PAPER

Discovery of 1,3-diyne compounds as novel and potent antidepressant agents: synthesis, cell-based assay and behavioral studies†

Kai-Qing Ma,*[a] Yan-Hong Miao,[ab] Xiao Li,[ab] Yu-Zhi Zhou,[a] Xiao-Xia Gao,[a] Xiang Zhang,[d] Jian-Bin Chao[c] and Xue-Mei Qin*[a]

Sixteen 1,3-diynes compounds were designed and synthesized. Their protective effects on corticosterone-injured PC12 cells were evaluated. Among them, five compounds (**7a**, **7c**, **7d**, **11**, **12**) displayed significant protective activity at 10 µmol. Moreover, the protective activity of compound **7a** was proved to be associated with the regulation of the apoptosis related proteins. The mice forced swim test showed that **7a** had a concentration-dependent antidepressant-like effect. Overall, our findings support that compound **7a** is a promising lead candidate that deserves further evaluation in the design of antidepressants.

1. Introduction

Natural product structures remain a rich source of inspiration in the discovery and development of novel small molecule modulators of bioactivity for chemical biology and medicinal chemistry research.[1,2] 1,3-Diynes are common functionalities in the skeletons of polyacetylenes natural products, such as panaxytriol (**1**) and falcarinol (**2**) (Fig. 1).[3] The diverse structures of these compounds, along with their interesting biological activities, which include anti-tumor,[4] anti-obesity,[5] anti-diabetic,[6]

immunosuppressive[7] and neurotoxicity effects[8] provide compelling reasons for the considerable interest of researchers in this class of natural products.[9] In our previous study, bupleurynol (**3**), RB-2 (**4**) and their isomers were isolated from *Radix Bupleuri* (RB). Furthermore, the polyacetylenes natural products appeared to be the key components responsible for antidepressant activity, which was determined primarily through pharmacokinetic studies.[10] However, to the best of our knowledge, structure–activity relationships (SARs) related to their antidepressant effects have not been studied due to the low abundance of these natural products.

Depression is a common psychiatric disorder, although the pathogenesis of depression is not well understood.[11] Up until now, antidepressants have functioned by increasing the levels of monoamine neurotransmitters (serotonin (5-HT), norepinephrine (NE) and dopamine (DA)) in the brain.[12]

However, treatment with these antidepressants shows modest efficacy and the majority of patients fail to achieve full remission.[13] Therefore, the discovery of new scaffolds with novel mechanisms of action as antidepressants has recently garnered significant interest.[14]

The PC12 cell line is derived from a pheochromocytoma of the rat adrenal medulla and is widely employed as a model system to investigate a variety of neuronal functions.[15] It has been demonstrated that different antidepressants drugs are capable of exhibiting protective effects against cytotoxicity in PC12 cells.[16,17] Hence, corticosterone-induced cytotoxicity in PC12 cells has been widely employed as a rapid *in vitro* screening tool for a primary assessment of neuroprotective activity.[18,19]

In a continuation of our efforts to explore the antidepressant activity of polyacetylenes natural products,[10,20] a novel series of compounds bearing the 1,3-diyne scaffold were designed and synthesized by using a convenient procedure. The anti
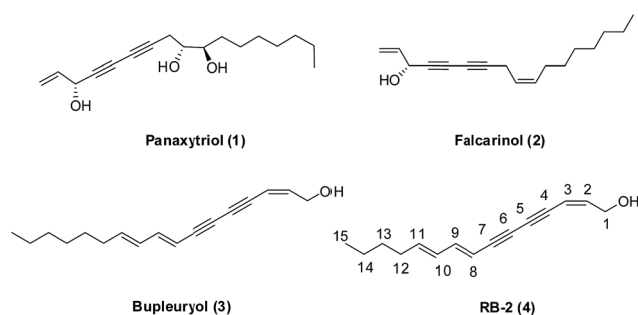

Fig. 1 The structures of several polyacetylenes natural products.

[a]*Modern Research Center for Traditional Chinese Medicine, Shanxi University, Taiyuan 030006, P. R. China. E-mail: makaiqing@sxu.edu.cn*

[b]*College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, P. R. China*

[c]*Scientific Instrument Center, Shanxi University, Taiyuan 030006, P. R. China*

[d]*Department of Chemistry, University of Louisville, Louisville, KY 40208, USA*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ra01268c

depression activity of these compounds were subsequently evaluated using corticosterone-injured PC12 cells, and we found that five compounds possess high neuroprotective activity. To elucidate the mechanism of this neuroprotective activity, the effect of compound **7a** on the expressions of apoptosis-related proteins (Bax, Bcl-2, Cyt-C and caspase-3) was investigated. Finally, we also assessed the effects of compound **7a** on mice through the forced swim tests (FST) to verify its antidepressant properties.
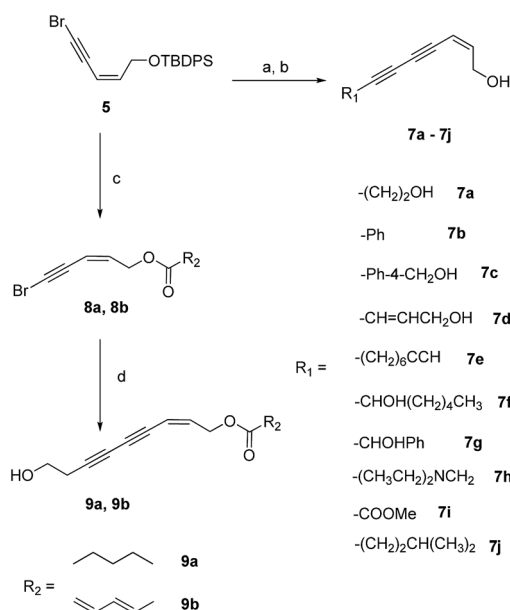
2. Results and discussion
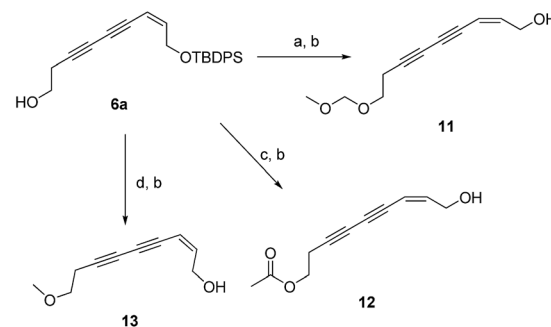
2.1 Chemistry

The key intermediate, compound **5**, was prepared as previously reported.[20] The final products (**7a–7j**) were prepared from the coupling of compound **5** and several different terminal alkynes using the Cadiot–Chodkiewicz reaction and subsequent removal of the TBDPS group (Scheme 1).

The structure–activity relationships (SARs) of compound **7a** were explored by decoration of the 1-OH group with different ester groups. As illustrated in Scheme 1, compound **5** was deprotected to give the free alcohol. The crude product reacted with hexanoic acid or (2E,4E)-hexa-2,4-dienoic acid to afford compounds **8a** or **8b**, respectively. The final compounds (**9a** and **9b**) were obtained from **8a** or **8b** via the Cadiot–Chodkiewicz coupling reaction with 3-butyn-1-ol (**10**).
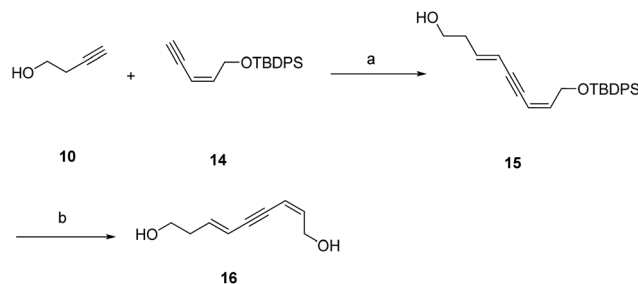
Additionally, the SARs of **7a** were explored by the decoration of the 9-OH group with different ester groups and alkyl groups. Compound **6** reacted with either dimethoxymethane, acetic anhydride or methyl iodide to give the corresponding ether or

Scheme 2 Synthetic route of compounds **11–13**. Reagent and conditions: (a) dimethoxymethane, DCM, P_2O_5, 16 h, 54%; (b) $Et_3N \cdot 3HF$, THF, rt, overnight, 43–59%; (c) acetic anhydride, Et_3N, DMAP, DCM, 43%; (d) CH_3I, NaH, THF, rt, 3 h, 46%.

