Published in final edited form as:

Planta Med. 2012 August ; 78(12): 1357–1362. doi:10.1055/s-0032-1315001.

Isoflavonoids from *Ficus benjamina* and their inhibitory activity on BACE1

Jingqiu Dai¹, Dai Shen², Wesley Y. Yoshida¹, Stephen M. Parrish¹, and Philip G. Williams^{1,3}

¹ Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii, USA, 96822

² Kenyon College, Gambier, Ohio, USA, 43022

³ University of Hawaii Cancer Center, 651 Ilalo Street, Honolulu, Hawaii, USA, 96813

Abstract

Examination of an active extract of the fruit of *Ficus benjamina* var. *nuda* (Miq.) Barrett (Moraceae) has led to the isolation of six new isoflavones and two coumarano-chroman-4-one, along with fifteen known compounds. The structures of the eight new compounds were elucidated on the basis of extensive NMR experiments and mass spectrometric measurements. The inhibitory activity of the compounds on the proteolytic cleavage of amyloid precursor protein by the aspartic protease BACE1 was evaluated. Both coumarano-chroman-4-ones and some isoflavones showed moderate activity in this assay.

Keywords

Ficus benjamina (Moraceae); Isoflavonoid; BACE1

Introduction

Plants and their products have played a central role in medicine for centuries [1]. In particular, the genus *Ficus*, which consists of approximately 800 species, is well-known as medicine in Ayurvedic and Chinese cultures [2]. The bark, roots, leaves, and fruits of this plant are frequently used for the treatment of illnesses ranging from anticancer to antiviral. In general, *Ficus* species are rich sources of polyphenolic compounds. In particular, flavonoids and isoflavonoids are responsible for the extract's strong antioxidant activity that may be useful in preventing diseases involving oxidative stress [2,3]. *Ficus benjamina* var. *nuda* (Miq.) Barrett (Moraceae) [4] commonly known as the weeping fig is an epiphytic evergreen tree [5]. While the fruit extract of *F. benjamina* has been reported to exhibit antitumor [6] and antibacterial activities [7], little is actually known about its chemical constituents [8-10]. As part of a program examining Hawaiian plants for bioactive

Correspondence Prof. Philip Williams, Department of Chemistry, University of Hawaii at Manoa, Honolulu, HI, 96822, USA, philipwi@hawaii.edu Phone: +1 808 956 5720 Fax: 1 808 956 5908.

Supporting Information

NMR spectra of all new compounds are available as Supporting Information. Structures of isolated known compounds and corresponding references are shown in Table S1.

Conflict of Interest: None.

components [3], we discovered that the EtOAc extract of the yellow fruits of *F. benjamina* inhibited the aspartic protease BACE1, which is relevant to Alzheimer's disease. At our initial test concentration of 30 µg/mL, the extract reduced BACE1 activity by approximately 50% (53±3%). Two coumarano-chroman-4-ones and six new isoflavonoids (**1-8**) were isolated from this extract (Fig. 1), in addition to 15 known compounds (See Table 1 in the Supporting Information for the structures). In this paper, we describe the isolation of these new compounds, their structure elucidation, and an assessment of their biological activity against BACE1.

Materials and Methods

General Experimental Procedures, Chemicals and Biochemicals

Optical rotations were measured on a polarimeter at the sodium line (589 nm). UV spectra were obtained on a Hewlett-Packard 8453 spectrophotometer and IR spectra were measured as a thin film on a CaF₂ disk. NMR spectra were acquired on a 500 MHz spectrometer operating at 500 (¹H) or 125 (¹³C) MHz using the residual solvent signals as an internal reference (CD₃OD δ_H 3.30 ppm, δ_C 49.0 ppm; CDCl₃ δ_H 7.26 ppm, δ_C 77.0 ppm). NMR samples were in 3mm Shigemi tubes during NMR analyses. High-resolution mass spectrometric data were obtained on a LC–MSTOF with ES ionization in the positive mode. Gradient separations were performed on a system consisting of solvent delivery modules, a photodiode array detector, an evaporating light scattering detector, and a system controller. RP-HPLC separations were performed on a Luna C₈ column (250 × 10 mm; 5 µm) using a linear gradient of 10–100% MeOH in H₂O over 40 min with a flow rate of 3 mL/min (PDA and ELSD detection) unless otherwise noted. TLC analyses were performed on Si₆₀F₂₅₄ plates and visualized under UV light or by heating after spraying with a 1% solution of anisaldehyde in acetic acid:H₂SO₄ (50:1). The positive control in the BACE1 assay was β-secretase inhibitor IV purchased from Calbiochem. The purity of this standard was greater than >95% as assessed by HPLC. Purity was assessed by LC-MS and NMR, and determined to be 95 % or greater for compounds **1-23** at the time of testing. BACE1 used in the assay was obtained from Calbiochem.

