127.9 (Ar–C), 125.9 (d, J = 15.4 Hz, Ar–C), 125.6 (4-C), 125.0 (Ar–C), 124.1 (d, J = 2.2 Hz, Ar–C), 123.6 (d, J = 4.0 Hz, Ar–C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.8 (d, J = 21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.1 (9-C), 51.4 (Ar-$\underline{C}H_2$), 51.1 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.8 (13-C), 20.0 (d, J = 2.6 Hz, CH_3). HR-ESIMS *m/z* 502.2903 $[M+H]^+$, calcd for $C_{33}H_{38}F_2NO$, 502.2921.

(R)-6-(3-(((2′-chloro-2-fluoro-[1,1′-biphenyl]-3-yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 p)

Yield 87.9%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (m, 2H, Ar–H), 7.32 (m, 3H, Ar–H), 7.25 (m, 1H, Ar–H), 7.19 (m, 2H, Ar–H), 7.11 (m, 2H, Ar–H), 6.92 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.69 (s, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.46 (t, J = 6.8 Hz, 2H, 9-H), 2.33 (d, J = 17.6 Hz, 1H, 2-H), 2.00 (d, J = 17.6 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.78 (t, J = 5.2 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.9 (5-C), 162.9 (d, J = 243.8 Hz, Ar–C), 158.1 (d, J = 246.4 Hz, Ar–C), 142.5 (d, J = 7.0 Hz, Ar–C), 135.0 (Ar–C), 133.6 (Ar–C), 131.5 (Ar–C), 131.0 (d, J = 4.6 Hz, Ar–C), 130.2 (d, J = 3.2 Hz, Ar–C), 129.6 (d, J = 8.1 Hz, Ar–C), 129.5 (Ar–C), 126.9 (d, J = 16.9 Hz, Ar–C), 126.6 (Ar–C), 126.1 (d, J = 15.1 Hz, Ar–C), 125.0 (4-C), 124.1 (d, J = 2.4 Hz, Ar–C), 123.5 (d, J = 4.1 Hz, Ar–C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.8 (d, J = 21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.0 (9-C), 51.2 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (11-C), 27.0 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 522.2346 $[M+H]^+$, calcd for $C_{32}H_{35}ClF_2NO$, 522.2375.

(R)-6-(3-(((3′,4′-dichloro-2-fluoro-[1,1′-biphenyl]-3-yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 q)

Yield 89.4%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H, Ar–H), 7.50 (d, J = 8.4 Hz, 1H, Ar–H), 7.45 (m, 1H, Ar–H), 7.36 (m, 1H, Ar–H), 7.27 (m, 2H, Ar–H), 7.19 (m, 1H, Ar–H), 7.10 (m, 1H, Ar–H), 6.92 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.69 (s, 2H, Ar-CH_2), 3.59 (s, 2H, Ar-CH_2), 2.45 (t, J = 6.8 Hz, 2H, 9-H), 2.33 (d, J = 17.2 Hz, 1H, 2-H), 2.00 (d, J = 17.2 Hz, 1H, 2-H), 1.91 (s, 3H, 13-H), 1.79 (t, J = 5.4 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.35 (m, 1H, 7-H), 1.01 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.9 (5-C), 162.9 (d, J = 243.8 Hz, Ar–C), 158.1 (d, J = 246.4 Hz, Ar–C), 142.5 (d, J = 7.0 Hz, Ar–C), 135.0 (Ar–C), 133.6 (Ar–C), 131.5 (Ar–C), 131.0 (d, J = 4.6 Hz, Ar–C), 130.2 (d, J = 3.2 Hz, Ar–C), 129.6 (d, J = 8.1 Hz, Ar–C), 129.5 (Ar–C), 126.9 (d, J = 16.9 Hz, Ar–C), 126.6 (Ar–C), 126.1 (d, J = 15.1 Hz, Ar–C), 125.0 (4-C), 124.1 (d, J = 2.4 Hz, Ar–C), 123.5 (d, J = 4.1 Hz, Ar–C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.8 (d, J = 21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.0 (9-C), 51.2 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (11-C), 27.0 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 556.1963 $[M+H]^+$, calcd for $C_{32}H_{34}Cl_2F_2NO$, 556.1986.