Scheme 3 Synthetic route of compound **16**. Reagent and conditions: (a) $ZrCp_2Cl_2$, $AlH(i\text{-}Bu)_2$, I_2, THF, overnight, $PdCl_2(PPh_3)_2$, CuI, $i\text{-}Pr_2NH$, THF, rt, 12 h, two steps 41%; (b) $Et_3N \cdot 3HF$, THF, rt, 44%.

ester, which was deprotected to give target molecules **11**, **12** and **13**, respectively (Scheme 2). Meanwhile, an enyne group was chosen to replace the 1,3-diyne group to furnish derivative **16** (Scheme 3). Intermediate **15** was prepared from 3-butyn-1-ol (**10**) through hydrozirconation–iodination followed by Sonogashira cross coupling.[21] The TBDPS group was removed to give compound **16**.

2.2 Protective activities on the corticosterone-injured PC12 cells

Preliminary screening of the synthesized compounds and natural product RB-2 was performed to evaluate their protective activities in corticosterone-injured PC12 cells. The protection rates of the tested compound were calculated[17] (Table 1).

Unfortunately, RB-2 did not show any protective effects on the corticosterone-injured PC12 cells. However, five analogues (**7a, 7c, 7d, 11, 12**) demonstrated potent protective effects on the PC12 cells at a concentration of 10 μM, which was equivalent to the protective effects of the positive control (agomelatine: 17.4 ± 3.6). Replacement of the diene moiety of RB-2 with an alkyl primary alcohol (**7a**) or allyl alcohol (**7d**) moiety showed promising and significant protective effects at 10 μM, while compounds with the secondary alcohol functional group displayed either no or weak protective effects (**7f** and **7g**). Replacement of the diene unit with phenyl and alkyl groups did not show any protective effects (**7b, 7j** and **7e**), while compound

Scheme 1 Synthetic route of compounds **7a–7j** and **9a, 9b**. Reagent and conditions: (a) CH≡CR, CuI, piperidine, rt, 3 h, 40–71% or CH≡CR, CuI, Pd(PPh₃)₄, $i\text{-}Pr_2NH$, THF, rt, 3 h, 44–52%; (b) $Et_3N \cdot 3HF$, THF, rt, overnight, 49–56% or TBAF, THF, 1.5 h, 55%; (c) $Et_3N \cdot 3HF$, THF, rt, overnight; RCOOH, DMAP, DCC, DCM, 5 h, rt, 40–41% (two steps); (d) but-3-yn-1-ol (**10**), CuI, Pd(PPh₃)₄, $i\text{-}Pr_2NH$, THF, rt, 3 h, 58–59%.

$R_1 =$
- -(CH₂)₂OH **7a**
- -Ph **7b**
- -Ph-4-CH₂OH **7c**
- -CH=CHCH₂OH **7d**
- -(CH₂)₆CCH **7e**
- -CHOH(CH₂)₄CH₃ **7f**
- -CHOHPh **7g**
- -(CH₃CH₂)₂NCH₂ **7h**
- -COOMe **7i**
- -(CH₂)₂CH(CH₃)₂ **7j**

$R_2 =$ **9a** **9b**

Table 1 The protection rates of the target compounds on cortico-sterone-injured PC12 cells

Compound	PR^a (%)		
	10 μM	20 μM	50 μM
2	—	—	—
7a	13.1 ± 2.9	17.8 ± 3.6	9.7 ± 3.4
7b	—	—	—
7c	13.9 ± 2.0	—	—
7d	14.5 ± 2.4	—	—
7e	—	—	—
7f	—	—	—
7g	3.9 ± 2.7	—	—
7h	—	4.6 ± 3.9	—
10a	—	—	—
10b	—	—	—
11	13.4 ± 1.9	19.6 ± 2.3	—
12	11.3 ± 1.6	16.7 ± 2.0	—
13	—	—	—
7i	—	—	—
7j	—	—	—
16	6.2 ± 2.1	6.4 ± 2.6	3.8 ± 2.1
17	—	—	—
Ago.	17.4 ± 3.6	22.5 ± 5.5	—

^{a} PR is the protection rate of the tested compound obtained from five independent experiments measured at 48 h after treatment with the test compound at three different concentrations (10 μM, 20 μM and 50 μM). PR = $(A_d - A_c)/A_c \times 100\%$ ± S.D., where A_c represents the mean absorbance value of five independent experiments of the control group treated only with corticosterone, A_d indicate the mean absorbance value of five independent experiments of the test group treated with corticosterone and the test drug. S.D. represents the standard deviation. Data are presented as mean ± S.D. '—' represents compounds did not exhibit protection effects on the corticosterone-injured PC12 cells.

7c, which featured a benzyl alcohol, showed protective effects at 10 μM. Incorporation of the di-substituted amino group into the structure afforded compound **6h**, which displayed weak protective activity at 20 μM. Conversion of the 1-OH group of **7a** to the corresponding ester removed the protective effects (**9a** and **9b**), demonstrating that the 1-OH group was necessary for the protective activity. When the 9-OH group was converted to either corresponding ethyl ester or methoxymethyl ether, the protective effect was retained. However, the methyl ether showed no protective effect. When the 1,3-diyne units were replaced with an enyne group (**16**), the protective effects were decreased, which indicated that the 1,3-diyne moiety was important. Hexa-2,4-diyne-1,6-diol (**17**), which was commercial available, was also selected to evaluate its protective effects. The results suggested that it displayed no protective effect. Comparing its structure to that of compound **7a** suggested that the vinyl alcohol was crucial to the activity.

2.3 The effects of compound 7a on the expressions of apoptosis-related proteins

To determine whether these 1,3-diyne compounds exerted their protective effects on inhibiting the apoptosis of the PC12 cells, the expression of apoptosis-related proteins in PC12 cells in response to corticosterone and treatment with compound **7a**
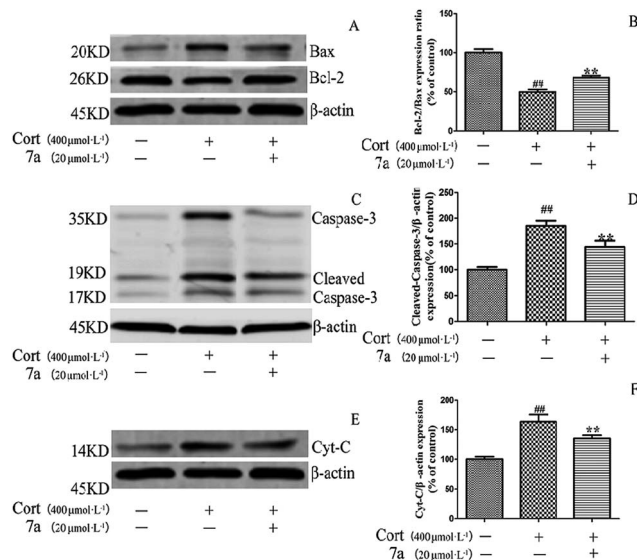

Fig. 2 Effect of compound **7a** on the expression levels of Bcl-2, Bax, Caspase-3 and Cyt-C in PC12 cells. PC12 cells were preincubated with **7a** before treatment with 400 μmol L^{-1} corticosterone for 24 h. Data are expressed as percentage of the control, and the results are expressed as the means ± SD (n = 5). **$P < 0.01$ vs. corticosterone group; ##$P < 0.01$ vs. control group. Cort: corticosterone. (A/B) The expression of Bcl-2 and Bax. (C/D) The expression of caspase-3. (E/F) The expression of Cyt-C.

were assessed. When PC12 cells were treated with corticosterone alone, the results showed that the expression of the pro-apoptotic proteins Bax, caspase-3 and cytochrome c were up-regulated, while the expression of the anti-apoptotic Bcl-2 protein was simultaneously down-regulated (Fig. 2).

These results indicated that corticosterone had induced apoptosis in the PC12 cells. However, when compound **7a** was added to the corticosterone-treated group, expression of the Bcl-2 protein increased remarkably, while the expression of Bax, caspase-3 and cytochrome c proteins decreased significantly compared to the group treated only with corticosterone. These results suggested that the alteration of the expression of the apoptosis-related proteins contributed to the protective effect of compound **7a** on corticosterone-induced apoptosis in PC12 cells.