Plant Material

The fruits of *Ficus benjamina* var. *nuda* were collected in Honolulu, Hawaii, in July 2010 adjacent to the University of Hawaii at Manoa campus. The sample was identified by Dr. J. Dai. A voucher specimen (No. 2101) is maintained in the Department of Chemistry at the University of Hawaii at Manoa.

Extraction and Isolation

The whole fresh fruits (1.5 kg) were extracted with EtOAc (3 × 2L, 12 h each time) at room temperature. The combined EtOAc extract (5.0 g) was separated over silica gel (50 g, 250 × 50 mm, 300–400 µm) with a step-gradient of increasing amounts of EtOAc in hexane to afford 20 fractions. The residue from fraction 16 (50 mg; 75% EtOAc in hexane fraction) was further purified by RP-HPLC to afford **5** (*t_R* 31.8 min, 0.6 mg), **3** (*t_R* 35.9 min, 0.5 mg), ficusin A (**10**, *t_R* 36.5 min, 0.8 mg) and gancaonin N (**13**, *t_R* 36.9 min, 3.0 mg). Fraction 17 (60 mg; 85% EtOAc in hexane fraction) was separated by RP-HPLC to yield **4** (*t_R* 23.0 min,

1.5 mg), 2'-methoxygenistein (t_R 28.0 min, 2.0 mg), versulin (t_R 30.2 min, 1.0 mg), **7** (t_R 34.2 min, 0.5 mg), **8** (t_R 34.9 min, 0.5 mg), carpachromene (t_R 35.8 min, 0.5 mg) and atalantoflavone (t_R 36.5 min, 0.5 mg). Separation of fraction 18 (35 mg; 90% EtOAc in hexane fraction) by RP-HPLC afforded **2** (t_R 35.5 min, 0.3 mg), lupiwighteone (**12**, t_R 36.0 min, 0.5 mg) and erysubin B (t_R 36.6 min, 2.5 mg). Separation of fraction 19 (50 mg; 95% EtOAc in hexane fraction) by RP-HPLC afforded parvisoflavone B (t_R 33.5 min, 1.2 mg), eriophorin C (t_R 34.1 min, 1.5 mg), alpinumisoflavone (t_R 35.0 min, 1.0 mg) and derrone (t_R 35.7 min, 3.0 mg). Fraction 20 (60 mg; 100% EtOAc in hexane fraction) was separated by RP-HPLC to yield **6** (t_R 25.0 min, 0.2 mg), erythrinin C (**11**, t_R 25.5 min, 0.4 mg), versulin (t_R 32.3 min, 1.0 mg), **1** (t_R 33.5 min, 0.7 mg) and lespedezol E1 (**9**, t_R 34.5 min, 1.2 mg) [11].

5,7,4'-Trihydroxy-6-(3,7-dimethyl-2,6-octadienyl)isoflavone (1)—light yellow powder; UV (MeOH): $\lambda_{\max}$ (log ϵ) 268 (4.00), 213 (4.07) nm; IR: $\nu_{\max}$ (film) 3380, 3174, 2906, 1657, 1603, 1512, 1431, 1248 cm^{-1} ; For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 407.1850 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{25}\text{H}_{27}\text{O}_5^+$ 407.1853).

5,7,2',4'-Tetrahydroxy-8-(3,7-dimethyl-2,6-octadienyl)isoflavone (2)—light yellow powder; UV (MeOH): $\lambda_{\max}$ (log ϵ) 265 (4.28), 213 (4.36) nm; IR: $\nu_{\max}$ (film) 3405, 1651, 1257 cm^{-1} ; For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 423.1818 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{25}\text{H}_{27}\text{O}_6^+$ 423.1802).

6-[(1*R,6*R**)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5,7,4'-trihydroxyisoflavone (3)**—light yellow powder; $[\alpha]_{\text{D}}^{22} +35$ (c 0.2, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 268 (3.84) nm; IR: $\nu_{\max}$ (film) 3384, 1655, 1611, 1570, 1518, 1426, 1247 cm^{-1} ; For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 405.1708 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_5^+$ 405.1697).

3,5,7-trihydroxy-4'-methoxycoumarano-chroman-4-one (4)—light yellow powder; $[\alpha]_{\text{D}}^{22} +18$ (c 0.2, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 324 (3.56), 299 (3.80) nm; IR: $\nu_{\max}$ (film) 3408, 1627, 1490, 1273, 1172, 1032 cm^{-1} ; For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 339.0476 [$\text{M}+\text{Na}$] $^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{O}_7\text{Na}^+$ 339.0475).

