Design and Synthesis of Dimeric Securinine Analogues with Neuritogenic Activities

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Ⓢ Supporting Information

ABSTRACT: Neurite outgrowth is crucial during neuronal development and regeneration, and strategies that aim at promoting neuritogenesis are beneficial for reconstructing synaptic connections after neuronal degeneration and injury. Using a bivalent analogue strategy as a successful approach, the current study identifies a series of novel dimeric securinine analogues as potent neurite outgrowth enhancers. Compounds **13**, **14**, **17−19**, and **21−23**, with different lengths of carbon chain of N,N-dialkyl substituting diacid amide linker between two securinine molecules at C-15 position, exhibited notable positive effects on both neuronal differentiation and neurite extension of neuronal cells. Compound **14**, one of the most active compounds, was used as a representative compound for mechanistic studies. Its action on neurite outgrowth was through phosphorylation/activation of multiple signaling molecules including Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), extracellular signal-regulated kinase (ERK) and Akt. These findings collectively identify a new group of beneficial compounds for neuritogenesis, and may provide insights on drug discovery of neural repair and regeneration.

KEYWORDS: Bivalent analogue strategy, neurite, neuronal differentiation, securinine

Neurite outgrowth is a crucial process during neuronal development and regeneration as it is a primarily necessary step to construct the neuronal network.[1] Collapse of this step may cause neuronal apoptosis that leads to neurodegenerative diseases. In Alzheimer's and Parkinson's diseases, neurite atrophy is among the typical early symptoms during the progressive degeneration of neurons.[2] Furthermore, Neurite loss is one of the typical features of neuronal injury. Reconstruction of the neuronal and synaptic network in the degenerated or injured brain is necessary for restoration of brain functions. Therefore, strategies that aim at promoting neurite outgrowth and reconstructing synaptic connections are essential for treatment of neurodegenerative diseases and various forms of neuronal injury.[3,4] So far, a wide variety of natural products and their derivatives have been found as neurite outgrowth promoting agents and are studied for neural regeneration.[5] Only in the recent 2 years, our group and others have reported a number of natural neuritogenic compounds including diarylheptanoids from *Alpinia officinarum*, isofuranodiene from wild celery, and a natural aroma compound maltol.[6−8]

Securinega alkaloids are a group of bridged tetracyclic natural products isolated from the plants of *Securinega*, *Phyllanthus*, and *Fluegggea* genera which belong to the Euphorbiaceae family.[9−11] (−)-Securinine (compound **1**, Figure 1), the most widespread
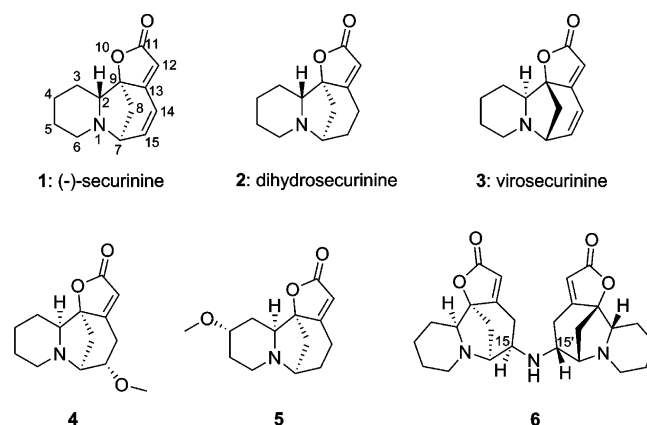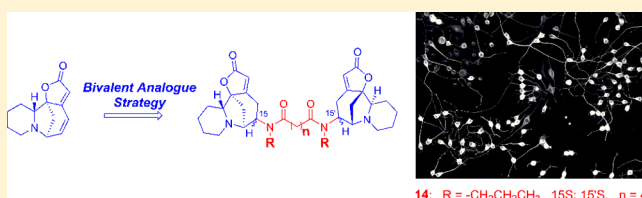

Figure 1. Chemical structures of natural securinine analogues.

and abundant *Securinega* alkaloid, has been attracting worldwide attention because of its strong neural activities and its property to cross the blood-brain barrier.[12−18] (−)-Securinine has been suggested to act as a γ-aminobutyric acid (GABA) receptor antagonist

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and may thus facilitating excitatory synaptic transmission, whereas its close relatives dihydrosecurinine and virosecurinine (compounds **2** and **3**, Figure 1) do not show similar activities.[19−21] It has also been reported that (−)-securinine shows neuroprotective activity against neurotoxicity induced by amyloid-β, a main toxic agent of Alzheimer's disease, and facilitates learning and memory.[22,23] So far, over 90 *Securinega* alkaloids have been isolated from natural source,[19−21] and several attempts have been made to obtain different securinine analogues which show antiproliferative effects on tumor cell lines and antioxidative/protective effects on neurons.[24−26] However, whether these alkaloids exert effects on neuronal development and neurite outgrowth has not been explored.

Our group has been focusing on the isolation and activity characterization of *Securinega* alkaloids.[27,28] In the present study, we compared the effects of (−)-securinine and several other natural securinine analogues (compounds **4**−**6**) on neurite development and identified that a dimeric securinine-type molecule linked by an amino group (compound **6**) showed neurite outgrowth-promoting activity. By using bivalent ligand approach,[29−32] a useful protocol in medicinal chemistry and drug design, a series of novel securinine-type dimeric analogues were synthesized based on the structure of compound **6**. Eight of these dimeric securinine analogues (compounds **13**, **14**, **17**−**19**, and **21**−**23**) successfully showed strong neuritogenic activities. One of the most active compounds, **14**, was selected as the lead compound and its molecular action was studied.

■ RESULTS AND DISCUSSION

A Natural Dimeric Securinine Analogue Shows Neuritogenic Activity. We used mouse neuroblastoma (Neuro-2a) cells, a neuronal cell line for studying neurite growth in vitro, to test the activities of (−)-securinine and other natural securinine analogues. We found (−)-securinine (compound **1**) and two monomeric securinine analogues (compounds **4** and **5**) showed minimal effects on neurite outgrowth (Figure 2). In contrast, a dimeric analogue (compound **6**) exhibited potent activities on promoting both neurite formation and extension (Figure 2). These results indicate that a structure containing two securinine moieties may possess potential activities on neurite outgrowth.

Synthesis of Dimeric Securinine Analogues. The basic design strategy of our further study is based on the natural dimeric securinine analogue compound **6** (Figure 3). To explore the potential of dimeric symmetrical ligands, the securinine portion was designed as an essential structure for our bivalent
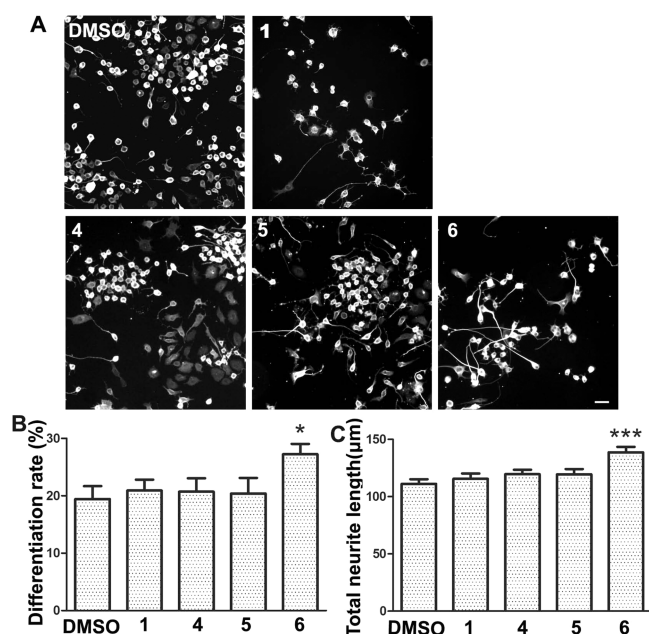
Figure 2. Natural securinine analogues were examined for neuritogenic activities. (A) Neuro-2a cells were treated with different compounds (25 μM) for 48 h. Scale bar, 50 μm. The differentiation rate (B) and the total neurite length of each differentiated cell (bearing at least one neurite longer than 40 μm; C) were analyzed. At least 400 cells/group were analyzed in each experiment ($n = 5$), and values were presented as mean ± SEM. *$P < 0.05$, ***$P < 0.001$, indicated compound vs DMSO, one-way analysis of variance (ANOVA) followed by Tukey's test.
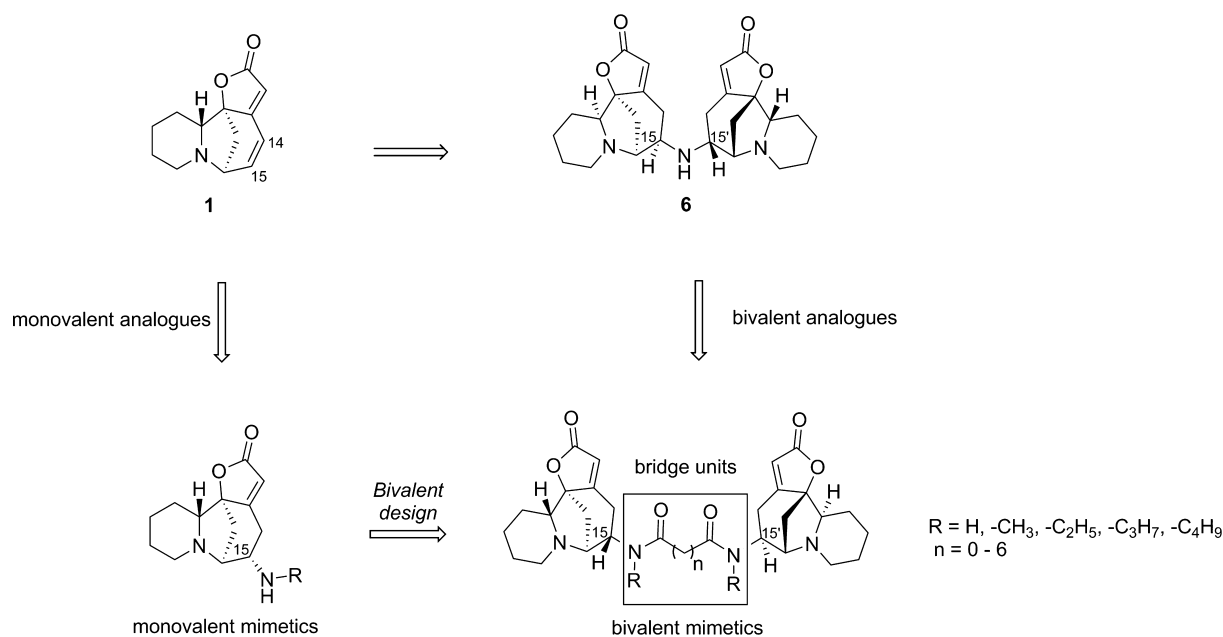
Figure 3. Design of the novel dimeric securinine analogues.