2.4 The behavioral studies

We then investigated whether the excellent potency of the 1,3-diyne analogues in PC12 cells translate into antidepressant-like efficacy in a behavioral model. The effects of compound **7a** was investigated in a mouse forced swim test, an assay in which mice are placed into a beaker of water and the time mouse spends passively floating in the water (immobility) is recorded. Most traditional antidepressants decrease the amount of time the mice spend immobile. Ten mice were administered intragastrically with venlafaxine as a positive control (50 mg kg^{-1}). Compound **7a** demonstrated a antidepressant-like effect in a concentration-dependent manner when administered intraperitoneally, with a significant reduction in immobility at the minimal dose of 0.0125 mg kg^{-1} (Fig. 3).
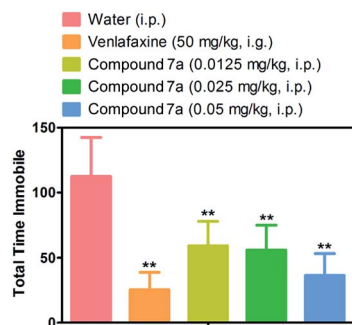
Fig. 3 Mouse forced swim data for compound **7a**. Compound **7a**, which shows the protective effects on corticosterone-injured PC12 cells, also produced the expected decrease in immobility. Data represent the mean ± S.D. of 10 mice per group. **$p < 0.001$.

3. Experimental

3.1 Chemistry

Unless otherwise noted, all reactions were carried out under an argon atmosphere with dry solvents. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker AV-600 spectrometers at ambient temperature with CDCl$_3$ as the solvent unless otherwise stated. High-resolution mass spectra (HRMS) were recorded at Shanxi University Mass Spectrometry Laboratory using a Thermo Scientific Q Exactive.

3.1.1 General procedure for the coupling reaction between terminal alkynes and bromoalkynes (6a–6f, 6h). In a 10 mL flask purged with argon, bromoalkyne (1 equiv.) and CuI (0.25 equiv.) were suspended in piperidine. The mixture was cooled in an ice bath, and then treated dropwise with terminal alkyne (1.3 equiv.). The reaction mixture was allowed to warm to room temperature over 4 hours before addition of saturation NH$_4$Cl solution. The resulting mixture was diluted with ether, and extracted with ether. The combined organic solution was dried over Na$_2$SO$_4$ and then concentrated under reduced pressure.

3.1.1.1 (Z)-9-((tert-Butyldiphenylsilyl)oxy)-7-nonlyene-3,5-diyn-1-ol (6a). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the 3-butyne-1-ol (12.3 μL, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **6a** (29.0 mg, 60%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.68 (m, 4H), 7.44–7.39 (m, 6H), 6.22–6.21 (m, 1H), 5.49 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 6.0 Hz, 2H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.3, 135.6, 133.4, 129.7, 127.7, 107.9, 81.8, 79.2, 71.4, 66.8, 62.5, 60.7, 26.8, 24.0, 19.1; HRMS (APCI) [M + H]$^+$ calculated for C$_{25}$H$_{29}$O$_2$Si: 389.1937, found: 389.1930.

3.1.1.2 (Z)-tert-Butyldiphenyl(7-phenyl-2-heptene-4,6-diyn-1-oxy)silane (6b). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the phenylacetylene (17.8 μL, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 1/0) to afford **6b** (26.6 mg, 51%) as

a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.71–7.70 (m, 4H), 7.49–7.47 (m, 2H), 7.45–7.40 (m, 6H), 7.38–7.31 (m, 3H), 6.28–6.24 (m, 1H), 5.60 (d, J = 10.8 Hz, 1H), 4.53 (d, J = 6.0 Hz, 2H); 1.08 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.3, 135.6, 133.4, 132.4, 129.7, 129.2, 128.4, 127.7, 121.8, 108.1, 82.4, 79.1, 77.5, 73.8, 62.6, 26.8, 19.2; HRMS (APCI) [M + H]$^+$ calculated for C$_{29}$H$_{28}$OSi: 421.1988, found: 421.1980.

3.1.1.3 (2E,8Z)-10-((tert-Butyldiphenylsilyl)oxy)-2,8-decadien-4,6-diyn-1-ol (6c). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the (E)-2-penten-4-yn-1-ol (34.4 mg, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **6c** (20.0 mg, 40%) as a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.68 (m, 4H), 7.44–7.39 (m, 6H), 6.41–6.37 (m, 1H), 6.24–6.21 (m, 1H), 5.84 (d, J = 15.6 Hz, 1H), 5.55 (d, J = 11.4 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 4.25 (d, J = 4.2 Hz, 2H), 1.26 (s, 1H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.3, 145.3, 135.6, 133.4, 129.7, 127.8, 109.0, 108.0, 80.7, 79.1, 77.3, 74.4, 62.8, 62.6, 26.8, 19.2; HRMS (APCI) [M + H]$^+$ calculated for C$_{26}$H$_{29}$O$_2$Si: 401.1937, found: 401.1927.

3.1.1.4 (Z)-(4-(7'-(tert-Butyldiphenylsilyl)oxy)-5-heptene-1,3-diyn)benzyl alcohol (6d). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the 4-ethynylbenzyl alcohol (21.5 mg, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 6/1) to afford **6d** (39.5 mg, 71%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69 (d, J = 7.8 Hz, 4H), 7.47 (d, J = 7.8 Hz, 2H), 7.44–7.39 (m, 6H), 7.33 (d, J = 7.8 Hz, 2H), 6.27–6.23 (m, 1H), 5.59 (d, J = 10.8 Hz, 1H), 4.71 (s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 1.55 (s, 1H), 1.07 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.4, 142.0, 135.6, 133.4, 132.6, 129.7, 127.7, 126.8, 120.9, 108.0, 82.2, 79.1, 77.6, 73.8, 64.8, 62.6, 26.8, 19.2; HRMS (APCI) [M + H]$^+$ calculated for C$_{30}$H$_{31}$O$_2$Si: 451.2093, found: 451.2086.

3.1.1.5 (Z)-(-2-Pentadecene-4,6,14-triyn-1-oxy)tert-butyldiphenylsilane (6e). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the 4-ethynylbenzyl alcohol (21.5 mg, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude oil, which was used directly in the next step.

3.1.1.6 (Z)-13-((tert-Butyldiphenylsilyl)oxy)-11-tridecene-7,9-diyn-6-ol (6f). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the 1-octyne-3-ol (23.7 μL, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 6/1) to afford **6f** (25.0 mg, 45%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.67 (m, 4H), 7.45–7.38 (m, 6H), 6.23 (dt, J = 11.4, 6.0 Hz, 1H), 5.51 (d, J = 10.8 Hz, 1H), 4.47 (d, 6.0 Hz, 3H), 1.75–1.68 (m, 2H), 1.56 (s, 1H), 1.47–1.42 (m, 2H), 1.31 (d, J = 1.8 Hz, 4H), 1.06 (s, 9H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.7, 135.6, 133.4, 129.7, 127.7, 107.7, 84.3, 78.5, 74.6, 69.4, 63.0, 62.5, 37.5, 31.4, 26.8, 24.7, 22.5, 19.2, 14.0; HRMS (APCI) [M + H]$^+$ calculated for C$_{29}$H$_{37}$O$_2$Si: 445.2563, found: 445.2552.

3.1.1.7 (Z)-8-((tert-Butyldiphenylsilyl)oxy)-N,N-diethyl-6-octene-2,4-diyn-1-amine (6h). Following the general procedure using the bromoalkyne (50 mg, 0.13 mmol), CuI (6.0 mg, 0.03 mmol), the N,N-diethylprop-2-yn-1-amine (22.5 μL, 0.17 mmol) and piperidine (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 2/1) to afford **6h** (26.1 mg, 48%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.68 (d, J = 7.2 Hz, 4H), 7.44–7.38 (m, 6H), 6.23–6.19 (m, 1H), 5.50 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 3.54 (s, 2H), 2.54 (q, J = 7.2 Hz, 4H), 1.08–1.06 (m, 15H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.2, 135.6, 133.5, 129.7, 127.7, 108.0, 80.0, 79.2, 71.9, 69.3, 62.6, 47.4, 41.4, 26.8, 19.2, 12.7; HRMS: (APCI) [M + H]$^+$ calculated for C$_{28}$H$_{36}$NOSi: 430.2566, found: 430.2563.