(R)-6-(3-(((2-fluoro-2′,3′-dimethyl-[1,1′-biphenyl]-3-yl) methyl) (3- fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 r)

Yield 88.6%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (m, 1H, Ar–H), 7.25 (m, 1H, Ar–H), 7.15 (m, 6H, Ar–H), 7.05 (m, 1H, Ar–H), 6.92 (m, 1H, Ar–H), 5.80 (s, 1H, 4-H), 3.58 (m, 4H, Ar-CH_2), 2.45 (t, J = 6.4 Hz, 2H, 9-H), 2.34 (s, 3H, Ar-CH_3), 2.33 (d, J = 17.2 Hz, 1H, 2-H), 2.07 (s, 3H, Ar-CH_3), 2.00 (d, J = 17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.78 (t, J = 5.2 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.8 (5-C), 162.9 (d, J = 243.8 Hz, Ar–C), 158.1 (d, J = 244.5 Hz, Ar–C), 142.6 (d, J = 6.9 Hz, Ar–C), 136.9 (Ar–C), 135.9 (Ar–C), 135.0 (Ar–C), 130.4 (d, J = 3.2 Hz, Ar–C), 130.1 (d, J = 4.4 Hz, Ar–C), 129.7 (d, J = 17.5 Hz, Ar–C), 129.5 (d, J = 10.5 Hz, Ar–C), 127.8 (Ar–C), 125.8 (d, J = 15.5 Hz, Ar–C), 125.2 (Ar–C), 125.0 (4-C), 124.1 (d, J = 2.3 Hz, Ar–C), 123.5 (d, J = 4.1 Hz, Ar–C), 115.3 (d, J = 21.2 Hz, Ar–C), 113.8 (d, J = 21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.1 (9-C), 51.4 (Ar-$\underline{C}H_2$), 51.1 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.2 (11-C), 27.0 (8-C), 24.8 (13-C), 20.0 (CH_3), 16.7 (CH_3). HR-ESIMS *m/z* 516.3050 $[M+H]^+$, calcd for $C_{34}H_{40}F_2NO$, 516.3078.

(R)-6-(3-(((2,2′-difluoro-3′-methoxy-[1,1′-biphenyl]-3-yl) methyl) (3- fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 s)

Yield 86.3%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (m, 1H, Ar–H), 7.26 (m, 2H, Ar–H), 7.15 (m, 4H, Ar–H), 7.00 (m, 1H, Ar–H), 6.92 (m, 2H, Ar–H), 5.79 (s, 1H, 4-H), 3.92 (s, 3H, OCH_3), 3.69 (m, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.45 (t, J = 6.6 Hz, 2H, 9-H), 2.34 (d, J = 17.2 Hz, 1H, 2-H), 2.07 (s, 3H, Ar-CH_3), 2.00 (d, J = 17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.78 (t, J = 5.2 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.57

(m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6 (3-C), 166.0 (5-C), 162.9 (d, J= 244.0 Hz, Ar–C), 158.2 (d, J=247.6 Hz, Ar–C), 150.9 (Ar–C), 148.4 (Ar–C), 141.9 (d, J=11.1 Hz, Ar–C), 142.5 (d, J=7.0 Hz, Ar–C), 130.9 (d, J=4.6 Hz, Ar–C), 130.3 (Ar–C), 129.6 (d, J=3.2 Hz, Ar–C), 126.2 (d, J=15.2 Hz, Ar–C), 124.9 (4-C), 124.4 (d, J=13.2 Hz, Ar–C), 124.1 (d, J=2.2 Hz, Ar–C), 123.7 (Ar–C), 123.3 (d, J=16.8 Hz, Ar–C), 122.7 (Ar–C), 115.3 (d, J=21.2 Hz, Ar–C), 113.8 (d, J=21.1 Hz, Ar–C), 112.9 (Ar–C), 58.3 (Ar-$\underline{C}H_2$), 56.3 (OCH_3), 54.0 (9-C), 51.3 (d, J=1.8 Hz, Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.7 (13-C). HR-ESIMS *m/z* 536.2744 $[M+H]^+$, calcd for $C_{33}H_{37}F_3NO_2$, 536.2776.