6-(3-methyl-2-buten-1-yl)-3,5,7-trihydroxy-4'-methoxycoumarano-chroman-4-one (5)—light yellow powder; $[\alpha]_{\text{D}}^{22} +3$ (c 0.2, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 325 (3.96), 304 (4.00) nm; IR: $\nu_{\max}$ (film) 3396, 1634, 1278 cm^{-1} ; For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 385.1291 [$\text{M}+\text{H}$] $^+$ (calcd for $\text{C}_{21}\text{H}_{21}\text{O}_7^+$ 385.1282).

5,4'-dihydroxy-2''-hydroxyisopropylidihydrofurano[4,5:7,8]-isoflavone (6)—light yellow powder; $[\alpha]_{\text{D}}^{22} -15$ (c 0.2, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 265 (4.02) nm;

For ^1H (CD_3OD , 500 MHz) and ^{13}C (CD_3OD , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 355.1178 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{19}\text{O}_6^+$ 355.1176).

5,4'-dihydroxy-2''-(2-hydroxy-6-methylhept-5-en-2-yl)dihydrofurano[4,5:7,8]-isoflavone (7)—light yellow powder; $[\alpha]_{\text{D}}^{22}$ -6 (c 0.2, MeOH); UV (MeOH): λ_{max} ($\log \epsilon$) 265 (3.85), 216 (4.01) nm; For ^1H (CDCl_3 , 500 MHz) and ^{13}C (CDCl_3 , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 423.1820 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{27}\text{O}_6^+$ 423.1802).

5,4'-dihydroxy-2''-(2-hydroxy-6-methylhept-5-en-2-yl)dihydrofurano[4,5:6,7]-isoflavone (8)—light yellow powder; $[\alpha]_{\text{D}}^{22}$ -8 (c 0.2, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 265 (3.85), 216 (4.01) nm; For ^1H (CDCl_3 , 500 MHz) and ^{13}C (CDCl_3 , 125 MHz) NMR spectroscopic data, see Tables 1 and 2; HR-ESI-TOF-MS m/z 423.1800 $[\text{M}+\text{H}]^+$, (calcd for $\text{C}_{25}\text{H}_{27}\text{O}_6^+$ 423.1802).

BACE1 Assay— β -Secretase mediated cleavage of amyloid precursor protein was determined as described by Naqvi [12] using an enzyme fragment complementation assay. Test compounds were solubilized in DMSO at the desired concentration and incubated in triplicate with the enzyme for 16 hours in 96-well plates. A DMSO control (1.5 μL) and a standard inhibitor (β -secretase inhibitor IV, Calbiochem, Purity >95% by HPLC) were also tested in triplicate as controls during the bioassays. The chemiluminescence signal was determined using a Fluostar Optima spectrophotometer. Data were analyzed using GraphPad Prism with BACE1 activity calculated as a percent of the positive control. IC_{50} values of pure compounds were calculated using a nonlinear regression analysis function that corresponded to the best one-fit model. The experimentally determined IC_{50} value for the positive control was consistent with the reported data [13].

Results and Discussion

Separation of the crude extract by a series of orthogonal techniques resulted in the isolation of compounds **1-8**, along with fifteen known metabolites. The known compounds were identified as ficusin A (**10**), gancaonin N-(**13**), 2'-methoxygenistein, versulin, carpachromene, atalantoflavone, lupiwighteone (**12**), erysubin B, parvisoflavone B, eriophorin C, alpinumisoflavone, derrone, erythrinin C (**11**), versulin, and lespedezol E1 (**9**) by comparison of their spectroscopic data with the literature (See Table S1 in the supporting information for a complete list of compounds including their structures and corresponding references). Analyses of the spectral data suggested that most of the compounds were isoflavonoids.

The molecular formula of compound **1** was defined as $\text{C}_{25}\text{H}_{26}\text{O}_5$ on the basis of the pseudo-molecular ion observed at m/z 407.1850 during ESI-TOF mass spectrometry analysis. Compound **1** displayed a complex UV spectrum, which included an absorbance at 268 nm with an extinction coefficient indicative of a medium intensity π - π^* transition of a conjugated system. This conclusion was supported by prominent vibrations in the IR spectrum attributable to a conjugated ketone (1657 cm^{-1}) and an aromatic ring (1603 and 1512 cm^{-1}), in addition to absorptions for hydrogen bonded alcohols (3380 cm^{-1}). Taken

together these data indicated that **1** was likely an isoflavonoid, which was supported by the number of oxygen atoms and the 13 degrees of unsaturation required by the molecular formula.