3.1.2 General procedure for the coupling reaction between terminal alkynes and bromoalkynes (6g, 6i, 6j). A solution of the bromoalkyne (1 equiv.) in THF (0.4 M) was sparged with argon over 20 min and then Pd(PPh$_3$)$_4$ (0.03 equiv.), CuI (0.03 equiv.) and diisopropylamine (2 equiv.) were added sequentially at room temperature, followed immediately by the terminal alkyne (1.2 equiv.). The reaction mixture was stirred at room temperature for 12 h and then concentrated under reduced pressure.

3.1.2.1 (Z)-8-((tert-Butyldiphenylsilyl)oxy)-1-phenyl-6-octene-2,4-diyn-1-ol (6g). Following the general procedure using the bromoalkyne (50.0 mg, 0.125 mmol), the 1-phenyl-2-propyn-1-ol (18.3 μL, 0.15 mmol), Pd(PPh$_3$)$_4$ (4.3 mg, 0.004 mmol), CuI (0.7 mg, 0.004 mmol), diisopropylamine (35.1 μL, 0.25 mmol) and THF (0.3 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **6g** (25.0 mg, 44%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.70–7.68 (m, 4H), 7.51 (d, J = 7.2 Hz, 2H), 7.44–7.34 (m, 9H), 6.26 (dt, J = 11.4, 6.0 Hz, 1H), 5.55–5.52 (m, 2H), 4.48 (d, J = 6.0 Hz, 2H), 2.26 (d, J = 6.0 Hz, 1H), 1.07 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 147.0, 139.6, 135.5, 133.3, 129.7, 128.7, 128.7, 127.7, 126.6, 107.6, 82.4, 78.3, 75.4, 71.1, 65.1, 62.5, 26.8, 19.1; HRMS: (APCI) [M + H]$^+$ calculated for C$_{30}$H$_{31}$O$_2$Si: 451.2093, found: 451.2089.

3.1.2.2 (Z)-Methyl-8-((tert-butyldiphenylsilyl)oxy)-6-octene-2,4-diynoate (6i). Following the general procedure using the bromoalkyne (50.0 mg, 0.125 mmol), the methyl propiolate (13.4 μL, 0.15 mmol), Pd(PPh$_3$)$_4$ (4.3 mg, 0.004 mmol), CuI (0.7 mg, 0.004 mmol), diisopropylamine (35.1 μL, 0.25 mmol) and THF (0.3 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 50/1) to afford **6i** (24.9 mg, 50%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.67 (d, J = 7.2 Hz, 4H), 7.46–7.39 (m, 6H), 6.40–6.36 (m, 1H), 5.53 (d, J = 11.4 Hz, 1H), 4.46 (d, J = 6.0 Hz, 2H), 3.80 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 153.3, 149.9, 135.5, 133.1, 129.8, 127.8, 127.8, 106.7, 79.5, 72.3, 71.0, 62.6, 53.0, 26.8, 19.2; HRMS (APCI) [M + H]$^+$ calculated for C$_{25}$H$_{27}$O$_3$Si: 403.1729, found: 403.1721.

3.1.2.3 (Z)-((10-Methyl-2-undecene-4,6-diyn)-1-oxyl)tert-butyl-diphenylsilane (6j). Following the general procedure using the bromoalkyne (50.0 mg, 0.125 mmol), the 5-methyl-hex-1-yne (19.7 μL, 0.15 mmol), Pd(PPh$_3$)$_4$ (4.3 mg, 0.004 mmol), CuI (0.7 mg, 0.004 mmol), diisopropylamine (35.1 μL, 0.25 mmol) and THF (0.3 mL), the resulting residue was purified by silica gel column chromatography (PE) to afford **6j** (27.0 mg, 52%) as

a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.68 (m, 4H), 7.45–7.38 (m, 6H), 6.18 (dt, J = 11.4, 6.0 Hz, 1H), 5.49 (d, J = 11.4 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 2.32 (t, J = 7.2 Hz, 2H), 1.72–1.65 (m, 1H), 1.43 (q, J = 7.2 Hz, 2H), 1.06 (s, 9H), 0.9 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.6, 135.6, 133.5, 129.6, 127.7, 108.2, 85.8, 79.7, 70.7, 64.9, 62.5, 37.0, 27.1, 26.8, 22.1, 19.2, 17.6; HRMS: (APCI) [M + H]$^+$ calculated for C$_{28}$H$_{35}$OSi: 415.2457, found: 415.2451.

3.1.3 General procedure for the deprotection of TBDPS (7a–7j, 11–13, 16). To a solution of a compound comprising TBDPS (1 equiv.) in THF was added either trihydrofluoride (12.3 equiv.) or TBAF (1.3 equiv.) at room temperature. The resulting solution was stirred at room temperature for 8 h or 1.5 h and quenched with H$_2$O. The reaction mixture was extracted with EtOAc, and the combined extracts were washed with saturated NaCl solution, dried over anhydrous Na$_2$SO$_4$ and the filtrate was concentrated under reduced pressure.

3.1.3.1 (Z)-2-Nonene-4,6-diyne-1,9-diol (7a). Following the general procedure using **6a** (17 mg, 0.044 mmol), Et$_3$N·3HF (87.7 μL, 0.54 mmol) and THF (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 2/1) to afford **7a** (3.5 mg, 54%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 6.24 (dt, J = 10.8 Hz, 1H), 5.61 (d, J = 11.4 Hz, 1H), 4.43 (t, J = 5.4 Hz, 2H), 3.78 (q, J = 6.0 Hz, 2H), 2.62 (t, J = 6.6 Hz, 2H), 1.56 (s, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.3, 109.4, 82.3, 79.6, 71.1, 66.6, 61.2, 60.7, 24.0; HRMS (APCI) [M + H]$^+$ calculated for C$_9$H$_{11}$O$_2$: 151.0759, found: 151.0752.

3.1.3.2 (Z)-7-Phenyl-2-hepten-4,6-diyn-1-ol (7b). Following the general procedure using **6b** (17.0 mg, 0.044 mmol), Et$_3$N·3HF (87.7 μL, 0.54 mmol) and THF (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 2/1) to afford **7b** (3.5 mg, 54%) as a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.51–7.50 (m, 2H), 7.38–7.32 (m, 3H), 6.30–6.26 (m, 1H), 5.71 (d, J = 11.4 Hz, 1H) 4.48 (d, J = 5.4 Hz, 2H), 1.26 (s, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.4, 132.5, 129.3, 128.4, 121.6, 109.5, 82.8, 79.5, 73.5, 61.2, 29.7; HRMS (APCI) [M + H]$^+$ calculated for C$_{13}$H$_{11}$O: 183.0810, found: 183.0803.

3.1.3.3 (2Z,8E)-2,8-Decadiene-4,6-diyne-1,10-diol (7c). Following the general procedure using **6c** (16.0 mg, 0.040 mmol), Et$_3$N·3HF (80.0 μL, 0.492 mmol) and THF (0.6 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 2/1) to afford **7c** (3.6 mg, 56%) as a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 6.44–6.42 (d, J = 15.0 Hz, 1H), 6.27–6.23 (m, 1H), 5.88 (d, J = 16.8 Hz, 1H), 5.76 (d, J = 10.8 Hz, 1H), 4.44 (s, 2H), 4.27 (s, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.6, 145.3, 109.5, 108.8, 81.0, 79.5, 62.7, 61.2, 29.7; HRMS (APCI) [M − H]$^-$ calculated for C$_{10}$H$_9$O$_2$: 161.0603, found: 161.0602.

3.1.3.4 (Z)-7-(4-(Hydroxymethyl)phenyl)-2-heptene-4,6-diyn-1-ol (7d). Following the general procedure using **6d** (34.0 mg, 0.075 mmol), Et$_3$N·3HF (151.3 μL, 0.928 mmol) and THF (1.1 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 3/1) to afford **7d** (9.2 mg, 58%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.50 (d, J = 7.8 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 6.29–6.25 (m, 1H), 5.71 (d, J = 10.8 Hz, 1H), 4.71 (s, 2H), 4.47 (d, J = 6.0 Hz, 2H), 1.25 (2, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.4, 142.2, 132.7, 126.8, 120.8,

109.5, 82.7, 79.5, 73.6, 64.8, 61.2, 29.7; HRMS (APCI) $[M - H]^-$ calculated for $C_{14}H_{11}O_2$: 211.0759, found: 211.0762.