Scheme 1. Synthesis of Compounds 7−33[a]

7: R = H; 15S; 15'S n = 0   15: R = n-C$_4$H$_9$; 15S; 15'S n = 4
8: R = H; 15S; 15'S n = 1   16: R = n-C$_5$H$_{11}$ 15S; 15'S n = 4
9: R = H; 15S; 15'S n = 2   17: R = n-C$_3$H$_7$; 15S; 15'S n = 2
10: R = H; 15S; 15'S n = 3   18: R = n-C$_3$H$_7$; 15S; 15'S n = 3
11: R = H; 15S; 15'S n = 4   19: R = n-C$_3$H$_7$; 15S; 15'S n = 5
12: R = CH$_3$; 15S; 15'S n = 4 20: R = n-C$_3$H$_7$; 15S; 15'S n = 6
13: R = C$_2$H$_5$; 15S; 15'S n = 4 21: R = n-C$_3$H$_7$; 15R; 15'R n = 4
14: R = n-C$_3$H$_7$;15S; 15'S n = 4 22: R = n-C$_3$H$_7$; 15R; 15'S n = 4

25: R = CH$_3$;   15S   28: R = n-C$_4$H$_9$; 15S
26: R = C$_2$H$_5$;   15S   29: R = n-C$_5$H$_{11}$; 15S
27: R = n-C$_3$H$_7$; 15S   30: R = n-C$_3$H$_7$; 15R

27: 15S
30: 15R

32: 15S
33: 15R

[a]Reagents and conditions: (a) azidotrimethylsilane, DBU, AcOH, CH$_2$Cl$_2$; (b) Pd/C, H$_2$, CH$_2$Cl$_2$; 42% in two steps; (c) amine, potassium phosphate, CH$_2$Cl$_2$, MeOH, 68%−78%; (d) diacyl chlorides, DIPEA, CH$_2$Cl$_2$, 24%−79%; (e) propylamine, potassium phosphate, CH$_2$Cl$_2$, MeOH, 66%; (f) adipoyl chloride, DIPEA, CH$_2$Cl$_2$, 73%; (g) propionyl chloride, DIPEA, CH$_2$Cl$_2$, 94% and 92%.

mimicking. Additional consideration on the high synthetic efficiency of amide-fragment, various diacid amide chains were embedded into the dimeric securinine analogues as the bridge units. Our hypothesis is that the bivalent mimetics may exhibit more potent neurite-promoting activities when compared to the monovalent mimetics.

Chemistry. The chemical synthesis of compounds 7−23 with a parallel synthetic approach is shown in Scheme 1. Compound 24 was prepared in two steps from (−)-securinine (1) in 42% yield. The Michael addition reaction of (−)-securinine (1) with appropriate primary amine in the presence of potassium phosphate afforded the intermediates 25−30 (68%−78% yields), respectively. While treatment of virosecurinine (3) with propylamine provided 31 in 66% yield. Subsequently, compounds 7−23 were synthesized by the acylation reaction of diacyl chlorides with the corresponding compounds 24−31 (24%−79% yields), respectively. Compounds 32 and 33 were obtained by the acylation reaction of propionyl chloride with 27 and 30 in 94% and 92% yields, respectively.

In Vitro Evaluation of Synthetic Compounds on Neurite Outgrowth. Neuro-2a cells were treated with compounds 7−23, 27, 32 and 33 (25 μM) for 48 h. The differentiation rate and the length of total neurites of each cell were analyzed by automated fluorescent microscopy and mathematical algorithms using high content screening technology.[34,35] Compounds 7−11 which possess different lengths of the carbon chain were found not obviously active for increasing neurite formation (Figure 4). The result suggests that modifying the length of carbon chain of the diacid amide linker alone is not sufficient to improve the activities of the dimeric securinine analogues. By introducing alkyl groups on the N atoms of compound 11, whose linker was envisioned as the most

appropriate space distance of the bivalent mimetic, we obtained compounds 12 (with methyl side chains) and 13 (with ethyl side chains). Interestingly, compound 13 with ethyl side chains showed significant activities on neurite outgrowth (37.5 ± 1.8% differentiated cells and 145.8 ± 4.6 μm total neurite length). We further increased the length of the side chains to 3, 4, or 5 carbons on N atoms of compound 11, leading to the discovery of compounds 14−16. Compound 14, the one with propyl side chains, showed even stronger activities on neurite outgrowth (40.8 ± 2.8% differentiated cells and 170.9 ± 7.2 μm total neurite length). Compound 15 with n-butyl side chains also showed activities on neurite extension but did not significantly induce more cells to differentiation. Interestingly, compound 16 with n-amyl side chains did not exhibit any activities (Figure 4). Collectively, the comparison among compounds 11−16 indicated that the optimal length of the side chains on N atoms is 2−4 carbons, and compound 14 with 3 carbons appeared to be the most active one in this series.

To further understand whether the linker length is important to compound 14's activity, we compared it with compounds 17−20 which bear different lengths of linkers while the N-propyl groups are retained. Compounds 17−19, which possess N^1,N^4-dipropylsuccinamide, N^1,N^5-dipropylglutaramide, and N^1,N^7-dipropylheptanediamide units, exhibited similar activities as 14. However, compound 20 with a N^1,N^8-dipropyloctanediamide unit lost both activities on differentiation and neurite extension. The results indicate that the linker length of two monomers is indeed important for neurite-inducing activity and the preferred length of the linker is 4−7 carbons (compounds 14, 17−19). Either shortening or elongating the linker dampened this activity. Interestingly, the specific stereoconformations were also examined by comparing compound 14 with its several stereoisomers
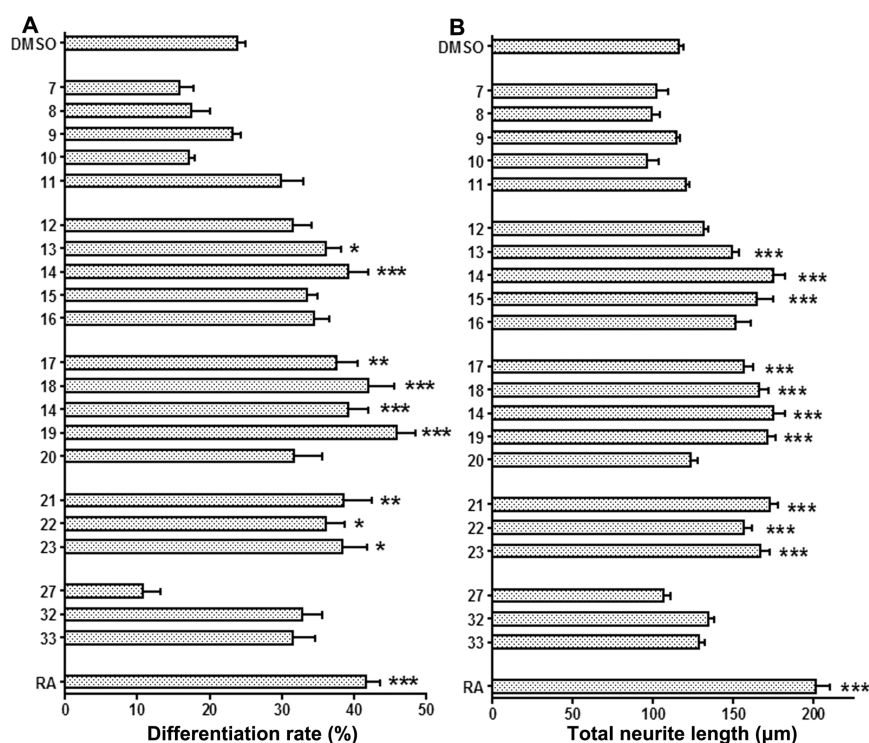
Figure 4. Evaluation of neuritogenic activities of synthetic bivalent securinine mimetics and their intermediates. Differentiation rate (A) and total neurite length (B) of the bivalent mimetics and monomer intermediates. All values were determined at 25 μM except the control compound retinoic acid (RA), which was used at 10 μM. Neuro-2a cells were incubated with different compound for 48 h. Neurites were visualized by immunostaining with anti-β-tubulin III antibody, and analyzed by high content technology. At least 400 cells/group were analyzed in each experiment ($n = 5$), and values were presented as mean $\pm$ SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, indicated compound vs DMSO, one-way ANOVA followed by Tukey's test.

(compounds 21−23), and similar activities were observed among these analogues. The intermediate compounds 27, 32, and 33 were confirmed with no activities, which were consistent with our hypothesis that the presence of bivalent mimetics is necessary for neurite-inducing activities of the dimeric securinine analogues.

Molecular Mechanism Study of Compound 14. To further dissect the molecular mechanism of compound 14, we tested different concentrations of 14 (1−100 μM) to determine its preferable dosage range on neuritogenesis. The results showed that a minimum of 10 μM was required to promote neuritogenesis, and increasing neurite length was observed when the concentration increased from 1 to 100 μM (Figure 5). However, 25 μM appeared to be enough for inducing differentiation, as increasing the concentration did not further induce more cells to differentiate (Figure 5). We thus used 25 μM in subsequent studies.