Analysis of the NMR spectra established the planar structure of **1**. Initial inspection of the ^1H NMR spectrum revealed several diagnostic signals, which in conjunction with the MS data, initially led us to suspect **1** was the known isoflavonoid lespedezol E1 (**9**) [14]. These signals included a characteristic singlet at $\delta_{\text{H-2}}$ 8.03, an A_2B_2 system ($\delta_{\text{H-5'}}$ 6.82 d, $^3J_{\text{H,H}} = 8.5$ Hz; $\delta_{\text{H-6'}}$ 7.39 d, $J_{\text{H,H}} = 8.5$ Hz), and an aromatic singlet at $\delta_{\text{H-8}}$ 6.38. While the reported spectroscopic data for **9** [14] were strikingly similar to **1**, careful analyses revealed minor differences that indicated we had isolated a constitutional isomer of **9**. In particular, analyses of the ^1H - ^{13}C HMBC spectrum of **1** (Fig. 2) revealed the presence of long-range HMBC couplings between the following nuclei inconsistent with **9**: C-8a from H-2 and H-8; C-6 from H-8 and H-1". On the basis of these data, **1** was 5,7,4'-trihydroxy-6-(3,7-dimethyl-2,6-octadienyl)isoflavone. Subsequent isolation of an authentic sample of **9** from this extract confirmed our conclusion that **1** was a different compound.

The structure of **2** was defined in a similar fashion. Based on the NMR data, **2** contained an isoflavonoid core similar to **1**. The ^1H NMR spectrum of **2** showed signals consistent with a geranyl group, which were also observed in the spectra of **1** and **9**. Compound **2** ($\delta_{\text{H-8}}$ 8.07) more closely resembled **9** ($\delta_{\text{H-8}}$ 8.07) than **1** ($\delta_{\text{H-8}}$ 8.03) with regards to the placement of the acyclic side chain based on the chemical shift of H-8. The other noticeable difference was the presence of an ABX system in **2** ($\delta_{\text{H-6'}}$ 7.06 d, $^3J_{\text{H,H}} = 8.2$ Hz; $\delta_{\text{H-5'}}$ 6.38 dd, $^3J_{\text{H,H}} = 8.2$, 2.3 Hz; $\delta_{\text{H-3'}}$ 6.40 d, $^3J_{\text{H,H}} = 2.3$ Hz) instead of an A_2B_2 spin system. A comparison of the molecular formulae of **2** and **9** established an additional oxygen atom was present in **2**, which defined the structure as depicted in Figure 1. This compound is an isomer of albanin B [15]; a conclusion that was supported by analyses of the 2D NMR data.

Compound **3** was obtained as a light yellow powder with the molecular formula $\text{C}_{25}\text{H}_{24}\text{O}_5$, as deduced from analysis of the HR-ESI-MS and ^{13}C NMR spectroscopic data. Comparison of the NMR spectra of **3** (Tables 1 and 2) with **1-2** revealed that **3** was also an isoflavonoid, although with an additional unit appended off of that core. Beginning with the vinyl signals observed at $\delta_{\text{H-10''}}$ 4.47 and 4.43, which indicated that a terminal olefin was present in **3**, a 2-isopropenylpentyl moiety (C-2'' to C-6'') was constructed based on detailed analyses of the ^1H - ^1H COSY and HMBC data. The allylic methyl group resonating at $\delta_{\text{H-7''}}$ 1.65 provided the HMBC correlations required to expand this 2-isopropenylpentyl moiety into a limonenyl ring that was substituted at C-3''. Given the isomers **1** and **2** previously described, the limonenyl ring could have been attached to either C-6 or C-8 of the isoflavonoid core of **3**. In the end, C-3'' was connected to C-6 of the isoflavonoid core based on an HMBC correlation (Fig. 3) observed to C-6 from H-2'' of the limonenyl ring. The *trans* orientation of H-3'' and H-4'' was then established on the basis of NOESY (Figure 3) correlations observed between the resonances for H-3'' and H₃-9''. Therefore, **3** was a new isoflavonoid that was a constitutional isomer of ficusin A (**10**) [16], also isolated from this extract, and of the flavanone cathayanon [17].