3.1.3.5 (Z)-2-Pentadecene-4,6,14-triyn-1-ol (7e). Following the general procedure using crude compound **6e** (16.0 mg, 0.035 mmol), $Et_3N \cdot 3HF$ (70.8 µL, 0.435 mmol) and THF (0.5 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 10/1) to afford **7e** (4.0 mg, 53%) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 6.23–6.19 (m, 1H), 5.61 (d, $J = 10.8$ Hz, 1H), 4.43 (d, $J = 6.0$ Hz, 2H), 2.35 (t, $J = 6.6$ Hz, 2H), 2.20 (t, $J = 7.2$ Hz, 2H), 1.95 (s, 1H), 1.42 (s, 4H), 1.25 (s, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 144.7, 109.8, 86.1, 86.0, 84.4, 68.2, 63.0, 61.2, 29.7, 28.3, 28.2, 28.1, 28.0, 19.5, 18.3; HRMS (APCI) $[M - H]^-$ calculated for $C_{15}H_{17}O$: 213.1279, found: 213.1282.

3.1.3.6 (Z)-2-Tridecene-4,6-diyne-1,8-diol (7f). Following the general procedure using **6f** (15.0 mg, 0.034 mmol), $Et_3N \cdot 3HF$ (67.6 µL, 0.415 mmol) and THF (0.5 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 3/1) to afford **7f** (3.5 mg, 51%) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.68 (m, 4H), 7.45–7.39 (m, 6H), 6.23 (dt, $J = 11.4, 6.0$ Hz, 1H), 5.51 (d, $J = 10.8$ Hz, 1H), 4.47 (d, $J = 6.0$ Hz, 3H), 1.75–1.68 (m, 2H), 1.56 (s, 1H), 1.47–1.42 (m, 2H), 1.31 (d, $J = 1.8$ Hz, 4H), 1.06 (s, 9H), 0.9 (t, $J = 6.6$ Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.8, 109.2, 84.7, 78.8, 74.3, 69.2, 63.1, 61.2, 37.5, 31.4, 24.7, 22.5, 14.0; HRMS (APCI) $[M - H]^-$ calculated for $C_{13}H_{17}O_2$: 205.1229, found: 205.1231.

3.1.3.7 (Z)-8-Phenylocta-2-octene-4,6-diyne-1,8-diol (7g). Following the general procedure using **6g** (25.0 mg, 0.055 mmol), $Et_3N \cdot 3HF$ (111.2 µL, 0.677 mmol) and THF (0.8 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 1/1) to afford **7g** (6.0 mg, 51%) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 7.53–7.52 (m, 2H), 7.41–7.36 (m, 3H), 6.27 (dt, $J = 11.4, 6.6$ Hz, 1H), 5.63 (d, $J = 10.8$ Hz, 1H), 5.58 (d, $J = 6.0$ Hz, 2H), 4.43 (s, 2H), 2.31 (d, $J = 6.0$ Hz, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.1, 139.5, 128.8, 128.8, 126.6, 109.1, 82.8, 78.7, 75.1, 70.9, 65.2, 61.2; HRMS (APCI) $[M - H]^-$ calculated for $C_{14}H_{11}O_2$: 211.0759, found: 211.0760.

3.1.3.8 (Z)-8-(Diethylamino)-2-octene-4,6-diyn-1-ol (7h). Following the general procedure using **6h** (25.0 mg, 0.058 mmol), TBAF (75.6 µL, 0.075 mmol) and THF (0.5 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 1/2) to afford **7h** (5.9 mg, 53%) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 6.26–6.22 (m, 1H), 5.62 (d, $J = 11.4$ Hz, 1H), 4.43 (d, $J = 6.6$ Hz, 2H), 3.59 (s, 2H), 2.59 (q, $J = 7.2$ Hz, 4H), 1.25 (s, 1H), 1.09 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.4, 132.1, 128.5, 109.4, 79.4, 71.8, 61.2, 47.5, 41.4, 29.7; HRMS (APCI) $[M + H]^+$ calculated for $C_{12}H_{18}NO$: 192.1388, found: 192.1381.

3.1.3.9 (Z)-Methyl-8-hydroxy-6-octene-2,4-diynoate (7i). Following the general procedure using **6i** (12.0 mg, 0.030 mmol), $Et_3N \cdot 3HF$ (59.1 µL, 0.369 mmol) and THF (0.5 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **7i** (2.4 mg, 49%) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 6.44–6.40 (m, 1H), 5.66 (d, $J = 10.8$ Hz, 1H), 4.45 (t, $J = 5.4$ Hz, 2H), 3.81 (s, 3H), 1.51 (s, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 151.1, 149.1, 107.9, 79.2, 72.6, 70.7, 61.3, 53.1, 29.7; HRMS (APCI) $[M + H]^+$ calculated for $C_9H_9O_3$: 165.0552, found: 165.0546.

3.1.3.10 (Z)-10-Methyl-2-undecaen-4,6-diyn-1-ol (7j). Following the general procedure using **6j** (24.0 mg, 0.058 mmol), Et_3N·3HF (116 µL, 0.713 mmol) and THF (0.8 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **7j** (5.6 mg, 55%) as a colorless oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 6.22–6.18 (m, 1H), 5.60 (d, $J = 10.8$ Hz, 1H), 4.42 (d, $J = 6.0$ Hz, 2H), 2.34 (t, $J = 7.2$ Hz, 2H), 1.73–1.66 (m, 1H), 1.44 (q, $J = 7.2$ Hz, 2H), 1.25 (s, 1H), 0.90 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 144.6, 109.8, 86.3, 80.1, 70.4, 64.6, 61.1, 37.0, 27.2, 22.1, 17.6; HRMS: (APCI) $[M - H]^-$ calculated for $C_{12}H_{15}O$: 175.1123, found: 175.1125.

3.1.4 Preparation of ester (10a, 10b)

3.1.4.1 (Z)-5-Bromo-2-pentene-4-yn-1-ol. To a solution of **5** (0.3 g, 0.75 mmol) in THF (11.0 mL) was added triethylamine trihydrofluride (1.5 mL, 9.23 mmol) at room temperature. The resulting solution was stirred at room temperature for 8 h and quenched with H$_2$O (3 mL). The reaction mixture was extracted with EtOAc (6 mL × 3), and the combined extracts were washed with saturated NaCl solution, dried over anhydrous Na$_2$SO$_4$ and the filtrate was concentrated under reduced pressure to give a crude oil, which was used directly in the next step.

3.1.4.2 General procedure for the DCC coupling reaction between the alcohols and acids (8a, 8b). Dicyclohexylcarbodiimide (1.1 equiv.) and N,N-dimethylaminopyridine (0.2 equiv.) were added to a stirred solution of acid (1.1 equiv.) in dichloromethane (0.15 M) at 0 °C. The reaction mixture was stirred for 30 min and then a solution of the crude compound alcohol (1 equiv.) in dichloromethane (0.25 M) was added dropwise. The mixture was allowed to warm to room temperature over 12 h, diluted with diethyl ether, and then sequentially washed with saturated aqueous solution of NH$_4$Cl, a saturated aqueous solution of NaHCO$_3$ and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure.

3.1.4.2.1 (2E,4E)-(Z)-5-Bromo-2-pentene-4-yn-1-oxylhexanoate (8a). Following the general procedure using dicyclohexylcarbodiimide (142.0 mg, 0.688 mmol), N,N-dimethylaminopyridine (15.3 mg, 0.125 mmol), hexanoic acid (69.4 mg, 0.616 mmol), the crude compound (60 mg, 0.63 mmol), and dichloromethane (8 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 20/1) to afford **8a** (40.0 mg, 41%, 2 steps) as a pale yellow oil. ^1H NMR (600 MHz, CDCl$_3$) δ: 6.07–6.03 (m, 1H), 5.64 (d, $J = 10.8$ Hz, 1H), 4.81 (d, $J = 6.0$ Hz, 2H), 2.32 (t, $J = 7.2$ Hz, 2H), 1.66–1.61 (m, 2H), 1.34–1.29 (m, 4H), 0.90 (t, $J = 6.6$ Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 173.6, 138.3, 112.1, 75.6, 62.0, 56.1, 34.1, 31.3, 24.6, 22.3, 13.9; HRMS: (APCI) $[M + H]^+$ calculated for $C_{11}H_{16}BrO_2$: 259.0334, found: 259.0323.