Neuronal differentiation requires activation of multiple signaling pathways, including mitogen-activated protein kinase (MAPK) pathways, phosphoinositide 3-kinase (PI3K)-Akt pathways and Ca^{2+} dependent pathways.[36−39] To understand what signaling pathways participate in compound 14 induced neuronal differentiation, we treated Neuro-2a cells with 14 for different time points (0−120 min) and examined the levels of activated/phosphorylated forms of different signaling molecules (Figure 6). Notably, compound 14 markedly activated extracellular signal regulated kinases 1/2 (ERK1/2) at 15 min treatment and thereafter. Examination of other two classical MAPK pathways revealed that c-Jun amino-terminal kinases (JNK) was only transiently activated at 5 min treatment, and p38 activity was not affected by compound 14. Besides upregulating ERK1/2 pathways, compound 14 also induced significant

activation of Akt and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) after 15 min treatment.

To further determine the important signaling molecules that are required for compound 14 promoted neurite outgrowth, we took advantage of a series of pharmacological inhibitors, including U0126 (MEK1/2 inhibitor), SP600125 (JNK inhibitor), SB202190 (p38 inhibitor), LY294002 (PI3K inhibitor), KN92 (CaMKII inhibitor control), KN93 (CaMKII inhibitor), NSC23766 (Rac1 inhibitor), H89 (protein kinase A; PKA inhibitor), and chelerythrine (protein kinase C; and PKC inhibitor). We preincubated Neuro-2a cells with each of these inhibitors for 1 h before the treatment of compound 14, and the differentiation rate and the total neurite length were examined (Figure 7). Consistent with the Western blot analysis that ERK, Akt, and CaMKII were activated by compound 14, the activities of ERK upstream kinases MEKs (MAPK/ERK kinase) and the Akt upstream kinase PI3K were both essentially required for compound 14 induced neurite outgrowth (Figure 7). Furthermore, Rac1 and JNK may also participate in compound 14's effect on neurite extension (Figure 7). By contrast, the activities of p38, PKA, and PKC were not important for compound 14's effect on promoting neurite outgrowth (Figure 7). These results collectively suggest that compound 14 induces neuronal differentiation and neurite extension through activation of MEK-ERK, PI3K-Akt and CaMKII pathways, although the identity of the direct molecular target of 14 awaits further investigation.

■ CONCLUSION

In summary, we have designed and synthesized a series of novel dimeric securinine analogues using bivalent ligand approach. Compounds 13, 14, 17−19, and 21−23 were identified as active
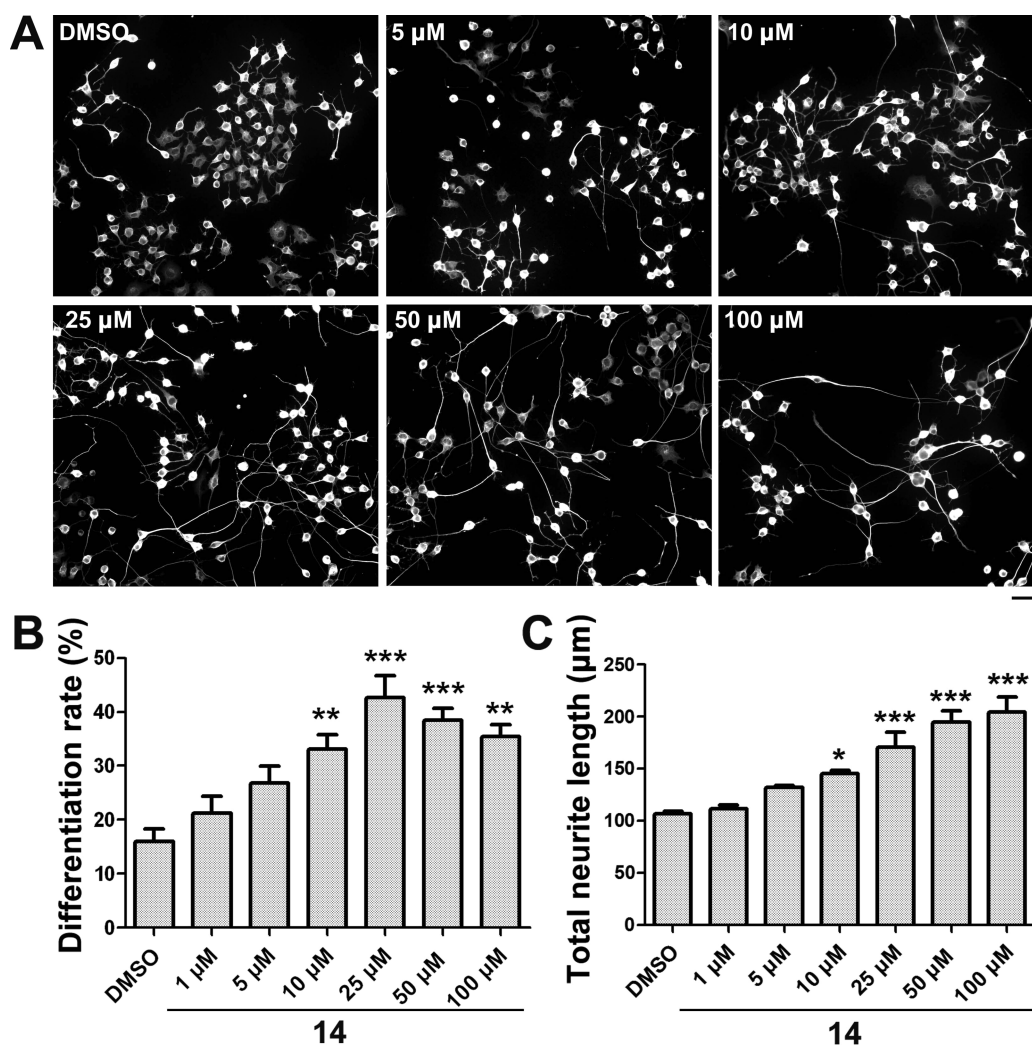
Figure 5. Effect of compound **14** at different concentrations on neurite outgrowth. Neuro-2a cells were treated with compound **14** at concentrations from 1 to 100 μM for 48 h. (A) Representative images of Neuro-2a cells after treatment with compound **14**. Scale bar, 50 μm. The differentiation rate (B) and the total neurite length of each differentiated cell (bearing at least one neurite longer than 40 μm; C) were analyzed. At least 400 cells/group were analyzed in each experiment ($n = 3$), and values were presented as mean $\pm$ SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, indicated compound vs DMSO, one-way ANOVA followed by Tukey's test.
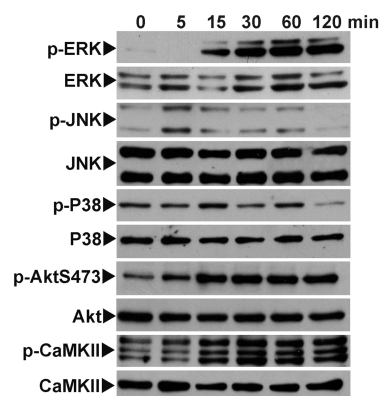
Figure 6. Compound **14** increases phosphorylation levels of ERK, Akt, and CaMKII in Neuro-2a cells. Compound **14** was added in Neuro-2a cells for indicated time points (0–120 min). Cells lysates were subjected to Western blot analysis for the phosphorylated and total forms of different signaling molecules.

compounds for promoting neurite outgrowth, and it was found that linker modifications of the bivalent mimetics should be

considered carefully in order to retain their activity. The preferred length of the linker between the two monomers is 4–7 carbons and the optimal side chain on N atoms is ethyl or propyl. Compound **13** with an N^1,N^6-diethyladipamide linker, and compounds **14**, **17–19** and **21–23** with different lengths of carbon chain of N,N-dipropyl diacid amide linker between two securinine molecules at C-15 position, exhibited notable positive effects on both neuronal differentiation and neurite extension of neuronal cells. Compound **14**, one of the most active compounds, was selected as the lead compound and its molecular action has been studied. Although detailed structure–activity relationship and the molecular targets of these analogues remain to be fully elucidated, the findings in this study have revealed a novel dimeric class of securinine analogues that exhibits strong beneficial effects on neurite outgrowth, which may provide a new avenue for drug design in treating neurodegenerative diseases.