Compound **4** provided a pseudo-molecular ion peak at m/z 339.0476 $[M+Na]^+$. In conjunction with the ^{13}C NMR spectroscopic data, this pseudo-molecular ion peak established the molecular formula of **4** as $C_{16}H_{12}O_7$, which indicated **4** was smaller than **1-3**. The most noticeable difference in the ^{13}C NMR spectrum of **4**, when compared to **1-3**, was the presence of a new resonance at 112.1 ppm (C-2) suggestive of a ketal functional group. This conclusion was supported by the $^1J_{CH}$ observed from C-2 to the singlet at δ_{H-2} 6.15, which was a chemical shift typical of a proton attached to a dioxygenated carbon. Analysis of the 2D NMR spectra established compound **4** as depicted, with two stereogenic centers at C-2 and C-3. While the relative configuration of similar structures has been assigned using an NOE correlation between H-2 and the bridgehead hydroxyl proton at C-3 [18], we were unable to observe a clear enhancement of either signal in a variety of solvents. Analysis of models indicated that the *cis* and *trans* diastereomers of **4** could not be distinguished using a *J*-based configurational analysis that utilizes proton-carbon couplings either [19]. In the case of the *cis* diastereomer, the bonds between C-2/H-2 and C-3/OH would be eclipsed, which results in the same magnitude of $^2J_{CH}$ coupling between H-2 and C-3 as would be observed for the *trans* diastereomer with a staggered conformation and an *anti* orientation of H-2 and the C-3 hydroxy group. As such, the relative configuration of C-2 and C-3 remains unassigned. A related compound **5**, differing only in the addition of a prenyl residue, was also isolated from this extract. Given the proton resonance for H-6 in **4** (δ_{H-6} 5.90) was absent in **5** (see Table 1) and HMBC correlations were readily observed from H-1'' of the prenyl moiety to C-5, -6, and -7 of the isoflavonoid core, the prenyl group in **5** was attached to C-6.

The structure of compound **6** was assigned by comparison with the other compounds' NMR data. The most obvious differences between **1** and **6** were four additional proton resonances present in **6** at $\delta_{H-2''}$ 4.81 (t, $^3J_{H,H}$ = 8.7 Hz), $\delta_{H-1''}$ 3.24 (d, $^3J_{H,H}$ = 8.7 Hz), $\delta_{H-4''}$ 1.24 (s), and $\delta_{H-5''}$ 1.29 (s). These signals were built into a substituted dihydrofuran ring based on the following HMBC correlations. HMBC correlations from H₂-1'' to C-7 and C-8a of the A-ring established a dihydrofuran ring containing C-7 and C-8a, as well as the oxygen attached to C-7. A series of HMBC correlations from two geminal methyl signals ($\delta_{H-4''}$ 1.24, s and $\delta_{H-5''}$ 1.29, s) to C-2'' of the dihydrofuran ring and to the oxygenated quaternary carbon C-3'', confirmed the presence of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran moiety in **6**. The constitutional isomer **11**, which has been previously reported [11], was also isolated from this extract, and identified based on comparison with the published spectral data.

Analysis of pure samples of compounds **7** and **8** indicated that these metabolites were constitutional isomers. Both compounds were white amorphous solids with the molecular formulae of $C_{25}H_{26}O_6$, as determined from HR-ESI-MS. Their UV spectra exhibited absorption maxima at λ_{max} 216 and 265 nm that are characteristic of the isoflavonoid chromophore. The 1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were comparable with those of **6** and **11**, suggesting both had tetracyclic cores. Rather than the two methyl singlets that were present in the spectra of **6** and **11**, two sets of three methyl singlets were evident in the spectra of **7** and **8** which suggested the acyclic tail was modified. Analysis of the 1H - 1H COSY spectra revealed correlations between two sets of methylene protons (H₂-5'' and H₂-6''), and from the latter to the only sp^2 proton resonance remaining

unassigned (H-7''). HMBC correlations to the C-7'' methine carbon from the methyl singlets (H₃-9''/H₃-10'') expanded this unit at one end of the chain into an isopropenyl moiety, while HMBC correlations from the other methyl singlet (H-4'') to C-5'', -3'' -2'' provided the remaining connectivities needed to secure the planar structures of **7** and **8**. The relative configuration of the side chains of these two molecules was presumably the same as for bonannione B (an isoflavanone derivative of **8**), whose configuration was elucidated by a combination of spectroscopic and quantum mechanical methods [20]. A comparison of the observed ¹³C chemical shifts at the relevant stereocenters of bonannione B with **7** and **8** supports this conclusion. In particular, the measured values of C-4'' and C-5'' for **7** and **8**, respectively (**7**: δ_{C-4''} 22.8; δ_{C-5''} 36.8; **8**: δ_{C-4''} 22.7 δ_{C-5''} 36.6), are consistent with the carbon chemical shifts of 23.1 and 37.3 ppm predicted to be characteristic of a 2*R**,3*R** configuration by DFT calculations (2*R**,3*S**: δ_{C-4''} 19.7; δ_{C-5''} 40.6) [20].