3.1.4.2.2 (2E,4E)-(Z)-5-Bromo-2-pentene-4-yn-1-oxyl-2,4-hexadienoate (8b). Following the general procedure using dicyclohexylcarbodiimide (127.7 mg, 0.618 mmol), N,N-dimethylaminopyridine (13.7 mg, 0.112 mmol), sorbic acid (69.4 mg, 0.616 mmol), the crude compound (60 mg, 0.63 mmol), and dichloromethane (8 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 10/1) to afford **8b** (3.8 mg, 40%, 2 steps) as a colorless oil. ^1H NMR (600 MHz,

CDCl$_3$) δ: 7.30–7.27 (m, 1H), 6.22–6.12 (m, 2H), 6.09 (dt, J = 10.8, 6.0 Hz, 1H), 5.78 (d, J = 15.0 Hz, 1H), 5.65 (d, J = 10.8 Hz, 1H), 4.88 (d, J = 6.6 Hz, 2H), 1.86 (d, J = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 166.9, 145.6, 139.8, 138.5, 129.7, 118.3, 112.0, 75.6, 62.0, 56.1, 18.7; HRMS: (APCI) [M + H]$^+$ calculated for C$_{11}$H$_{12}$BrO$_2$: 255.0021, found: 255.0013.

3.1.4.3 General procedure for the coupling reaction between terminal alkynes and bromoalkynes (9a, 9b). A solution of the bromoalkyne (1 equiv.) in THF (0.4 M) was sparged with argon over 20 min and then PdCl$_2$(PPh$_3$)$_2$ (0.03 equiv.), CuI (0.03 equiv.) and diisopropylamine (2 equiv.) were added sequentially at room temperature, followed immediately by the terminal alkyne (1.2 equiv.). The reaction mixture was stirred at room temperature for 12 h and then concentrated under reduced pressure.

3.1.4.3.1 (Z)-9-Hydroxy-2-nonene-4,6-diyn-1-oxyl hexanoate (9a). Following the general procedure using the bromoalkyne ester **8a** (20 mg, 0.077 mmol), the 3-butyn-1-ol (7.1 μL, 0.093 mmol), PdCl$_2$(PPh$_3$)$_2$ (1.6 mg, 0.002 mmol), CuI (0.4 mg, 0.002 mmol), diisopropylamine (21.6 μL, 0.154 mmol) and THF (0.7 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **9a** (11.2 mg, 59%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 6.16–6.12 (m, 1H), 5.68 (d, J = 10.8 Hz, 1H), 4.83 (d, J = 6.6 Hz, 2H), 3.78 (t, J = 6.0 Hz, 2H), 2.62 (t, J = 6.0 Hz, 2H), 2.32 (t, J = 7.2 Hz, 2H), 1.64 (dt, J = 15.6, 7.8 Hz, 2H), 1.34–1.27 (m, 4H), 1.25 (s, 1H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 173.5, 140.2, 111.5, 82.6, 80.3, 70.6, 66.6, 62.2, 60.7, 34.1, 31.3, 24.6, 24.0, 22.3, 13.9; HRMS: (APCI) [M − H]$^-$ calculated for C$_{15}$H$_{19}$O$_3$: 247.1334, found: 247.1339.

3.1.4.3.2 (2E,4E)-(Z)-9-Hydroxy-2-nonene-4,6-diyn-1-oxyl-2,4-hexadienoate (9b). Following the general procedure using the bromoalkyne ester **8b** (21.0 mg, 0.082 mmol), the 3-butyn-1-ol (7.5 μL, 0.099 mmol), PdCl$_2$(PPh$_3$)$_2$ (1.7 mg, 0.003 mmol), CuI (0.5 mg, 0.003 mmol), diisopropylamine (23.2 μL, 0.164 mmol) and THF (0.8 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 4/1) to afford **9b** (11.0 mg, 58%) as a pale colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.30–7.27 (m, 1H), 6.22–6.13 (m, 3H), 5.78 (d, J = 15.0 Hz, 1H), 5.68 (d, J = 10.8 Hz, 1H), 4.90 (d, J = 6.0 Hz, 2H), 3.78 (d, J = 6.0 Hz, 2H), 2.62 (d, J = 6.0 Hz, 2H), 1.86 (d, J = 6.0 Hz, 3H), 1.83 (s, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 166.9, 145.7, 140.3, 139.8, 129.7, 118.3, 111.4, 82.6, 80.3, 70.7, 66.6, 62.2, 60.7, 24.0, 18.7; HRMS: (APCI) [M + H]$^+$ calculated for C$_{15}$H$_{17}$O$_3$: 245.1178, found: 245.1171.

3.1.5 Preparation of ether 11

3.1.5.1 (Z)-16,16-Dimethyl-15,15-diphenyl-2,4,14-trioxa-15-silanyl-11-heptadecene-7,9-diyne (11-1). To a stirred solution of **6a** (25 mg, 0.064 mmol) in dry DCM (2 mL) was added dimethoxymethane (57.0 μL, 0.643 mmol), then the reaction mixture was cooled to 0 °C, phosphorous pentoxide (45.7 mg, 0.322 mmol) was added to the reaction mixture. The mixture was warmed to room temperature and stirred for 16 h. The resulting mixture was diluted with DCM, washed with saturated NaHCO$_3$, dried with anhydrous K$_2$CO$_3$, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel

column chromatography (PE/EtOAc = 20/1) to afford **11-1** (15.0 mg, 54%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.68–7.67 (m, 4H), 7.44–7.38 (m, 6H), 6.21–6.17 (m, 1H), 5.48 (d, J = 10.8 Hz, 1H), 4.64 (s, 2H), 4.47 (d, J = 5.4 Hz, 2H), 3.65 (t, J = 7.2 Hz, 2H), 3.36 (s, 3H), 2.62 (t, J = 6.6 Hz, 2H), 1.05 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 146.1, 135.6, 133.5, 129.7, 127.7, 108.0, 96.4, 82.0, 79.5, 71.2, 66.0, 65.4, 62.5, 55.3, 26.8, 21.2, 19.2; HRMS: (APCI) [M + H]$^+$ calculated for C$_{27}$H$_{33}$O$_3$Si: 433.2199, found: 433.2192.

3.1.5.2 (Z)-9-(Methoxymethoxy)nona-2-en-4,6-diyn-1-ol (11). Following the general procedure using **11-1** (15.0 mg, 0.035 mmol), triethylamine trihydrofluoride (69.6 μL, 0.431 mmol) and THF (0.5 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 4/1) to afford **11** (2.9 mg, 43%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 6.24–6.20 (m, 1H), 5.60 (d, J = 11.4 Hz, 1H), 4.66 (s, 2H), 4.41 (d, J = 4.2 Hz, 2H), 3.68 (t, J = 6.6 Hz, 2H), 3.39 (s, 3H), 2.65 (t, J = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 145.1, 109.6, 96.5, 82.6, 79.8, 70.9, 65.8, 65.3, 61.2, 55.4, 29.7; HRMS: (APCI) [M − H]$^-$ calculated for C$_{11}$H$_{13}$O$_3$: 193.0865, found: 193.0866.

3.1.6 Preparation of ester 12

3.1.6.1 (Z)-9-((tert-Butyldiphenylsilyl)oxy)-7-nonene-3,5-diyn-1-oxyl acetate (12-1). Acetic anhydride (0.32 mL, 3.42 mmol) was added dropwise to a solution of **5a** (0.2 g, 2.85 mmol), DMAP (34.9 mg, 0.28 mmol) and triethylamine (0.8 mL, 5.71 mmol) in 30 mL of dry DCM. After stirring for 16 h at room temperature, the reaction mixture was poured into a separatory funnel containing 30 mL of 10% NH$_4$Cl. The organic phase was isolated, and the aqueous phase was extracted with CH$_2$Cl$_2$ (30 mL × 3). All the organic phases were combined, washed with brine, dried over anhydrous Na$_2$SO$_4$, and the filtrate was concentrated under reduced pressure to give a crude oil. The resulting residue was purified by silica gel column chromatography (PE/EtOAc = 30/1) to afford **12-1** (95.3 mg, 43%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69–7.67 (m, 4H), 7.45–7.38 (m, 6H), 6.21 (dt, J = 11.4, 6.0 Hz, 1H), 5.49 (d, J = 10.8 Hz, 1H), 4.47 (d, J = 6.0 Hz, 2H), 4.16 (t, J = 6.6 Hz, 2H), 2.66 (t, J = 6.6 Hz, 2H), 2.07 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 170.7, 146.4, 135.6, 133.4, 129.7, 127.7, 107.9, 80.7, 79.2, 71.5, 66.4, 62.5, 61.7, 26.8, 20.8, 20.1, 19.1; HRMS: (APCI) [M + H]$^+$ calculated for C$_{27}$H$_{31}$O$_3$Si: 431.2042, found: 431.2036.