(R)-6-(3-((3-fluorobenzyl) ((2,2′,3′-trifluoro-[1,1′-biphenyl]-3- yl) methyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 t)

Yield 87.8%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (m, 1H, Ar–H), 7.26 (m, 2H, Ar–H), 7.20 (m, 2H, Ar–H), 7.13 (m, 4H, Ar–H), 6.92 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.70 (s, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.46 (t, J=6.7 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 2.00 (d, J=17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.79 (t, J=5.2 Hz, 1H, 6-H), 1.66 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.8 (5-C), 163.0 (d, J=243.9 Hz, Ar–C), 158.2 (d, J=248.0 Hz, Ar–C), 150.8 (dd, J_1=246.5 Hz, J_2=13.1 Hz, Ar–C), 148.0 (dd, J_1= 248.7 Hz, J_2=12.9 Hz, Ar–C), 142.4 (d, J=6.7 Hz, Ar–C), 131.4 (d, J= 4.6 Hz, Ar–C), 130.1 (Ar–C), 129.6 (d, J=8.1 Hz, Ar–C), 126.5 (d, J= 15.1 Hz, Ar–C), 126.2 (d, J=1.3 Hz, Ar–C), 125.9 (d, J=12.4 Hz, Ar–C), 125.0 (4-C), 124.1 (d, J=2.0 Hz, Ar–C), 123.9 (dd, J_1=4.7 Hz, J_2=6.9 Hz, Ar–C), 122.3 (dd, J_1=2.5 Hz, J_2=16.6 Hz, Ar–C), 116.8 (d, J=17.1 Hz, Ar–C), 115.3 (d, J=21.2 Hz, Ar–C), 113.8 (d, J=21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.0 (9-C), 51.3 (d, J=1.9 Hz, Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.7 (13-C). HR-ESIMS *m/z* 524.2547 $[M+H]^+$, calcd for $C_{32}H_{34}F_4NO$, 524.2577.

(R)-6-(3-(((2,2′-difluoro-3′-methyl-[1,1′-biphenyl]-3-yl) methyl) (3- fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 u)

Yield 89.5%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (m, 1H, Ar–H), 7.25 (m, 3H, Ar–H), 7.17 (m, 3H, Ar–H), 7.10 (m, 3H, Ar–H), 6.92 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.69 (s, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.46 (t, J=6.7 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 2.33 (s, 3H, CH_3), 1.99 (d, J=17.2 Hz, 1H, 2-H), 1.89 (s, 3H, 13-H), 1.78 (t, J=5.2 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 166.0 (5-C), 163.0 (d, J=244.0 Hz, Ar–C), 158.4 (d, J=247.0 Hz, Ar–C), 158.3 (d, J=245.5 Hz, Ar–C), 142.6 (d, J= 6.9 Hz, Ar–C), 131.3 (d, J=5.1 Hz, Ar–C), 130.8 (d, J=4.6 Hz, Ar–C), 130.4 (d, J=2.2 Hz, Ar–C), 129.7 (d, J=8.1 Hz, Ar–C), 128.9 (d, J= 2.0 Hz, Ar–C), 126.2 (d, J=15.2 Hz, Ar–C), 152.2 (d, J=17.7 Hz, Ar–C), 125.0 (4-C), 124.2 125.0 (d, J=23.8 Hz, Ar–C), 123.9 (d, J= 23.0 Hz, Ar–C), 123.7 (d, J=4.0 Hz, Ar–C), 123.6 (d, J=4.3 Hz, Ar–C), 123.3 (d, J=16.6 Hz, Ar–C), 115.4 (d, J=21.2 Hz, Ar–C), 113.9 (d, J= 21.1 Hz, Ar–C), 58.4 (Ar-$\underline{C}H_2$), 54.0 (9-C), 51.4 (d, J=1.7 Hz, Ar-$\underline{C}H_2$), 51.1 (6-C), 47.1 (2-C), 36.4 (1-C), 28.7 (12-C), 28.0 (7-C), 27.2 (11-C), 27.0 (8-C), 24.8 (13-C), 14.8 (d, J=4.3 Hz, CH_3). HR-ESIMS *m/z* 520.2814 $[M+H]^+$, calcd for $C_{33}H_{37}F_3NO$, 520.2827.