All compounds were screened against BACE1 (Table 3), which is one of the proteases responsible for one of the hallmarks of Alzheimer's Disease, i.e., the buildup of amyloid plaques. Compound **2** was the most active of the new metabolites with an IC₅₀ value at 45 μM, while **4** and **5** had IC₅₀ values of 139 and 91 μM, respectively. Lupiwighteone (**12**), isolated from the extract and identified based on comparison of its NMR data, was the most active of all the compounds tested with an IC₅₀ value of 27 μM [21] (see Table 3; Table S1 in the supporting information). These levels of activity are consistent with results reported for other flavonoid inhibitors of BACE1, in particular myricetin, quercetin, kaempferol, morin and apigenin [22,23]. Comparing the activity of the original crude extract to the results obtain with the pure compounds, suggests that these compounds may not be ultimately responsible for the original activity observed. Whether the initial activity was the result of promiscuous aggregation-based inhibition [24] or a compound that did not survive the purification process, is not known at this time.

To-date, the only other compounds reported from *F. benjamina* are a series of triterpenes [8]. These molecules were not detected in our extract, and so would not explain the activity of the crude extract. Investigation of other *Ficus* spp. have yielded similar isoflavonoids, including ficosin A [16] that was also isolated from this extract, which may suggest that this molecule could be a marker for *Ficus* spp..

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This work was funded by grants from the Alzheimer's Drug Discovery Foundation (281204) and the National Institute of Aging (5R21AG032405-02; 1R01AG039468-01). Funds for the upgrades of the NMR instrumentation were provided by the CRIF program of the National Science Foundation (CH E9974921) and the Elsa Pardee Foundation. The purchase of the Agilent LC-MS was funded by grant W911NF-04-1-0344 from the Department of Defense.

References and Notes

1. Kinghorn AD, Pan L, Fletcher JN, Chai H. The Relevance of Higher Plants in Lead Compound Discovery Programs. J Nat Prod. 2011; 74:1539–1555. [PubMed: 21650152]