3.1.6.2 (Z)-9-Hydroxy-7-nonene-3,5-diyn-1-oxyl acetate (12). Following the general procedure using **12-1** (22.0 mg, 0.051 mmol), triethylamine trihydrofluoride (0.1 mL, 0.627 mmol) and THF (0.7 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **12** (4.8 mg, 49%) as a pale yellow oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 6.26–6.22 (m, 1H), 5.60 (d, J = 10.8 Hz, 1H), 4.43 (d, J = 6.0 Hz, 2H), 4.19 (t, J = 6.6 Hz, 2H), 2.69 (t, J = 6.6 Hz, 2H), 2.09 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ: 170.8, 145.4, 109.4, 81.2, 79.6, 71.2, 66.2, 61.6, 61.2, 29.7, 20.8; HRMS: (APCI) [M + H]$^+$ calculated for C$_{11}$H$_{13}$O$_3$: 193.0865, found: 193.0858.

3.1.7 Preparation of ether 13

3.1.7.1 (Z)-((9-Methoxy-2-nonene-4,6-diyn)-1-oxyl)tert-butyldiphenylsilane (13-1). A solution of **6a** (25 mg, 0.064 mmol) and methyl iodide (80.1 μL, 1.287 mmmol) in dry THF (0.5 mL) was cooled to 0 °C, then sodium hydride (60% dispersion in mineral

oil, 3 mg, 0.077 mmol) was added portionwise over 10 min. After 5 min more, the cooling was removed and the reaction mixture was stirred at room temperature for 3 h, H_2O (1 mL) was added to the reaction flask and the resulting solution was extracted with EtOAc (1 mL × 3), the combined extracts were washed with saturated NaCl solution (1 mL × 2), dried over anhydrous Na_2SO_4 and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE/EtOAc = 20/1) to afford **13-1** (12.0 mg, 46%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.69–7.68 (m, 4H), 7.45–7.39 (m, 6H), 6.19 (dt, J = 11.4, 6.0 Hz, 1H), 5.48 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 3.51 (t, J = 7.2 Hz, 2H), 3.37 (s, 3H), 2.59 (t, J = 6.6 Hz, 2H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 146.1, 135.6, 133.5, 129.7, 127.7, 108.0, 82.0, 79.5, 71.2, 70.1, 65.9, 62.5, 58.7, 266.8, 20.9, 19.1; HRMS: (APCI) [M − H]$^-$ calculated for $C_{26}H_{29}O_2Si$: 401.1937, found: 401.1929.

3.1.7.2 (Z)-9-Methoxy-2-nonene-4,6-diyn-1-ol (13). Following the general procedure using **13-1** (20.0 mg, 0.050 mmol), triethylamine trihydrofluoride (0.1 mL, 0.615 mmol) and THF (0.7 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 4/1) to afford **13** (4.0 mg, 49%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.24–6.20 (dt, J = 11.4, 6.6 Hz, 1H), 5.60 (d, 10.8 Hz, 1H), 4.42 (d, J = 6.0 Hz, 2H), 3.53 (t, J = 6.6 Hz, 2H), 3.38 (s, 3H), 2.62 (t, J = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 145.1, 109.6, 82.5, 79.8, 70.9, 70.1, 65.7, 61.2, 58.8, 29.7; HRMS: (APCI) [M + H]$^+$ calculated for $C_{10}H_{13}O_2$: 165.0916, found: 165.0909.

3.1.8 Preparation of enyne analogue 16

3.1.8.1 (3E,7Z)-9-((tert-Butyldiphenylsilyl)oxy)-3,7-nonadien-5-yn-1-ol (15). In a two-neck flask filled with argon, Cp_2ZrCl_2 (1.25 g, 4.276 mmol) was placed in of dry THF (7 mL). The mixture was cooled in an ice bath, and DIBAL-H (1 M, 3.4 mL, 3.4 mmol) was added. The reaction mixture turned a pale pink and it was allowed to stir for 1 h. In a second flask filled with argon, a solution of 3-butyn-1-ol (0.2 g, 2.85 mmol) in dry THF (2.3 mL) was chilled in an ice bath, and DIBAL-H (1 M, 2.9 mL, 2.9 mmol) was added to this solution. The resulting yellow solution was allowed to stirred under argon for 1 h. Then the 3-butyn-1-ol solution was added *via* syringe into the prepared zirconium hydride reagent. The reaction mixture was allowed to warm to room temperature, and then to stirred for 5 hours. The mixture was cooled to −78 °C, and to that, crystalline iodine (869 mg, 3.424 mmol) was added. The reaction mixture stirred overnight and then water (4 mL) was added. After stirring for 2.5 hours, the mixture was poured into an aqueous solution prepared from sat. $Na_2S_2O_3$: sat. $NaHCO_3$ (2 : 1). The aqueous layer was extracted with EtOAc (2 mL × 3), and the combined extracts were washed with saturated NaCl solution (3 mL × 2), dried over anhydrous Na_2SO_4 and the filtrate was concentrated under reduced pressure to give a crude oil, which was used directly in the next step.

A solution of the crude compound vinyl iodide (43 mg, 0.217 mmol) in THF (1 mL) was sparged with argon over 10 min and then $PdCl_2(PPh_3)_2$ (1.5 mg, 0.002 mmol), CuI (0.4 mg, 0.002 mmol) and triethylamine (60.2 μL, 0.434 mmol) were sequentially added at 0 °C. Followed immediately by (Z)-(2-pentene-4-yn-1-oxyl)*tert*-butyldiphenylsilane (76.7 mg, 0.239 mmol). The

stirred reaction mixture was allowed to warm to room temperature over 12 h and then filtered through silica and washed with diethyl ether. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE/EtOAc = 5/1) to afford **15** (35.0 mg, 41%, 2 steps) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.70–7.66 (m, 4H), 7.44–7.36 (m, 6H), 6.09–6.05 (m, 1H), 5.97–5.92 (m, 1H), 5.63 (d, J = 10.8 Hz, 1H), 5.59 (d, J = 12.6 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H), 3.67 (t, J = 6.0 Hz, 2H), 2.36 (q, J = 6.6 Hz, 2H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 141.8, 140.1, 135.6, 135.4, 133.6, 129.6, 127.7, 127.6, 112.4, 109.2, 62.5, 61.5, 36.4, 26.8, 19.2; HRMS: (APCI) [M + H]$^+$ calculated for $C_{25}H_{31}O_2Si$: 391.2093, found: 391.2083.

3.1.8.2 (2Z,6E)-2,6-Nonadien-4-yne-1,9-diol (16). Following the general procedure using **15** (30.0 mg, 0.077 mmol), triethylamine trihydrofluoride (0.15 mL, 0.947 mmol) and THF (1.1 mL), the resulting residue was purified by silica gel column chromatography (PE/EtOAc = 2/1) to afford **16** (5.1 mg, 44%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.15 (dt, J = 15.6, 7.2 Hz, 1H), 6.07 (dt, 10.8, 6.0 Hz, 1H), 5.72 (dd, J = 16.2, 10.8 Hz, 2H), 4.41 (d, J = 5.4 Hz, 2H), 3.71 (t, J = 6.0 Hz, 2H), 2.41 (q, J = 6.0 Hz, 2H), 1.56 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 140.8, 140.8, 112.2, 110.8, 93.7, 84.3, 61.5, 61.1, 36.4; HRMS (APCI) [M − H]$^-$ calculated for $C_9H_{11}O_2$: 151.0759, found: 151.0752.

3.2 Biology assay

3.2.1 Materials.
Corticosterone (purity $\geqq$ 98%) was purchased from TCI Shanghai (Shanghai, China). Fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco (Grand Island, USA), RPMI-1640 was purchased from HyClone (GE, USA). Primary antibodies for β-actin, cytochrome C, Caspase-3, Bcl-2, Bax were purchased from Cell Signaling (CST, USA). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), poly-L-polylysine (PLL) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Agomelatine was purchased from Energy Chemical.