■ METHODS

General Experimental Methods. All nonaqueous reactions were performed under an argon atmosphere in flame-dried glassware with rigid exclusion of moisture from the reaction setup. (−)-Securinine was purchased from Sigma-Aldrich, and virosecurinine was isolated and
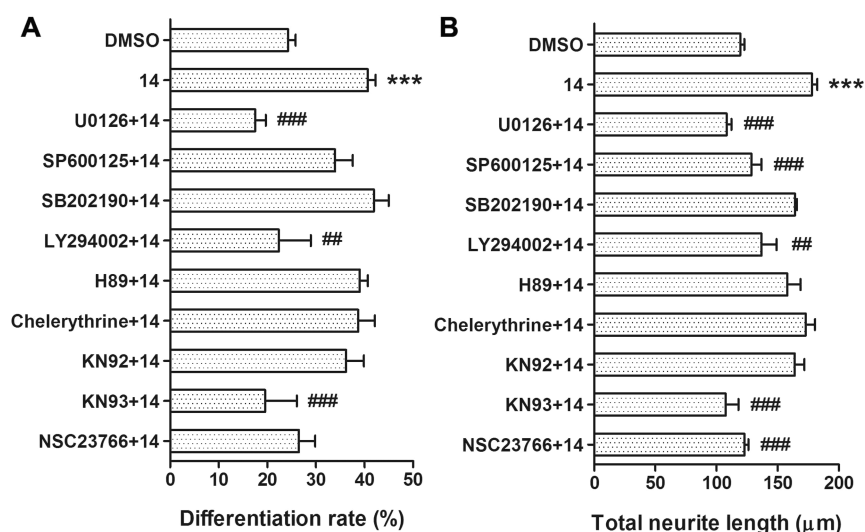
Figure 7. Activation of MEK-ERK, PI3K-Akt and CaMKII signaling pathways is required for compound **14** induced neurite outgrowth. Neuro-2a cells were pretreated with different inhibitors, including MEK inhibitor (U0126, 10 μM), JNK inhibitor (SP600125, 5 μM), p38 inhibitor (SB202190, 10 μM), PI3K inhibitor (LY290042, 10 μM), PKA inhibitor (H89, 0.5 μM), PKC inhibitor (chelerythrine chloride, 0.5 μM), KN92 (CaMKII inhibitor control, 1 μM), KN93 (CaMKII inhibitor, 1 μM), or Rac1 inhibitor (NSC2376, 10 μM) for 1 h, followed by compound **14** treatment (25 μM) for 48 h. Neurites were visualized by immunostaining with anti-β-tubulin III antibody, and analyzed by high content technology. Cell differentiation rate (A) and average length of total neurites per differentiated cell (B) were quantified. At least 400 cells/group were analyzed in each experiment ($n = 5$), and values were presented as mean $\pm$ SEM. ***$P < 0.001$, compound **14** vs DMSO; ##$P < 0.01$, ###$P < 0.001$, inhibitor and compound **14** cotreatment vs **14** single treatment.

purified from the twigs and leaves of the plant *Flueggea virosa* from Southern China. All other reagents were obtained from commercial sources and were used without any extra purification. Anhydrous CH_2Cl_2 were obtained by distillation under nitrogen atmosphere from appropriate drying agents. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD on Bruker AV-300 (^1H: 300 MHz, ^{13}C: 75 MHz) spectrometer. Chemical shifts (δ in ppm) and coupling constants (J in Hz) were determined by reference to residual solvent resonances. IR spectra were recorded on a Jasco FT/IR-480 plus Fourier transform infrared spectrometer using KBr pellets. HR-ESI-MS spectra were acquired on Agilent 6210 LC/MSD TOF mass spectrometer. All melting points were obtained on an X-5 micro melting point apparatus without correction. TLC analyses were carried out using precoated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, P. R. China). Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Plant, Qingdao, P. R. China). Compounds were visualized by UV absorbance at 254 nm and by staining with aqueous potassium permanganate. The purity of compounds used in pharmacology testing was 95% or higher.

N^1,N^2-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-oxalamide (7). Oxalyl chloride (0.55 equiv, diluent in 1 mL of anhydrous CH_2Cl_2) was added to a solution of (−)-securinine derivatives **24** (1.0 equiv) and DIPEA (1.5 equiv) in anhydrous CH_2Cl_2 and then stirred for 6−7 h under the nitrogen atmosphere at −10 °C. The reaction was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2 (4 × 30 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue that was purified by column chromatography using silica gel to give the desired product **7** as a white solid (55% yield). mp: 153−154 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 8.85 (2H, N−H), 5.75 (s, 2H), 4.08−3.94 (m, 2H), 3.31−3.25 (m, 2H), 3.11−2.97 (m, 2H), 2.87 (d, J = 12.6 Hz, 6H), 2.75 (d, J = 9.5 Hz, 2H), 2.49−2.41 (m, 2H), 1.77 (d, J = 10.9 Hz, 4H), 1.38 (d, J = 10.6 Hz, 10H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.0, 172.7, 160.3, 110.9, 90.9, 62.1, 59.9, 50.8, 48.5, 32.9, 28.8, 26.3, 24.1, 22.2; IR: 3327, 2935, 2852, 1753, 1671, 1498 cm^{-1}; ESI-MS: m/z 523.5 [M + H]$^+$; HRMS(ESI): m/z 523.2553 [M + H]$^+$, calcd for $C_{28}H_{35}N_4O_6$ = 523.2551.

N^1,N^3-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-malonamide (8). Reaction of malonyl chloride with **24** as described for **7** gave the title compound **8** as a white solid (74% yield). mp: 162−163 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.68 (2H, N−H), 5.65 (d, J = 1.9 Hz, 2H), 4.21 (dd, J = 9.1, 5.6 Hz, 2H), 3.41 (d, J = 3.0 Hz, 2H), 3.15 (s, 2H), 2.99 (s, 6H), 2.71−2.61 (m, 2H), 2.57 (dd, J = 11.3, 5.9 Hz, 2H), 1.87 (s, 2H), 1.67−1.56 (m, 4H), 1.48−1.31 (m, 8H), 1.22 (d, J = 11.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 173.1, 166.9, 111.3, 91.1, 61.6, 59.8, 50.8, 48.5, 41.6, 33.3, 29.6, 26.0, 23.8, 21.7; IR: 3276, 2936, 2851, 1754, 1648, 1541 cm^{-1}; ESI-MS: m/z 537.8 [M + H]$^+$; HRMS(ESI): m/z 537.2709 [M + H]$^+$, calcd for $C_{29}H_{37}N_4O_6$ = 537.2708.

N^1,N^4-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-succinamide (9). Reaction of succinyl chloride with **24** as described for **7** gave the title compound **9** as a white solid (70% yield). mp: 163−164 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.84 (2H, N−H), 5.66 (d, J = 2.1 Hz, 2H), 4.23 (dd, J = 5.8, 3.7 Hz, 2H), 3.41 (s, 2H), 3.22−3.07 (m, 2H), 2.94 (m, 6H), 2.69−2.44 (m, 8H), 1.89 (s, 2H), 1.68−1.57 (m, 4H), 1.39 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 172.3, 172.1, 111.1, 91.2, 61.7, 59.7, 50.7, 48.5, 33.3, 31.5, 29.7, 26.2, 23.9, 21.7; IR: 3289, 2935, 2852, 1753, 1671, 1498 cm-1; ESI-MS: m/z 551.8 [M + H]$^+$; HRMS(ESI): m/z 551.2862 [M + H]$^+$, calcd for $C_{30}H_{39}N_4O_6$ = 551.2864.

N^1,N^5-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-glutaramide (10). Reaction of glutaryl chloride with **24** as described for **7** gave the title compound **10** as a white solid (75% yield). mp: 167−169 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (2H, N−H), 5.54 (s, 2H), 4.16 (s, 2H), 3.34 (s, 2H), 3.04 (dd, J = 3.8, 11.0 Hz, 2H), 2.92 (s, 6H), 2.52 (dd, J = 12.4, 10.5 Hz, 4H), 2.17 (s, 4H), 1.82 (s, 4H), 1.61 (d, J = 11.0 Hz, 2H), 1.50−1.24 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 173.4, 172.4, 110.7, 91.3, 61.6, 59.6, 50.5, 48.3, 34.9, 33.0, 29.6, 26.1, 23.9, 22.0, 21.7; IR: 3308, 2937, 2851, 1753, 1644, 1539 cm^{-1}; ESI-MS: m/z 565.9 [M + H]$^+$; HRMS(ESI): m/z 565.3023 [M + H]$^+$, calcd for $C_{31}H_{41}N_4O_6$ = 565.3021.

N^1,N^6-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-adipamide (11). Reaction of adipoyl chloride with **24** as described for **7** gave the title compound **11** as a white solid (79% yield). mp: 170−172 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.76 (2H, N−H), 5.59 (d, J = 1.8 Hz, 2H), 4.22 (dd, J = 11.4, 7.4 Hz, 2H), 3.44−3.33 (m, 2H), 3.15−3.03

(m, 2H), 2.93 (dd, J = 15.3, 4.2 Hz, 6H), 2.65–2.55 (m, 2H), 2.52–2.45 (m, 2H), 2.24–2.15 (m, 4H), 1.85 (s, 2H), 1.69–1.52 (m, 8H), 1.37 (m, 8H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 173.4, 172.7, 110.8, 91.4, 61.6, 59.7, 50.6, 48.4, 35.4, 33.1, 29.7, 26.2, 24.6, 23.9, 21.8; IR: 3356, 2938, 2851, 1753, 1644, 1543 cm^{-1}; ESI-MS: m/z 579.5 [M + H]$^+$; HRMS(ESI): m/z 579.3178 [M + H]$^+$, calcd for C$_{32}$H$_{43}$N$_4$O$_6$ = 579.3177.

N^1,N^6-Dimethyl-N^1,N^6-bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido-[1,2-a]azepin-5-yl)adipamide (12). Reaction of adipoyl chloride with **25** as described for **7** gave the title compound **12** as a yellow solid (61% yield). mp: 144–146 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.07 (s, 2H), 4.56–4.60 (m, 2H), 3.44–3.47 (m, 2H), 3.12–3.21 (m, 2H), 2.92–3.05 (m, 4H), 2.88 (s, 6H), 2.72–2.78 (d, J = 11.7 Hz, 4H), 2.53–2.58 (m, 2H), 2.31 (s, 4H), 1.24–1.87 (m, 18H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.8, 172.8, 172.6, 110.5, 91.2, 61.7, 59.9, 58.0, 48.4, 33.9, 33.7, 32.4, 26.7, 26.4, 24.4, 24.2, 22.1; IR: 2925, 2851, 1757, 1637, 1384 cm^{-1}; ESI-MS: m/z 607.5 [M + H]$^+$; HRMS(ESI): m/z 607.3468 [M + H]$^+$, calcd for C$_{34}$H$_{47}$N$_4$O$_6$ = 607.3490.