(R)-6-(3-(((2′-chloro-2-fluoro-3′-methoxy-[1,1′-biphenyl]-3- yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 v)

Yield 84.9%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (m, 1H, Ar–H), 7.27 (m, 2H, Ar–H), 7.18 (m, 2H, Ar–H), 7.11 (m, 2H, Ar–H), 6.98 (m, 1H, Ar–H), 6.92 (m, 2H, Ar–H), 5.79 (s, 1H, 4-H), 3.94 (s, 3H, OCH_3), 3.60 (s, 2H, Ar-CH_2), 3.60 (s, 2H, Ar-CH_2), 2.46 (t, J=6.6 Hz, 2H, 9-H), 2.33 (d, J=17.2 Hz, 1H, 2-H), 2.33 (s, 3H, Ar-CH_3), 1.99 (d, J= 17.2 Hz, 1H, 2-H), 1.90 (s, 3H, 13-H), 1.78 (t, J=5.2 Hz, 1H, 6-H), 1.66 (m, 1H, 7-H), 1.57 (m, 2H, 8-H), 1.34 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.97 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6 (3-C), 166.0 (5-C), 163.0 (d, J=243.8 Hz, Ar–C), 158.0 (d, J=246.3 Hz, Ar–C), 155.3 (Ar–C), 142.6 (d, J=6.9 Hz, Ar–C), 136.7 (Ar–C), 131.0 (d, J=4.5 Hz, Ar–C), 130.1 (d, J=2.8 Hz, Ar–C), 129.7 (d, J=8.1 Hz, Ar–C), 127.1 (d, J=17.0 Hz, Ar–C), 127.0 (Ar–C), 126.1 (d, J=15.1 Hz, Ar–C), 125.0 (4-C), 124.2 (d, J=2.4 Hz, Ar–C), 123.5 (d, J=4.0 Hz, Ar–C), 123.3 (Ar–C), 122.2 (Ar–C), 115.3 (d, J=21.2 Hz, Ar–C), 113.8 (d, J= 21.1 Hz, Ar–C), 111.4 (Ar–C), 58.2 (Ar-$\underline{C}H_2$), 56.3 (OCH_3), 54.0 (9-C), 51.3 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 552.2465 $[M+H]^+$, calcd for $C_{33}H_{37}ClF_2NO_2$, 552.2481.

(R)-2′-fluoro-3′-(((3-fluorobenzyl) (3-(1,1,5-trimethyl-3-oxocyclohex-4-en-6-yl) propyl) amino) methyl)-[1,1′-biphenyl]-2-carbonitrile (11 w)

Yield 83.6%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, J= 8.4 Hz, 1H, Ar–H), 7.66 (m, 1H, Ar–H), 7.50 (m, 3H, Ar–H), 7.26 (m, 3H, Ar–H), 7.11 (m, 2H, Ar–H), 6.92 (m, 1H, Ar–H), 5.79 (s, 1H, 4-H), 3.71 (s, 2H, Ar-CH_2), 3.62 (s, 2H, Ar-CH_2), 2.48 (t, J=6.8 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 1.99 (d, J=17.2 Hz, 1H, 2-H), 1.91 (s, 3H, 13-H), 1.80 (t, J=5.2 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.58 (m, 2H, 8-H), 1.35 (m, 1H, 7-H), 1.01 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4 (3-C), 165.7 (5-C), 163.0 (d, J=244.0 Hz, Ar–C), 157.9 (d, J=247.1 Hz, Ar–C), 142.4 (d, J=7.8 Hz, Ar–C), 139.7 (Ar–C), 133.1 (Ar–C), 132.6 (Ar–C), 132.0 (d, J=4.8 Hz, Ar–C), 130.9 (Ar–C), 130.0 (d, J=2.3 Hz, Ar–C), 129.6 (d, J=8.1 Hz, Ar–C), 128.2 (Ar–C), 126.7 (d, J=15.1 Hz, Ar–C), 125.9 (d, J=16.1 Hz, Ar–C), 125.0 (4-C), 124.2 (d, J=2.5 Hz, Ar–C), 124.0 (d, J=6.2 Hz, Ar–C), 118.0 (CN), 115.3 (d, J=21.2 Hz, Ar–C), 113.8 (d, J=21.1 Hz, Ar–C), 113.0 (Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.1 (9-C), 51.2 (Ar-$\underline{C}H_2$), 51.0 (6-C), 47.2 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0 (8-C), 24.8 (13-C). HR-ESIMS *m/z* 513.2695$[M+H]^+$, calcd for $C_{33}H_{35}F_2N_2O$, 513.2717.