3.2.2 Cell culture and treatment.
The differentiated PC12 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and were cultured in RPMI-1640 contained 10% (v/v) heat-inactivated FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Cells were cultured in a 5% CO_2 atmosphere at 37 °C. Cells in the exponential phase of growth were used for all experiments. To study the cytoprotective effects of compounds, PC12 cells were divided into non-treated control, corticosterone (400 μmol L^{-1}) and corticosterone (400 μmol L^{-1}) plus compound groups for all experiments. Compounds were applied 3 h prior to treatment with corticosterone.

3.2.3 Cell viability assay.
Cell viability was measured by MTT assay. PC12 cells (2 × 10^4 per well) were seeded in 96-well plates, which were coated with poly-L-lysine (PLL, 0.01%). After 24 h incubation, PC12 cells were treated with corticosterone (400 μmol L^{-1}) or corticosterone (400 μmol L^{-1}) plus various concentrations of compounds (10/20/50 μmol L^{-1}), for 24 h. Following incubation, 10 μL MTT (5 mg mL^{-1}) were added to each well. After 4 h at 37 °C, the culture medium was removed

and 100 μL DMSO was added to dissolve the formazan crystals. Absorbance was measured at a test wavelength of 570 nm with a microplate reader (BioTek, USA), and the cell viability was expressed as a percentage of the value against the no treated control group.

3.2.4 Western blot analyses. PC12 cells (2×10^6 per dishes) were seeded on 100 mm dishes. At the end of the treatments, the PC12 cells were harvested and washed twice with cold PBS. The cells were lysed with RIPA lysis buffer (Beyotime, China) containing 1% phenylmethylsulfonylfluoride (PMSF, Beyotime, China). The whole-cell lysates were centrifuged at 12 000 rpm min^{-1} for 15 min at 4 °C, and the supernatants were collected. Protein concentrations were determined by bicinchoninic acid assay. Equal amounts of protein (50 μg) were separated by electrophoresis on 12% sodium dodecyl sulphate poly-acrylamide gels and transferred onto PVDF membranes. These membranes were incubated with 5% (w/v) non-fat milk powder in tris-buffered saline containing 0.1% (v/v) Tween-20 (TBST) for 2 h to block nonspecific binding sites. The membranes were then incubated overnight at 4 °C with the primary antibodies. After washing with TBST, the membranes were incubated for 2 h at room temperature with the fluorescent secondary antibodies. After rewashing with TBST, the membranes were scanning by fluorescent scanner (Odclyssey CLX, Gene Company limited, USA).

3.3 General procedures for behavioral studies

Kunming mice were obtained from the experimental animal center of Military Medical Science Academy of the PLA, China. All animal experiments were done in accordance with the National Guidelines for Experimental Animal Welfare (MOST, PR China, 2006), which had full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC Intl.). This study was approved by the Committee on the Ethics of Animal Experiments of Shanxi University. Maximal effort was made to minimize animal suffering and the number of animals necessary for the acquisition of reliable data. All mice in this study were treated humanely throughout the experimental period. Compound **7a** was synthesized as described above, and Venlafaxine hydrochloride capsules were purchased from Chengdu Kanghong Pharmaceutical Group Co., Ltd. The Venlafaxine hydrochloride was administered intragastrically (IG). Compound **7a** was dissolved with a small amount of DMSO and diluted with sterile water for injection before use. The final concentration of DMSO is less than 0.1%. Compound **7a** was administered by intra-peritoneal (IP) injection. The purified water was administered by intraperitoneal (IP) injection. The mice were assigned into 5 groups ($n = 10$); venlafaxine hydrochloride group (50 mg kg^{-1}), group, **7a** group (0.0125 mg kg^{-1}), **7a** group (0.025 mg kg^{-1}), **7a** group (0.05 mg kg^{-1}). Mice were individually placed into clear glass cylinders (diameter 20 cm, height 50 cm) containing 25 ± 1 °C water 10 cm deep. Mice were placed in the water and forced to swim for 6 min. The time the animal spent immobile was recorded over a 4 min trial. Immobility was defined as the postural position of floating in the water.

3.4 Statistical analysis

All values were expressed as mean ± S.D. A two-tailed unpaired t-test by SPSS 16.0 (Chicago, IL, USA) was applied to analyze those significant differences between two groups, and the significance threshold was considered at $p < 0.001$.

4. Conclusions

In summary, based on the structure of natural product RB-2, sixteen 1,3-diynes compounds were designed and synthesized. Subsequently, their protective effects on corticosterone-injured PC12 cells were evaluated. Five compounds showed significant protective activity in this assay. The SAR studies suggested that the 1,3-diyne and the allyl alcohol motifs were crucial to the protective activity. Moreover, the protective activity of compound **7a** was shown to be associated with the regulation of apoptosis-related proteins. Finally, the mice forced swim test showed that **7a** had a concentration-dependent antidepressant-like effect. Together, our findings supported that compound **7a** is a promising lead candidate that deserves further evaluation in the design of antidepressants with unique scaffolds.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Notes and references

1 T. Rodrigues, D. Reker, P. Schneider and G. Schneider, *Nat. Chem.*, 2016, **8**, 531–541.

2 D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2016, **79**, 629–661.

3 A. L. Shi Shun and R. R. Tykwinski, *Angew. Chem., Int. Ed. Engl.*, 2006, **45**, 1034–1057.

4 W. Heydenreuter, E. Kunold and S. A. Sieber, *Chem. Commun.*, 2015, **51**, 15784–15787.

5 L. F. Liang, T. Wang, Y. S. Cai, W. F. He, P. Sun, Y. F. Li, Q. Huang, O. Taglialatela-Scafati, H. Y. Wang and Y. W. Guo, *Eur. J. Med. Chem.*, 2014, **79**, 290–297.

6 H. Kim, J. Chin, H. Choi, K. Baek, T. G. Lee, S. E. Park, W. Wang, D. Hahn, I. Yang, J. Lee, B. Mun, M. Ekins, S. J. Nam and H. Kang, *Org. Lett.*, 2012, **15**, 100–103.

7 B. Zhang, Y. Wang, S. P. Yang, Y. Zhou, W. B. Wu, W. Tang, J.-P. Zuo, Y. Li and J. M. Yue, *J. Am. Chem. Soc.*, 2012, **134**, 20605–20608.

8 Z. Zhang, C. Lu, X. Liu, J. Su, W. Dai, S. Yan, A. Lu and W. Zhang, *J. Proteome Res.*, 2013, **13**, 925–933.

9   Z. F. Zhou, M. Menna, Y. S. Cai and Y. W. Guo, *Chem. Rev.*, 2015, **115**, 1543–1596.

10 J. Liu, Y. Fang, L. Yang, X. Qin, G. Du and X. Gao, *J. Pharm. Biomed. Anal.*, 2015, **111**, 257–265.

11 O. Berton and E. J. Nestler, *Nat. Rev. Neurosci.*, 2006, **7**, 137–151.

12 B. Haenisch and H. Bönisch, *Pharmacol. Ther.*, 2011, **129**, 352–368.

13 F. Artigas, *ACS Chem. Neurosci.*, 2013, **4**, 5–8.

14 D. M. Gerhard, E. S. Wohleb and R. S. Duman, *Drug Discovery Today*, 2016, **21**, 454–464.

15 D. Vaudry, P. Stork, P. Lazarovici and L. Eiden, *Science*, 2002, **296**, 1648–1649.

16 Y. F. Li, Y. Q. Liu, W. C. Huang and Z. P. Luo, *Acta Pharmacol. Sin.*, 2003, **24**, 996–1000.

17 B. Zeng, Y. Li, B. Niu, X. Wang, Y. Cheng, Z. Zhou, T. You, Y. Liu, H. Wang and J. Xu, *J. Mol. Neurosci.*, 2016, **59**, 567–578.

18 W. Ang, G. Chen, L. Xiong, Y. Chang, W. Pi, Y. Liu, C. Li, J. Zheng, L. Zhou, B. Yang, Y. Deng, S. Yang, Y. Luo and Y. Wei, *Eur. J. Med. Chem.*, 2014, **82**, 263–273.

19 Y. Yang, W. Ang, H. Long, Y. Chang, Z. Li, L. Zhou, T. Yang, Y. Deng and Y. Luo, *Sci. Rep.*, 2016, **6**, 34711.

20 K. Q. Ma, Y. H. Miao, X. X. Gao, J. B. Chao, X. Zhang and X. M. Qin, *Chin. Chem. Lett.*, 2017, DOI: 10.1016/j.cclet.2016.11.032.

21 D. J. Galler and K. A. Parker, *Org. Lett.*, 2015, **17**, 5544–5546.