N^1,N^6-Diethyl-N^1,N^6-bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido-[1,2-a]azepin-5-yl)adipamide (13). Reaction of adipoyl chloride with **26** as described for **7** gave the title compound **13** as a yellow solid (63% yield). mp: 146–148 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.65 (s, 2H), 4.29–4.31 (m. 2H), 3.44 (m, 2H), 3.27–3.34 (m, 2H), 3.02–3.18 (m, 4H), 2.95–3.00 (m, 2H), 2.80–2.92 (m, 6H), 2.45–2.51 (m, 2H), 2.20–2.39 (m, 4H), 1.84 (m, 2H), 1.55–1.62 (m, 4H), 1.06–1.25 (m, 18H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.5, 172.9, 172.4, 110.0, 91.1, 61.8, 60.1, 57.2, 48.5, 48.1, 33.4, 30.0, 26.9, 26.4, 24.8, 24.1, 22.1, 11.0; IR: 2924, 2852, 1761, 1637, 1371 cm^{-1}; ESI-MS: m/z 635.6 [M + H]$^+$; HRMS(ESI): m/z 635.3784 [M + H]$^+$, calcd for C$_{36}$H$_{51}$N$_4$O$_6$ = 635.3787.

N^1,N^6-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^6-dipropyladipamide (14); N^1-((5R,6S,11aR,11bS)-2-Oxo-4,5,6,8,9,-10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]-azepin-5-yl)-N^6-((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^6-dipropyladipamide (21); N^1,N^6-Bis((5R,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido-[1,2-a]azepin-5-yl)-N^1,N^6-dipropyladipamide (22). Reaction of adipoyl chloride with **27** and **30** as described for **7** gave the title compounds **14**, **21**, and **22** in one port, which were further purified by HPLC as three yellow solids (24%, 42%, and 24% yield, respectively).

Compound 14. mp: 151–152 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (s, 2H), 4.25 (d, J = 6.3 Hz, 2H), 3.42 (s, 2H), 3.18–3.06 (m, 4H), 2.87 (m, 10H), 2.44 (dd, J = 11.0, 5.8 Hz, 2H), 2.24 (d, J = 3.7 Hz, 4H), 1.80 (s, 2H), 1.55 (s, 12H), 1.34 (m, 8H), 0.81 (t, J = 7.3 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 172.8, 172.7, 109.8, 90.9, 61.8, 59.9, 57.1, 48.2, 48.1, 33.4, 33.3, 26.7, 26.2, 24.9, 24.9, 24.0, 21.9, 11.0; IR: 2923, 2851, 1760, 1637, 1384 cm^{-1}; ESI-MS: m/z 663.7 [M + H]$^+$; HRMS(ESI): m/z 663.4126 [M + H]$^+$, calcd for C$_{38}$H$_{55}$N$_4$O$_6$ = 663.4116.

Compound 21. mp: 150–151 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (s, 2H), 4.49 (t, J = 8.7 Hz, 1H), 4.25 (s, 1H), 3.55–3.40 (m, 2H), 3.20–3.06 (m, 4H), 3.00–2.82 (m, 8H), 2.77 (t, J = 7.3 Hz, 3H), 2.62 (dd, J = 10.9, 6.6 Hz, 1H), 2.45 (dd, J = 10.9, 5.8 Hz, 1H), 2.33–2.21 (m, 4H), 1.80 (s, 2H), 1.46 (m, 19H), 0.83 (dd, J = 7.0, 4.7 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.1, 172.9, 172.6, 172.4, 172.0, 171.2, 110.8, 109.8, 90.7, 90.4, 65.5, 64.5, 60.4, 59.9, 57.0, 55.8, 49.4, 49.1, 48.2, 44.7, 37.0, 36.6, 33.7, 33.4, 33.3, 33.2, 27.1, 26.7, 25.4, 25.0, 24.9, 24.8, 23.7, 23.6, 20.9, 20.7, 11.2, 11.0; IR: 2938, 2862, 1759, 1637, 909 cm^{-1}; ESI-MS: m/z 663.6 [M + H]$^+$; HRMS(ESI): m/z 663.4166 [M + H]$^+$, calcd for C$_{38}$H$_{55}$N$_4$O$_6$ = 663.4116.

Compound 22. mp: 150–152 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.65 (s, 2H), 4.54 (t, J = 8.7 Hz, 2H), 3.60–3.48 (m, 2H), 3.27 (dd, J = 11.1, 5.0 Hz, 2H), 3.16 (d, J = 6.4 Hz, 2H), 3.03 (dd, J = 10.8, 4.1 Hz, 2H), 2.98–2.76 (m, 10H), 2.67 (dd, J = 10.8, 6.6 Hz, 2H), 2.40–2.27 (m, 4H), 1.85 (s, 2H), 1.60–1.31 (m, 18H), 0.89 (d, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.3, 172.8, 172.1, 111.0, 90.9, 64.7, 60.6, 49.5, 44.9, 36.8, 33.5, 29.6, 27.3, 25.5, 25.3, 25.0, 23.7, 20.8, 11.3;

IR: 2937, 2862, 1759, 1636, 909 cm^{-1}; ESI-MS: m/z 663.6 [M + H]$^+$; HRMS(ESI): m/z 663.4150 [M + H]$^+$, calcd for C$_{38}$H$_{55}$N$_4$O$_6$ = 663.4116.

N^1,N^6-Dibutyl-N^1,N^6-bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido-[1,2-a]azepin-5-yl)adipamide (15). Reaction of adipoyl chloride with **28** as described for **7** gave the title compound **15** as a yellow solid (69% yield). mp: 159–161 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.66 (s, 2H), 4.32 (s, 2H), 3.48 (s, 2H), 3.18 (d, J = 10.4 Hz, 6H), 3.02–2.85 (m, 10H), 2.51 (dd, J = 10.3, 5.8 Hz, 2H), 2.30 (s, 4H), 1.87 (s, 2H), 1.63 (s, 12H), 1.37–1.27 (m, 10H), 0.94 (t, J = 7.3 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.2, 172.7, 172.5, 109.7, 90.8, 61.6, 59.8, 57.0, 48.1, 46.1, 33.5, 33.3, 33.2, 26.5, 26.1, 24.8, 23.9, 21.9, 19.9, 13.5; IR: 2927, 2851, 1743, 1637, 1381 cm^{-1}; ESI-MS: m/z 691.3 [M + H]$^+$; HRMS(ESI): m/z 691.4421 [M + H]$^+$, calcd for C$_{40}$H$_{59}$N$_4$O$_6$ = 691.4429.

N^1,N^6-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^6-dipentyladipamide (16). Reaction of adipoyl chloride with **29** as described for **7** gave the title compound **16** as a yellow solid (60% yield). mp: 167–168 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.66 (s, 2H), 4.43–4.17 (m, 2H), 3.48 (s, 2H), 3.17 (d, J = 10.1 Hz, 4H), 2.90 (m, 10H), 2.51 (dd, J = 10.5, 5.8 Hz, 2H), 2.44–2.11 (m, 6H), 1.87 (s, 2H), 1.62 (s, 10H), 1.39–1.21 (m, 16H), 0.91 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 172.7, 172.6, 109.7, 90.9, 61.6, 59.8, 57.0, 48.2, 46.3, 33.4, 33.2, 31.3, 28.8, 26.5, 26.1, 24.8, 23.9, 22.0, 21.9, 13.8; IR: 2926, 2850, 1761, 1643, 1384 cm^{-1}; ESI-MS: m/z 719.3 [M + H]$^+$; HRMS(ESI): m/z 719.4761 [M + H]$^+$, calcd for C$_{42}$H$_{63}$N$_4$O$_6$ = 719.4742.

N^1,N^4-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^4-dipropylsuccinamide (17). Reaction of succinyl chloride with **27** as described for **7** gave the title compound **17** as a yellow solid (42% yield). mp: 166–168 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.63 (s, 2H), 4.27–4.29 (m, 2H), 3.22–3.40 (m, 4H), 3.06–3.14 (m, 2H), 2.79–2.99 (m, 8H), 2.55–2.63 (m, 6H), 2.43–2.48 (m, 2H), 1.74–1.84 (m, 4H), 1.50–1.61 (m, 4H), 1.22–1.40 (m, 10H), 0.87 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.4, 172.8, 172.8, 109.9, 91.0, 61.1, 60.0, 57.1, 48.4, 48.0, 33.6, 33.6, 28.3, 26.8, 24.8, 24.1, 22.0, 11.2; IR: 2924, 2850, 1761, 1644, 1384 cm^{-1}; ESI-MS: m/z 635.2 [M + H]$^+$; HRMS(ESI): m/z 635.3850 [M + H]$^+$, calcd for C$_{36}$H$_{51}$N$_4$O$_6$ = 635.3803.

N^1,N^5-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^5-dipropylglutaramide (18). Reaction of glutaryl chloride with **27** as described for **7** gave the title compound **18** as a yellow solid (70% yield). mp: 172–174 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.65 (s, 2H), 4.25 (d, J = 5.8 Hz, 2H), 3.46 (s, 2H), 3.17 (d, J = 10.7 Hz, 4H), 2.92 (m, 10H), 2.53–2.46 (m, 2H), 2.36 (d, J = 7.3 Hz, 4H), 1.89 (d, J = 9.2 Hz, 4H), 1.62 (m, 6H), 1.30 (d, J = 6.7 Hz, 10H), 0.88 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.2, 172.6, 172.6, 109.7, 90.8, 61.7, 59.8, 57.0, 48.1, 48.0, 33.2, 32.5, 26.6, 26.1, 24.7, 23.9, 21.9, 20.8, 11.0; IR: 2926, 2851, 1752, 1633, 1384 cm^{-1}; ESI-MS: m/z 649.3 [M + H]$^+$; HRMS(ESI): m/z 649.3956 [M + H]$^+$, calcd for C$_{37}$H$_{53}$N$_4$O$_6$ = 649.3959.