2. Sirisha N, Sreenivasulu M, Sangeeta K, Madhusudhana C. Antioxidant Properties of Ficus Species – A Review. *Int J PharmTech Res.* 2010; 2:2174–2182.
3. Dai J, Sorribas A, Yoshida WY, Williams PG. Sebestenoids A-D, BACE1 inhibitors from *Cordia sebestena*. *Phytochemistry.* 2010; 71:2168–2173. [PubMed: 20952040]
4. The plant is known locally as *Ficus benjamina* var *comosa*..
5. Anonymous. [October 17, 2011] Flora of North America. Available at: <http://www.fna.org>
6. Mousa O, Vuorela P, Kiviranta J, Wahab SA, Hiltunen R, Vuorela H. Bioactivity of certain Egyptian *Ficus* species. *J Ethnopharmacol.* 1994; 41:71–76. [PubMed: 8170162]
7. Werfel S, Ruëff F, Przybilla B. [Anaphylactic reaction to *Ficus benjamina* (weeping fig)]. *Der Hautarzt; Zeitschrift Für Dermatologie, Venerologie, Und Verwandte Gebiete.* 2001; 52:935–937.
8. Parveen M, Ghalib RM, Mehdi SH, Rehman SZ, Ali M. A new triterpenoid from the leaves of *Ficus benjamina* (var. *comosa*). *Nat Prod Res.* 2009; 23:729–736. [PubMed: 19418356]
9. Parveen M, Ghalib RM, Mehdi SH, Ul Hasan Mattu R, Ali M. A novel antimicrobial triterpenic acid from the leaves of *Ficus benjamina* (var. *comosa*). *Journal of Saudi Chemical Society.* 2009; 13:287–290.
10. Simo CCF, Kouam SF, Poumale HMP, Simo IK, Ngadjui BT, Green IR, Krohn K. Benjaminamide: A new ceramide and other compounds from the twigs of *Ficus benjamina* (Moraceae). *Biochem Syst Ecol.* 2008; 36:238–243.
11. Deshpande V, Pendse A,RP. Erythrins A, B and C, three new isoflavones from the bark of *Erythrina variegata*. *Indian Journal of Chemistry B.* 1977; 15B:205–207.
12. Naqvi T. Galactosidase Enzyme Fragment Complementation as a High-Throughput Screening Protease Technology. *J Biomol Screen.* 2004; 9:398–408. [PubMed: 15296639]
13. Stachel SJ, Coburn CA, Steele TG, Jones KG, Loutzenhiser EF, Grego AR, Rajapakse HA, Lai M-T, Crouthamel M-C, Xu M, Tugusheva K, Lineberger JE, Pietrak BL, Espeseth AS, Shi X-P, Chen-Dodson E, Holloway MK, Munshi S, Simon AJ, Kuo L, Vacca JP. Structure-Based Design of Potent and Selective Cell-Permeable Inhibitors of Human β -Secretase (BACE-1). *J Med Chem.* 2004; 47:6447–6450. [PubMed: 15588077]
14. Miyasea T, Sanoa M, Yoshinob K, K N. Antioxidants from *Lespedeza homoloba* (II). *Phytochemistry.* 1999; 52:311–319. [PubMed: 10513404]
15. Fukai T, Nomura T. NMR spectra of isoprenoid substituted phenols. 4. Revised structures of albanins D and E, geranylated flavones from *Morus alba*. *Heterocycles.* 1991; 32:499–510.
16. Barm F, Aida M, Hano Y, Nomura T. Ficusins A and B, two new cyclic-monoterpene- substituted isoflavones from *Ficus septic*. *Heterocycles.* 1995; 41:2761–2768.
17. Ni G, Zhang Q-J, Wang Y-H, Chen R-Y, Zheng Z-F, Yu D-Q. Chemical constituents of the stem bark of *Morus cathayana*. *J Asian Nat Prod Res.* 2010; 12:505–515. [PubMed: 20552491]
18. Tahara S, Moriyama M, Orihara S, Ingham J, Kawabata J, Mizutani J. Naturally Occurring Coumaranochroman-4-ones: a New Class of Isoflavonoids from Lupins and Jamaican Dogwood. *Z Naturforsch, C: Biosci.* 1991; 46C:331–340.
19. Matsumori N, Kaneno D, Murata M, Nakamura H, Tachibana K. Stereochemical Determination of Acyclic Structures based on Carbon-Proton Spin-Coupling Constants. A Method of Configuration Analysis for Natural Products. *J Org Chem.* 1999; 64:866–876. [PubMed: 11674159]
20. Rosselli S, Bruno M, Maggio A, Bellone G, Formisano C, Mattia C, Di Micco S, G B. Two New Flavonoids from Bonanna graeca: A DFT-NMR Combined Approach in Solving Structures. *Eur J Org Chem.* 2007; 15:2504–2510.
21. Hashidoko Y, Tahara S, Mizutani J. Isoflavonoids of yellow lupine. Part I. New complex isoflavones in the root of yellow lupine (*Lupinus luteus* L., cv. Barpine). *Agric Biol Chem.* 1986; 50:1797–1807.
22. Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H. Flavonols and flavones as BACE-1 inhibitors: Structure-activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. *BBA-Gen Subjects.* 2008; 1780:819–825.
23. Williams P, Sorribas A, Howes M-JR. Natural products as a source of Alzheimer's drug leads. *Nat Prod Rep.* 2011; 28:48–77. [PubMed: 21072430]

24. McGovern SL, Caselli E, Grigorieff N, Shoichet BK. A Common Mechanism Underlying Promiscuous Inhibitors from Virtual and High-Throughput Screening. *J Med Chem.* 2002; 45:1712–1722. [PubMed: 11931626]

- 1** $R_1 = R_2 = R_4 = H$ $R_3 =$
2 $R_1 = OH$ $R_3 = R_4 = H$ $R_2 =$
9 $R_1 = R_3 = R_4 = H$ $R_2 =$
13 $R_1 = OH$ $R_2 = H$ $R_3 = CH_2CHC(CH_3)_2$ $R_4 = CH_3$

- 3** $R_1 = H$ $R_2 =$
10 $R_2 = H$ $R_1 =$

- 4** $R_1 = H$
5 $R_1 =$

- 6** $R_1 = C(OH)Me_2$
7 $R_1 =$

- 8** $R_1 =$
11 $R_1 = C(OH)Me_2$

- 12** $R_1 =$

Fig. 1.
Chemical structures of compounds **1-8**.

Fig. 3.
Selected HMBC ($H \rightarrow C$) and ROESY ($\leftrightarrow$) correlations of **3**.