(R)-6-(3-(((2-fluoro-2′-nitro-[1,1′-biphenyl]-3-yl) methyl) (3-fluorobenzyl) amino) propyl)-1,1,5-trimethylcyclohex-4-en-3-one (11 x)

Yield 80.7%, yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (m, 1H, Ar–H), 7.68 (m, 1H, Ar–H), 7.55 (m, 1H, Ar–H), 7.45 (m, 2H, Ar–H), 7.25 (m, 3H, Ar–H), 7.10 (m, 2H, Ar–H), 6.92 (m, 1H, Ar–H), 5.78 (s, 1H, 4-H), 3.64 (s, 2H, Ar-CH_2), 3.58 (s, 2H, Ar-CH_2), 3.60 (s, 2H, N-CH_2), 2.43 (t, J=6.8 Hz, 2H, 9-H), 2.34 (d, J=17.2 Hz, 1H, 2-H), 1.99 (d, J= 17.2 Hz, 1H, 2-H), 1.91 (s, 3H, 13-H), 1.80 (t, J=5.4 Hz, 1H, 6-H), 1.67 (m, 1H, 7-H), 1.56 (m, 2H, 8-H), 1.33 (m, 1H, 7-H), 1.00 (s, 3H, 11-H), 0.98 (s, 3H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (3-C), 165.9 (5-C), 162.9 (d, J=243.8 Hz, Ar–C), 157.8 (d, J=245.2 Hz, Ar–C), 149.1 (Ar–C), 142.4 (d, J=6.9 Hz, Ar–C), 132.9 (Ar–C), 132.5 (Ar–C), 131.5 (d, J=4.5 Hz, Ar–C), 130.8 (Ar–C), 129.6 (d, J=8.2 Hz, Ar–C), 128.9 (Ar–C), 128.8 (d, J=2.7 Hz, Ar–C), 126.0 (d, J=15.0 Hz, Ar–C), 125.6 (d, J=16.9 Hz, Ar–C), 125.0 (4-C), 124.5 (Ar–C), 124.3 (d, J=4.0 Hz, Ar–C), 124.1 (d, J=2.5 Hz, Ar–C), 115.3 (d, J=21.2 Hz, Ar–C), 113.8 (d, J=21.1 Hz, Ar–C), 58.3 (Ar-$\underline{C}H_2$), 54.0 (9-C), 51.1 (6-C), 51.0 (Ar-$\underline{C}H_2$), 47.1 (2-C), 36.3 (1-C), 28.7 (12-C), 27.9 (7-C), 27.1 (11-C), 27.0

(8-C), 24.7 (13-C). HR-ESIMS *m/z* 533.2485 $[M+H]^+$, calcd for $C_{32}H_{35}F_2N_2O_3$, 533.2616.

Biological evaluation

Chemotaxis assay

The chemotaxis assay was performed 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. Old medium and 10%FBS were loaded into the lower chemotaxis chamber. Control (cells only) and pretreated cells were resuspended in serum-free medium at a density of 5×10^4 cells/mL and placed into the upper chamber (50 μL/well). The transwell were inserted between the upper and lower chambers. The cells were incubated at 37 °C in 5% CO2 for 6 h; then the filter membrane was rinsed, fixed, and stained. The number of migrating cells in three separate fields was counted using light microscopy. 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 compounds were expressed as the median inhibitory concentration (IC_{50}) values. LY294002 (Camarillo, CA, USA) was used as a positive control substance for this assay.[29]

Invasion assay

Invasion of cells was measured in Matrigel (BD, Franklin Lakes, NJ, USA) -coated transwell inserts (Costar, Manassas, VA, USA) containing polycarbonate filters with 8 μM pores. The inserts were coated with 50 μL of Matrigel matrix according to the manufacturer's recommendations. 5×10^4 cells in 100 μL of cell suspension with various concentrations of **11g** were plated in the upper chamber, whereas 750 μL of medium with 10% fetal bovine serum were added to lower well. After incubating for 24 h, cells that migrated to the lower surface of the membrane were fixed and stained. For each membrane, three random fields were counted at 10 magnification. Relative invasion rate (%) = number of migrated cells in sample/number of migrated cells in control × 100%.

Wound healing assay

The assay was carried out as previously described.[30] MDA-MB-231 cells were seeded in 6-well plates and grown to full confluence. The cell monolayer was wounded with a sterile micropipette tip to generate a clean wound area across the center of the well. Subsequently, cellular debris was rinsed away with PBS, and then incubated in medium with or without compound for 24 h. Cell migrated into the wound was monitored and photographed under microscope. Relative migration rate (%) = migration distance in sample/ migration distance in control × 100%.