N^1,N^7-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^7-dipropylheptanediamide (19). Reaction of pimeloyl chloride with **27** as described for **7** gave the title compound **19** as a yellow solid (74% yield). mp: 177–178 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.67 (s, 2H), 4.28 (d, J = 12.1 Hz, 2H), 3.50 (s, 2H), 3.16 (d, J = 9.6 Hz, 4H), 2.91 (m, 10H), 2.55–2.48 (m, 2H), 2.27 (t, J = 6.9 Hz, 4H), 1.88 (s, 2H), 1.63 (d, J = 6.4 Hz, 12H), 1.35 (d, J = 6.9 Hz, 10H), 0.89 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 173.1, 172.7, 109.8, 90.9, 61.7, 59.8, 56.9, 48.2, 48.0, 33.3, 33.2, 28.9, 26.6, 26.2, 24.9, 24.9, 23.9, 21.9, 11.0; IR: 2924, 2846, 1772, 1655, 909 cm^{-1}; ESI-MS: m/z 677.4 [M + H]$^+$; HRMS(ESI): m/z 677.4310 [M + H]$^+$, calcd for C$_{39}$H$_{57}$N$_4$O$_6$ = 677.4272.

N^1,N^8-Bis((5S,6S,11aR,11bS)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^8-dipropyloctanediamide (20). Reaction of suberoyl chloride with **27** as described for **7** gave the title compound **20** as a yellow solid (52% yield). mp: 176–177 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.57 (s, 2H), 4.21 (d, J = 5.6 Hz, 2H), 3.38 (s, 2H), 3.07 (dt, J = 15.1, 9.2 Hz, 4H), 2.90–2.76 (m, 10H), 2.40 (dd, J = 10.8, 5.7 Hz, 2H), 2.21–2.10 (m, 4H), 1.76 (s, 2H), 1.51 (d, J = 10.7 Hz, 12H), 1.23 (s, 12H), 0.79 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 173.1, 172.5, 109.6, 90.8, 61.6,

59.7, 56.8, 48.1, 48.0, 33.3, 33.1, 29.0, 26.5, 26.0, 25.0, 24.8, 23.8, 21.8, 10.9; IR: 2925, 2846, 1771, 1655, 909 cm^{-1}; ESI-MS: m/z 691.8 [M + H]$^+$; HRMS(ESI): m/z 691.4459 [M + H]$^+$, calcd for C$_{40}$H$_{59}$N$_4$O$_6$ = 691.4429.

N^1,N^6-Bis((5R,6R,11aS,11bR)-2-oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N^1,N^6-dipropyladipamide (23). Reaction of adipoyl chloride with **31** as described for **7** gave the title compound **23** as a yellow solid (73% yield). mp: 153−154 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.66 (s, 2H), 4.31 (s, 2H), 3.48 (s, 2H), 3.15 (d, J = 10.6 Hz, 4H), 3.02−2.83 (m, 10H), 2.54−2.46 (m, 2H), 2.30 (s, 4H), 1.87 (s, 2H), 1.62 (s, 12H), 1.36 (s, 8H), 0.88 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.3, 172.8, 172.7, 109.8, 90.9, 61.8, 59.9, 57.1, 48.2, 48.1, 33.4, 33.3, 26.7, 26.2, 24.9, 24.9, 24.0, 21.9, 11.0; IR: 2924, 2851, 1761, 1637, 1384 cm^{-1}; ESI-MS: m/z 663.7 [M + H]$^+$; HRMS(ESI): m/z 663.4094 [M + H]$^+$, calcd for C$_{38}$H$_{55}$N$_4$O$_6$ = 663.4116.

(5S,6S,11aR,11bS)-5-Amino-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (24). Azidotrimethylsilane (4.0 equiv) was added to a mixed solution of (−)-securinine (1.0 equiv), AcOH (1.0 equiv), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.6 equiv) in CH$_2$Cl$_2$ and then stirred for 8−10h at rt. The reaction was quenched with saturated NaHCO$_3$ solution and extracted with CH$_2$Cl$_2$ (4 × 10 mL). The combined organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo to give a residue that was purified by column chromatography using silica gel to give **34** (C15-R diastereoisomer was abandoned). Then catalytic Pd/C was added to a solution of **34** in CH$_2$Cl$_2$ and stirred for 3−5 h with a hydrogen balloon. The Pd/C was filtrated by silica gel in sintered discs and the organic layer was concentrated in vacuo to give a residue that was purified by column chromatography using silica gel to give **24** as a yellow liquid (42% in two steps). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.46 (d, J = 2.4 Hz, 1H), 3.04−2.75 (m, 6H), 2.59 (dd, J = 10.8, 6.5 Hz, 1H), 2.20 (ddd, J = 14.9, 8.9, 2.4 Hz, 1H), 1.83−1.68 (m, 1H), 1.57−1.44 (m, 3H), 1.42−1.22 (m, 5H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.6, 173.2, 109.6, 91.0, 66.7, 60.5, 55.9, 50.4, 35.6, 33.2, 25.6, 23.8, 21.1; IR: 3368, 3292, 2939, 2856, 1740 cm^{-1}; ESI-MS: m/z 235.3 [M + H]$^+$; HRMS(ESI): m/z 235.1449 [M + H]$^+$, calcd for C$_{13}$H$_{19}$N$_2$O$_2$ = 235.1441.

(5S,6S,11aR,11bS)-5-(Methylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (25). Potassium phosphate (0.3 equiv) was added to a solution of (−)-securinine (1.0 equiv) and 25% methylamine (25 wt % solution in H$_2$O, 2.0 equiv) in CH$_2$Cl$_2$ and MeOH (10:1) and then stirred for 28 h at rt. The reaction was quenched with saturated NH$_4$Cl solution and extracted with CH$_2$Cl$_2$ (4 × 30 mL). The combined organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo to give a residue that was purified by column chromatography using silica gel to give the desired product **25** (C15-R diastereoisomer was trace) as a yellow liquid (68% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (d, J = 2.1 Hz, 1H), 3.20−3.23 (m, 1H), 2.88−2.94 (m, 4H), 2.77−2.85 (m, 1H), 2.57−2.62 (m, 1H), 2.42−2.49 (dd, J = 6.0, 10.8 Hz, 1H), 2.36 (s, 3H), 1.82−1.87 (m, 2H), 1.55−1.59 (m, 2H), 1.31−1.43 (m, 4H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.2, 173.0, 110.9, 91.3, 62.7, 61.5, 59.7, 48.7, 34.4, 32.3, 29.7, 25.7, 23.7, 21.5; IR: 2935, 2851, 1723, 1643, 1435 cm^{-1}; ESI-MS: m/z 249.3 [M + H]$^+$; HRMS(ESI): m/z 249.1591 [M + H]$^+$, calcd for C$_{14}$H$_{21}$N$_2$O$_2$ = 249.1598.

(5S,6S,11aR,11bS)-5-(Ethylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (26). Reaction of (−)-securinine with ethylamine as described for **25** gave the title compound **26** (the minor C15-R diastereoisomer was abandoned) as a yellow liquid (69% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.59 (d, J = 2.4 Hz, 1H), 3.18−3.21 (m, 1H), 3.02−3.06 (m, 1H), 2.88−2.94 (m, 3H), 2.79−2.86 (m, 1H), 2.51−2.64 (m, 3H), 2.46 (dd, J = 6.0, 12.0 Hz, 1H), 1.87 (d, 1H, J = 10.1 Hz), 1.80−1.85 (m, 1H), 1.55−1.61 (m, 1H), 1.15−1.50 (m, 5H), 1.05 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.4, 173.1, 110.9, 91.4, 63.0, 59.8, 59.2, 48.8, 42.1, 32.3, 30.3, 25.8, 23.8, 21.6, 15.4; IR: 2935, 2852, 1743, 1644, 898 cm^{-1}; ESI-MS: m/z 263.3 [M + H]$^+$; HRMS(ESI): m/z 263.1754 [M + H]$^+$, calcd for C$_{15}$H$_{23}$N$_2$O$_2$ = 263.1742.

(5S,6S,11aR,11bS)-5-(Propylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (27) and (5R,6S,11aR,11bS)-5-(Propylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (30).

Reaction of (−)-securinine with propylamine as described for **25** gave a mixture of the title compounds **27** and **30** (Rf = 0.34 and 0.36; CHCl$_3$/MeOH = 8:1) which were separated by column chromatography silica gel (CHCl$_3$/MeOH = 40:1) to offer two yellow liquids (69% and 17% yield). **27**: ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.57 (d, J = 2.1 Hz, 1H), 3.22−3.16 (m, 1H), 3.01 (dd, J = 6.3, 4.2 Hz, 1H), 2.92−2.75 (m, 3H), 2.58−2.40 (m, 4H), 1.89−1.79 (m, 2H), 1.59−1.25 (m, 9H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.6, 173.1, 110.8, 91.4, 63.0, 59.8, 59.7, 49.7, 48.7, 32.37, 30.4, 25.8, 23.8, 23.4, 21.6, 11.7; IR: 2939, 2860, 1744, 1628, 1451 cm^{-1}; ESI-MS: m/z 277.5 [M + H]$^+$; HRMS(ESI): m/z 277.1901 [M + H]$^+$, calcd for C$_{16}$H$_{25}$N$_2$O$_2$ = 277.1911. **30**: ^{1}H NMR (300 MHz, CDCl3) δ 5.55 (s, 1H), 3.34 (d, J = 6.3 Hz, 1H), 3.07−2.84 (m, 4H), 2.72−2.47 (m, 4H), 2.32−2.22 (m, 1H), 1.84 (s, 1H), 1.62−1.11 (m, 9H), 0.92−0.86 (m, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.6, 173.3, 109.9, 91.3, 61.8, 61.0, 60.6, 50.1, 48.6, 35.7, 31.3, 25.8, 23.8, 23.5, 21.2, 11.9; IR: 2934, 2851, 1744, 1635, 1442 cm^{-1}; ESI-MS: m/z 277.5 [M + H]$^+$; HRMS(ESI) m/z 277.1905 [M + H]$^+$, calcd for C$_{16}$H$_{25}$N$_2$O$_2$ = 277.1911.