Table 1

¹H NMR Spectroscopic Data (500 MHz, δ_{H} (J in Hz)) for Compounds 1-8

H#	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^b	8 ^b
2	8.03, s	8.07, s	8.01, s	6.15, s	6.12, s	8.09, s	7.82, s	7.84, s
6		6.27, s		5.90, brs		6.26, s	6.32, s	
8	6.38, s		6.32, s	5.90, brs	5.93, s			6.36, s
2'	7.39, d (8.5)		7.35, d (8.4)			7.37, d (8.5)	7.39, d (8.2)	7.39, d (7.4)
3'	6.82, d (8.5)	6.40, d (2.3)	6.84, d (8.4)	6.52, d (2.1)	6.52, d (2.3)	6.84, d (8.5)	6.93, d (8.2)	6.90, d (7.4)
5'	6.82, d (8.5)	6.38, dd (8.2, 2.3)	6.84, d (8.4)	6.58, dd (8.4, 2.1)	6.56, dd (8.4, 2.3)	6.84, d (8.5)	6.93, d (8.2)	6.90, d (7.4)
6'	7.39, d (8.5)	7.06, d (8.2)	7.35, d (8.4)	7.31, d (8.4)	7.32, d (8.4)	7.37, d (8.5)	7.39, d (8.2)	7.39, d (7.4)
1''	3.36, d (7.0)	3.42, d (7.0)			3.16, d (7.1)	3.24, d (8.7)	3.21, d (8.7)	3.17, d (8.7)
2''	5.24, t (7.0)	5.21, t (7.0)	5.19, brs		5.13, t (7.1)	4.81, t (8.7)	4.82, t (8.7)	4.81, t (8.7)
3''			4.02, d (9.6)					
4''	1.78, s	1.79, s	3.08, m		1.61, s	1.24, s	1.33, s	1.31, s
5''	1.95, m	1.97, m	1.76, m		1.72, s	1.29, s	1.54, m	1.54, m
6''	2.04, m	2.05, m	2.01, m; 2.20, m				2.10, m	2.12, m
7''	5.02, m	5.03, m	1.65, s				5.13, t (6.3)	5.12, t (6.3)
8''								
9''	1.55, s	1.53, s	1.67, s				1.64, s	1.63, s
10''	1.60, s	1.57, s	4.43, s; 4.47, s				1.69, s	1.69, s
OCH ₃				3.78, s	3.78, s			

^a data recorded in CD₃OD^b data recorded in CDCl₃

Table 2

¹³C NMR Spectroscopic Data for Compounds **1-8** (125 MHz, δ ppm)

Position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^b	8 ^b
2	154.4	156.6	154.5	112.1	112.1	154.5	152.1	152.5
3	124.5	122.2	124.5	79.0	79.0	124.5	123.6	123.5
4	182.1	182.9	182.4	192.8	192.9	182.5	181.2	180.9
4a	106.0	104.4	105.7	100.5	100.5	106.1	106.0	106.4
5	160.4	161.2	161.2	165.8	162.8	164.5	163.5	157.5
6	113.4	100.0	115.5	97.2	111.0	94.9	94.4	109.3
7	165.0	164.5	164.6	170.4	167.8	168.2	166.5	166.4
8	94.2	108.2	94.5	97.2	96.5	104.9	103.5	88.9
8a	157.7	155.3	157.6	160.8	158.7	154.2	153.1	158.4
1'	123.6	111.0	123.5	119.6	119.8	123.0	123.0	123.2
2'	131.4	158.0	131.5	162.2	162.2	131.5	130.4	130.3
3'	116.3	104.5	116.3	97.7	97.7	116.2	115.5	115.5
4'	158.7	160.2	158.7	164.5	164.5	159.0	155.5	155.9
5'	116.3	108.2	116.3	109.7	109.7	116.2	115.5	115.5
6'	131.4	133.1	131.5	126.7	126.8	131.5	130.4	130.3
1''	22.2	22.3	150.3		21.8	27.5	26.6	26.4
2''	123.6	123.7	126.1		123.5	92.9	91.0	91.4
3''	135.6	135.9	38.0		131.9	71.8	73.7	73.8
4''	16.3	16.2	44.5		25.9	25.2	22.8	22.7
5''	40.9	40.8	30.7		17.8	25.4	36.8	36.6
6''	27.7	27.6	31.7				21.9	21.9
7''	125.4	125.3	23.7				123.8	123.9
8''	132.0	132.1	149.5				132.5	132.4
9''	17.7	17.7	19.1				17.5	17.7
10''	25.8	25.8	110.7				25.7	25.7
OCH ₃				56.1	56.1			

^a data recorded in CD₃OD

data recorded in CDCI3

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3

BACE1 Inhibitory Activity

Compound	IC ₅₀ Values (μM)
1	>200
2	45 ± 3
3	>200
4	139 ± 10
5	91 ± 13
6	> 90
7	> 90
8	> 90
9	> 90
10	> 90
11	> 90
Lupiwighteone (12)	27 ± 4
Gancaonin N (13)	42 ± 4
Positive Control	9 ± 2 nM (Lit: 11 nM [13])

The other known compounds were inactive (IC₅₀ > 90 μM)