Adhesion assay

Cell adhesion assay was performed as described[31] with modifications. The 96-well plates (BD Biosciences, Bedford, MA, USA) were coated with fibronectin (20 μg/mL) at 4 °C for overnight and then blocked in BSA (1%) for 1 h. MDA-MB-231 cells were exposed to **11g** (0.1, 1, 10 or 25 μM) for 24 h before seeding. Target cells were suspended in medium. Cells (5×10^4 mL^{-1}) were seeded to fibronectin coated plates and then incubated for 1 h at 37 °C. Non-adherent cells were removed by gentle washing with PBS. Then, crystal violet staining assay was employed to analyze the adhesion ability of cells. The OD value was measured using microplate reader at 570 nm. Relative adhesion rate(%) = OD value in sample/ OD value cells in control × 100%.

Construction of the hypoxic cell model

To construct the hypoxic cell model, cells were incubated for 24 h in culture dishes for adherence and pre-starved for 12 h with L15 starvation medium (containing only 1% FBS), which was replaced with fresh starvation medium containing different concentrations of **11g**. Thereafter, the cells were placed into an anoxic culture cassette, which was filled with high-purity nitrogen (purity of nitrogen, > 99.999%), sealed, and cultured in an incubator for different durations.[32]

Western blotting

MDA-MB-231 cells were cultured in 6-well plates and lysed on ice in 200 μL of RIPA buffer (containing 1% (v/v) PMSF) (Solarbio, Beijing, China). The samples were electrophoresed on 10% SDS-polyacrylamide gels, transferred to polyvinylidene fluoride membranes, blocked for 1 h in 2% (w/v) skim milk, 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.

Real-time PCR (RT-PCR) analysis

Total RNA from MDA-MB-231 cells was extracted using TRIzol (Tiangen Biotechnology, Beijing, China). And the same amount of RNA was taken for reverse transcription and RT-PCR using One Step TB Green PrimeScript RT-PCR kit (Takara, Beijing, China) and 7500 Real Time PCR System (ThermoFisher Scientific, Waltham, MA, USA). The human-specific primers were: HIF-1α (sense primer: 5′-CTGGATGCTGGTGATTTGGA-3′; antisense primer: 5′-TGTCACCATCATCTGTGAG-3′); GAPDH (sense primer: 5′-GTCATTGAGAGCAATGCCAG-3′; antisense primer: 5′-GTGTTCCTACCCCCAATGTG-3′). All primers were purchased from GENEWIZ (Tianjin, China). GAPDH was employed as the internal control.

Enzyme-linked immunosorbent assay (ELISA)

The supernatant of breast cancer cells cultured with the compound was collected and centrifuged, and the human VEGF ELISA kit (RD Systems, USA) was used to detect the concentration of VEGF in accordance with the protocol. The optical density was measured at 450 nm using an enzyme marker (BioTek, Winooski, USA). The results are expressed as pg/mL. All the experiments were done three times.

Statistical analysis

The results were expressed as means ± SD from at least three independent experiments. Statistical analyses were conducted by GraphPad Prism. P value <0.05 was considered statistically significant.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Chiral ionone alkaloid derivatives · Antitumor effect · HIF-1α · VEGFR2 · Akt

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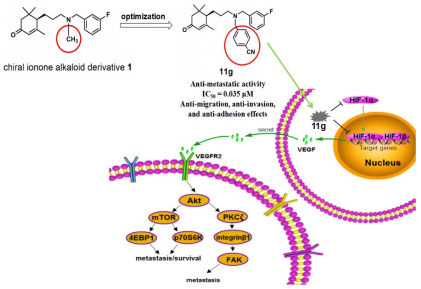
FULL PAPERS

Novel chiral ionone alkaloid derivatives were synthesized, and compound **11 g** with higher antichemotactic migration activity than the lead compound was screened. Compound **11 g** exerted inhibitory effects on the adhesion, migration and invasion of MDA-MB-231 cells. The mechanisms for the antitumor metastatic effects of **11 g** might be that it inhibited HIF-1α expression to down regulate the secretion of VEGF and the phosphorylation of VEGFR2, and then suppressed the downstream pathways, including Akt1/mTOR/p70S6K and Akt2/PKCζ/integrin β1.



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