(5S,6S,11aR,11bS)-5-(Butylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (28). Reaction of (−)-securinine with butylamine as described for **25** gave the title compound **28** (C15-R diastereoisomer was abandoned) as a yellow liquid (78% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.51 (s, 1H), 3.13 (s, 1H), 2.94 (dt, J = 9.9, 5.1 Hz, 1H), 2.87−2.69 (m, 4H), 2.53−2.33 (m, 4H), 1.84−1.71 (m, 2H), 1.48 (d, J = 11.9 Hz, 1H), 1.44−1.11 (m, 9H), 0.83−0.77 (m, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.5, 173.0, 110.7, 91.3, 62.9, 59.7, 59.7, 48.6, 47.4, 32.3, 32.2, 30.3, 25.7, 23.7, 21.5, 20.3, 13.9; IR: 2937, 2851, 1744, 1634, 898 cm^{-1}; ESI-MS: m/z 291.2 [M + H]$^+$; HRMS(ESI): m/z 291.2060 [M + H]$^+$, calcd for C$_{17}$H$_{27}$N$_2$O$_2$ = 291.2067.

(5S,6S,11aR,11bS)-5-(Pentylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (29). Reaction of (−)-securinine with amylamine as described for **25** gave the title compound **29** (C15-R diastereoisomer was abandoned) as a yellow liquid (76% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.50 (d, J = 2.2 Hz, 1H), 3.16−3.08 (m, 1H), 2.93 (dt, J = 9.6, 4.8 Hz, 1H), 2.86−2.69 (m, 4H), 2.52−2.33 (m, 4H), 1.83−1.71 (m, 2H), 1.49 (dd, J = 10.2, 6.7 Hz, 1H), 1.42−1.21 (m, 7H), 1.21−1.12 (m, 4H), 0.78 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.5, 173.0, 110.7, 91.3, 62.9, 59.7, 59.7, 48.6, 47.7, 32.2, 30.3, 29.8, 29.3, 25.7, 23.7, 22.4, 21.5, 13.9; IR: 2936, 2855, 1744, 1635, 898 cm^{-1}; ESI-MS: m/z 305.0 [M + H]$^+$; HRMS(ESI): m/z 305.2225 [M + H]$^+$, calcd for C$_{18}$H$_{29}$N$_2$O$_2$ = 305.2224.

(5R,6R,11aS,11bR)-5-(Propylamino)-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-2-one (31). Reaction of virosecurinine with propylamine as described for **25** gave the title compound **31** (C15-S diastereoisomer was abandoned) as a yellow liquid (62% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.63−5.54 (m, 1H), 3.26−3.13 (m, 1H), 3.02 (dt, J = 12.4, 6.3 Hz, 1H), 2.95−2.75 (m, 4H), 2.50 (tdd, J = 11.2, 13.9, 7.9 Hz, 4H), 1.92−1.79 (m, 2H), 1.62−1.50 (m, 1H), 1.52−1.23 (m, 7H), 0.86 (q, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ: 174.5, 173.2, 110.9, 91.4, 63.1, 59.8, 59.7, 49.7, 48.8, 32.3, 30.5, 25.8, 23.8, 23.4, 21.6, 11.7; IR: 2937, 2851, 1744, 1625, 1456 cm^{-1}; ESI-MS: m/z 277.5 [M + H]$^+$; HRMS(ESI): m/z 277.1907 [M + H]$^+$, calcd for C$_{16}$H$_{25}$N$_2$O$_2$ = 277.1911.

N-((5S,6S,11aR,11bS)-2-Oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N-propylpropionamide (32). Reaction of propionyl chloride with **27** as described for **7** gave the title compound **32** as a yellow thick liquid (94% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (s, 1H), 4.24 (s, 1H), 3.43 (s, 1H), 3.10 (dd, J = 9.4, 7.1 Hz, 2H), 2.84 (dd, J = 7.9, 14.0 Hz, 4H), 2.48−2.39 (m, 1H), 2.23 (ddd, J = 9.3, 6.8, 3.0 Hz, 3H), 1.78 (s, 1H), 1.60 (d, J = 11.1 Hz, 1H), 1.51 (d, J = 11.7 Hz, 2H), 1.41−1.25 (m, 4H), 1.08−1.01 (m, 4H), 0.81 (t, J = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 177.8, 174.1, 172.6, 109.7, 90.7, 62.0, 59.8, 56.8, 48.2, 47.9, 33.0, 26.7, 26.6, 25.5, 24.6, 23.3, 21.2, 11.0, 9.3; IR: 2927, 2862, 1760, 1635, 909 cm^{-1}; ESI-MS: m/z 333.4 [M + H]$^+$; HRMS(ESI): m/z 333.2198 [M + H]$^+$, calcd for C$_{19}$H$_{29}$N$_2$O$_3$ = 333.2172.

N-((5R,6S,11aR,11bS)-2-Oxo-4,5,6,8,9,10,11,11a-octahydro-2H-6,11b-methanofuro[2,3-c]pyrido[1,2-a]azepin-5-yl)-N-propylpro-

pionamide (33). Reaction of propionyl chloride with **30** as described for **7** gave the title compound **33** as a yellow thick liquid (92% yield). ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.67 (s, 1H), 4.54−4.40 (m, 1H), 3.52 (dd, J = 8.6, 7.9 Hz, 1H), 3.36−3.20 (m, 2H), 3.11 (dd, J = 11.2, 4.1 Hz, 1H), 2.89 (ddd, 8.0 Hz, 4H), 2.70 (dd, J = 11.1, 6.5 Hz, 1H), 1.86 (s, 1H), 1.60 (dd, J = 6.5, 4.2 Hz, 2H), 1.46 (d, J = 12.5 Hz, 3H), 1.39−1.32 (m, 2H), 1.13 (dd, J = 7.4, 1.8 Hz, 6H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 175.5, 172.7, 171.7, 111.2, 90.6, 65.0, 60.7, 56.7, 49.2, 46.0, 36.4, 27.0, 26.9, 24.8, 24.5, 23.1, 20.1, 11.3, 8.9; IR: 2941, 2875, 1765, 1639, 905 cm^{-1}; ESI-MS: m/z 333.6 [M + H]$^+$; HRMS(ESI): m/z 333.2198 [M + H]$^+$, calcd for C$_{19}$H$_{29}$N$_2$O$_3$ = 333.2172.

Biological Assays. *Cell Culture.* Neuro-2a cells were cultured in MEM medium with 10% fetal bovine serum. Neuro-2a cells were purchased from American type culture collection (ATCC) company and maintained at 37 °C under 5% CO$_2$ atmosphere. To study neurite outgrowth, the culture medium was switched to modified Eargle's medium (MEM) supplemented with 0.5% fetal bovine serum (FBS) in the presence of indicated compounds. For neurite morphology or Western blot analysis, Neuro-2a cells were seeded in 12-well plates (1 × 10^4/well) or 35 mm dishes (6 × 10^4/dish), respectively.

Immunocytochemistry. Differentiated Neuro-2a cells were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 min, and permeablized with 0.4% Triton X-100 (Sigma-Aldrich). Immunostaining with mouse anti-β-tubulin III (Sigma-Aldrich) antibody was followed by incubation with Alexa Fluor-546 goat anti-mouse IgG antibody (Life Technologies) and DAPI (Life Technologies).

Neurite Outgrowth Assay and Quantification of Neurite Bearing Cells. Automated fluorescent microscopic images were captured and analyzed by high content technology using the Thermo Scientific Array-ScanVTI HCS Reade.[34,35] Briefly, 49 pictures around the center of each well were photographed using a 10× objective. Then more than 400 cells in each well were analyzed by mathematical algorithms used Cellomics view software for neurite outgrowth counting. Neurite were defined to be longer than double cell body diameters (40 μm).[33] The differentiation rate and the total neurite average length were analyzed for statistically.

Western Blotting. Neuro-2a cells were seeded in 35 mm dish (6 × 10^4/dish) overnight and then incubated with compound **14** for different time (0−120 min) at 37 °C. Cell extract was prepared by 2× SDS sample buffer. Those cell lysates were subsequently separated on 10% SDS-PAGE, and transferred to PVDF membranes. Membranes were probed with phosphorylated forms or total CaMKII, Akt, ERK, P38, and JNK (Cell Signaling Technology) antibodies and then subsequently with HRP-conjugated anti-rabbit IgG. Immunoreactive proteins were detected using ECL Plus (GE Healthcare) detect system.

Statistical Analysis. The results are expressed as the mean ± standard error of the mean (SEM). Data were subjected to Student's t test or one-way analysis of variance (ANOVA) followed by Tukey's test to assess the differences between the relevant control and each experimental group. A value of P < 0.05 was considered statistically significant.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.6b00188.

^{1}H and ^{13}C NMR spectra for compounds **7−32** (PDF)

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■ ABBREVIATIONS USED

Akt, protein kinase B; ANOVA, analysis of variance; ATCC, American type culture collection; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; DIPEA, *N,N*-diisopropylethylamine; DMSO, dimethyl sulfoxide; ERK1/2, extracellular signal regulated kinases 1/2; FBS, fetal bovine serum; GABA, γ-aminobutyric acid; JNK, c-Jun NH2-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MEM, modified Eagle's medium; Neuro-2a cells, mouse neuroblastoma N2a cells; RA, retinoic acid; PI3K, phosphoinositide 3-kinase; SEM, standard error of the mean; PKA, protein kinase A; PKC, protein kinase C

■ REFERENCES

(1) Polleux, F., and Snider, W. (2010) Initiating and growing an axon. *Cold Spring Harbor Perspect. Biol. 2*, a001925.

(2) Krstic, D., and Knuesel, I. (2013) Deciphering the mechanism underlying late-onset Alzheimer disease. *Nat. Rev. Neurol. 9*, 25−34.

(3) Ofengeim, D., Shi, P., Miao, B., Fan, J., Xia, X., Fan, Y., Lipinski, M. M., Hashimoto, T., Polydoro, M., Yuan, J., Wong, S. T., and Degterev, A. (2012) Identification of small molecule inhibitors of neurite loss induced by Abeta peptide using high content screening. *J. Biol. Chem. 287*, 8714−8723.

(4) Whitlon, D. S., Grover, M., Dunne, S. F., Richter, S., Luan, C. H., and Richter, C. P. (2015) Novel High Content Screen Detects Compounds That Promote Neurite Regeneration from Cochlear Spiral Ganglion Neurons. *Sci. Rep. 5*, 15960.

(5) More, S. V., Koppula, S., Kim, I. S., Kumar, H., Kim, B. W., and Choi, D. K. (2012) The role of bioactive compounds on the promotion of neurite outgrowth. *Molecules 17*, 6728−6753.

(6) Tang, G., Dong, X., Huang, X., Huang, X. J., Liu, H., Wang, Y., Ye, W. C., and Shi, L. (2015) A natural diarylheptanoid promotes neuronal differentiation via activating ERK and PI3K-Akt dependent pathways. *Neuroscience 303*, 389−401.

(7) Mustafa, A. M., Maggi, F., Papa, F., Kaya, E., Dikmen, M., and Ozturk, Y. (2016) Isofuranodiene: A neuritogenic compound isolated from wild celery (Smyrnium olusatrum L., Apiaceae). *Food Chem. 192*, 782−787.

(8) Hong, S., Iizuka, Y., Lee, T., Kim, C. Y., and Seong, G. J. (2014) Neuroprotective and neurite outgrowth effects of maltol on retinal ganglion cells under oxidative stress. *Mol. Vision 20*, 1456−1462.

(9) Saito, S., Kotera, K., Shigematsu, N., Ide, A., Sugimoto, N., Horii, Z., Hanaoka, M., Yamawaki, Y., and Tamura, Y. (1963) Structure of securinine. *Tetrahedron 19*, 2085−2099.

(10) Nakano, T., Yang, T. H., and Terao, S. (1963) Studies on the alkaloids of Securinega virosa Pax. et Hoffom. I. Structure of virosecurinine. *Tetrahedron 19*, 609−619.

(11) Chirkin, E., Atkatlian, W., and Poree, F. H. (2015) The Securinega alkaloids. *Alkaloids. Chem. Biol. 74*, 1−120.

(12) Iketubosin, G. O., and Mathieson, D. W. (1963) The Isolation of Hordenine and Norsecurinine from Securinega Virosa. The Structure of Norsecurinine. *J. Pharm. Pharmacol. 15*, 810−815.

(13) Beutler, J. A., and Brubaker, A. N. (1987) The chemistry and pharmacology of the securinine alkaloids. *Drugs Future 12*, 957−976.

(14) Dehmlow, E. V., Guntenhöner, M., and Van Ree, T. (1999) A novel alkaloid from Fluggea virosa: 14, 15-epoxynorsecurinine. *Phytochemistry 52*, 1715−1716.

(15) Gan, L. S., Fan, C. Q., Yang, S. P., Wu, Y., Lin, L. P., Ding, J., and Yue, J. M. (2006) Flueggenines A and B, two novel C,C-linked dimeric indolizidine alkaloids from Flueggea virosa. *Org. Lett. 8*, 2285−2288.

(16) Qin, S., Liang, J. Y., Gu, Y. C., and Guo, Y. W. (2008) Suffruticosine, a novel octacyclic alkaloid with an unprecedented skeleton from Securinega suffruticosa (Pall.) Rehd. *Tetrahedron Lett. 49*, 7066−7069.

(17) Zhang, W., Li, J. Y., Lan, P., Sun, P. H., Wang, Y., Ye, W. C., and Chen, W. M. (2011) Chemical synthesis and biological activities of securibega alkaloids. *J. Chin. Pharm. Sci. 20*, 203−217.

(18) Zhang, H., Zhang, C. R., Zhu, K. K., Gao, A. H., Luo, C., Li, J., and Yue, J. M. (2013) Fluevirosines A-C: a biogenesis inspired example in the discovery of new bioactive scaffolds from Flueggea virosa. *Org. Lett. 15*, 120−123.

(19) Beutler, J. A., Karbon, E. W., Brubaker, A. N., Malik, R., Curtis, D. R., and Enna, S. J. (1985) Securinine alkaloids: a new class of GABA receptor antagonist. *Brain Res. 330*, 135−140.

(20) Qu, Z. W., and Zhang, J. T. (1987) Influence of securinine on 5 kinds of neurotransmitter receptors and membrane potential of the frog spinal cord. *Yao Xue Xue Bao 22*, 305−307.

(21) Rognan, D., Boulanger, T., Hoffmann, R., Vercauteren, D. P., Andre, J. M., Durant, F., and Wermuth, C. G. (1992) Structure and molecular modeling of GABAA receptor antagonists. *J. Med. Chem. 35*, 1969−1977.

(22) Lin, X., Guolin, W., and Juntian, Z. (2002) Intracellular calcium involved in the long-term potentiation induced by securinine in dentate gyrus of anesthetized rats. *Planta Med. 68*, 752−753.

(23) Lin, X., and Jun Tian, Z. (2004) Neuroprotection by D-securinine against neurotoxicity induced by beta-amyloid (25−35). *Neurol. Res. 26*, 792−796.

(24) Perez, M., Ayad, T., Maillos, P., Poughon, V., Fahy, J., and Ratovelomanana-Vidal, V. (2016) Synthesis and biological evaluation of new securinine analogues as potential anticancer agents. *Eur. J. Med. Chem. 109*, 287−293.

(25) Perez, M., Ayad, T., Maillos, P., Poughon, V., Fahy, J., and Ratovelomanana-Vidal, V. (2016) Synthesis and Antiproliferative and Metabolic Evaluations of Novel Securinine Derivatives. *ACS Med. Chem. Lett. 7*, 403−407.

(26) Neganova, M. E., Klochkov, S. G., Afanasieva, S. V., Serkova, T. P., Chudinova, E. S., Bachurin, S. O., Reddy, V. P., Aliev, G., and Shevtsova, E. F. (2016) Neuroprotective Effects of the Securinine-Analogues: Identification of Allomargaritarine as a Lead Compound. *CNS Neurol. Disord.: Drug Targets 15*, 102−107.

(27) Zhao, B. X., Wang, Y., Zhang, D. M., Jiang, R. W., Wang, G. C., Shi, J. M., Huang, X. J., Chen, W. M., Che, C. T., and Ye, W. C. (2011) Flueggines A and B, two new dimeric indolizidine alkaloids from Flueggea virosa. *Org. Lett. 13*, 3888−3891.

(28) Wu, Z. L., Zhao, B. X., Huang, X. J., Tang, G. Y., Shi, L., Jiang, R. W., Liu, X., Wang, Y., and Ye, W. C. (2014) Suffrutines A and B: a pair of Z/E isomeric indolizidine alkaloids from the roots of Flueggea suffruticosa. *Angew. Chem., Int. Ed. 53*, 5796−5799.

(29) Gestwicki, J. E., Cairo, C. W., Strong, L. E., Oetjen, K. A., and Kiessling, L. L. (2002) Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. *J. Am. Chem. Soc. 124*, 14922−14933.

(30) Li, F., Jas, G. S., Qin, G., Li, K., and Li, Z. (2010) Synthesis and evaluation of bivalent, peptidomimetic antagonists of the alphavbeta3 integrins. *Bioorg. Med. Chem. Lett. 20*, 6577−6580.

(31) Xiao, Q., Liu, Y., Qiu, Y., Yao, Z., Zhou, G., Yao, Z., and Jiang, S. (2011) Design, synthesis of symmetrical bivalent mimetics of annonaceous acetogenins and their cytotoxicities. *Bioorg. Med. Chem. Lett. 21*, 3613−3615.

(32) Liu, Y., Li, Z., Zhou, G. B., Yao, Z. J., and Jiang, S. (2014) Identification of novel bivalent mimetics of annonaceous acetogenins via a scaffold-hopping strategy. *Bioorg. Med. Chem. Lett. 24*, 1650−1653.

(33) Xiao, Y., Peng, Y., Wan, J., Tang, G., Chen, Y., Tang, J., Ye, W. C., Ip, N. Y., and Shi, L. (2013) The atypical guanine nucleotide exchange factor Dock4 regulates neurite differentiation through modulation of Rac1 GTPase and actin dynamics. *J. Biol. Chem. 288*, 20034−20045.

(34) Radio, N. M., Breier, J. M., Shafer, T. J., and Mundy, W. R. (2008) Assessment of chemical effects on neurite outgrowth in PC12 cells using high content screening. *Toxicol. Sci. 105*, 106−118.

(35) Radio, N. M. (2012) Neurite outgrowth assessment using high content analysis methodology. In *Neurotrophic Factors* (Skaper, S. D., Ed.), pp 247−260, Springer, New York.

(36) Perron, J. C., and Bixby, J. L. (1999) Distinct neurite outgrowth signaling pathways converge on ERK activation. *Mol. Cell. Neurosci. 13*, 362−378.

(37) Hansen, T. O., Rehfeld, J. F., and Nielsen, F. C. (2000) Cyclic AMP-induced neuronal differentiation via activation of p38 mitogen-activated protein kinase. *J. Neurochem. 75*, 1870−1877.

(38) Borodinsky, L. N., Coso, O. A., and Fiszman, M. L. (2002) Contribution of Ca2+ calmodulin-dependent protein kinase II and mitogen-activated protein kinase kinase to neural activity-induced neurite outgrowth and survival of cerebellar granule cells. *J. Neurochem. 80*, 1062−1070.

(39) Sánchez, S., Jiménez, C., Carrera, A. C., Diaz-Nido, J., Avila, J., and Wandosell, F. (2004) A cAMP-activated pathway, including PKA and PI3K, regulates neuronal differentiation. *Neurochem. Int. 44*, 231−242.