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Preparation of 7-Substituted Ginkgolide Derivatives: Potent Platelet Activating Factor (PAF) Receptor Antagonists

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Ginkgolides are structurally unique constituents of *Ginkgo biloba* extracts and are known antagonists of the platelet-activating factor (PAF) receptor. Ginkgolide C is 25-fold less potent than ginkgolide B as a PAF receptor antagonist, due to the presence of the 7*β*-OH. Recently, we found that 7α-fluoro ginkgolide B was equipotent to ginkgolide B underlining the critical importance of the 7-position of ginkgolides for PAF receptor activity. Herein we describe the synthesis of a series of ginkgolide B derivatives with modifications at the 7-position and the pharmacological evaluation of these derivatives as assayed by cloned PAF receptors. In two cases nucleophilic attack on a 7*β*-*O*-triflate ginkgolide B did not lead to the expected products, but gave rise to two unprecedented ginkgolide derivatives, one with a novel rearranged skeleton. Furthermore, standard reduction of 7α-azido ginkgolide B did not give the expected primary amine, but instead yielded alkylated amines depending on the solvent employed. Pharmacological testing with cloned PAF receptors showed that ginkgolides with 7α-substitutents had increased affinity compared to 7*β*-substituents, in particular 7α-chloro ginkgolide B, the most potent nonaromatic ginkgolide derivative described to date with a K_i value of 110 nM.

Introduction

Ginkgo biloba L., the last surviving member of a family of trees (*Ginkgoaceae*) that appeared more than 250 million years ago, has been mentioned in the Chinese Materia Medica for more than 2500 years.[1] A standardized *G. biloba* extract (EGb 761) containing terpene trilactones (5−7%) and flavonoids (22−24%) has demonstrated neuromodulatory properties.[2,3] Several clinical studies using EGb 761 have reported positive effects on various neurodegenerative diseases, including Alzheimer's disease,[4−6] and recent studies on healthy volunteers have shown positive effects of EGb 761 on short-term working memory.[7,8]

A number of *G. biloba* constituents have been isolated, including the unique terpene trilactones, i.e., ginkgolides A, B, C, J and M and bilobalide.[9−12] Ginkgolides are diterpenes with a cage skeleton consisting of six five-membered rings, the difference between the five ginkgolides being in the variation in the number and positions of hydroxyl groups on the spirononane framework. Although the molecular basis for the action of *G. biloba* terpene trilactone constituents in the central nervous system (CNS) is only poorly understood, it is known that ginkgolides, particularly ginkgolide B (GB, **1**, Figure 1), is a potent in vitro antagonist of the platelet-activating factor receptor (PAFR).[13,14] The PAFR is a potential target for neurodegenerative diseases,[15] while PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) has been suggested as a retrograde messenger in long-term potentiation (LTP),[16] thus indicating the importance of the PAFR as a target for ginkgolides.

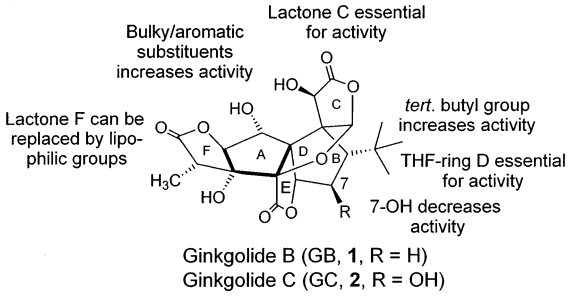

Figure 1. Summary of structure−activity relationship (SAR) studies of ginkgolides as PAFR antagonists. GB (**1**) is ca. 25-fold more potent than GC (**2**).

Structure−activity relationship (SAR) studies of ginkgolides on the PAFR have primarily focused on GB (**1**) (Figure 1) derivatives[17−21] as outlined in Figure 1, e.g., the importance of lactones and the *tert*-butyl group has been investigated, whereas the effect of stereochemistry of hydroxyl groups remains to be examined. Ginkgolide C (GC, **2**, Figure 1), having a hydroxyl group at C-7, is significantly less potent than GB (**1**),[22] which has been explained by the hydrophilic 7*β*-OH of GC (**2**) being next to the lipophilic *tert*-butyl group, which is believed to interact with a lipophilic pocket in the PAFR.[14] Moreover, substitution at 7-OH further decreases antagonistic activity, as demonstrated by 7-*O*-(4-methylphenyl)-GB that was devoid of PAFR activity[23]

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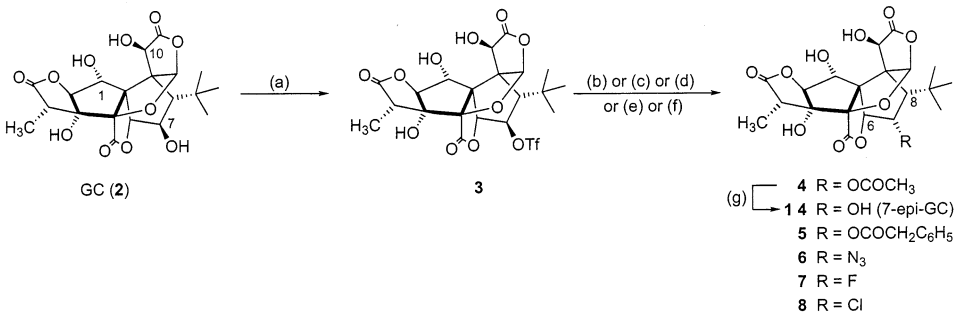
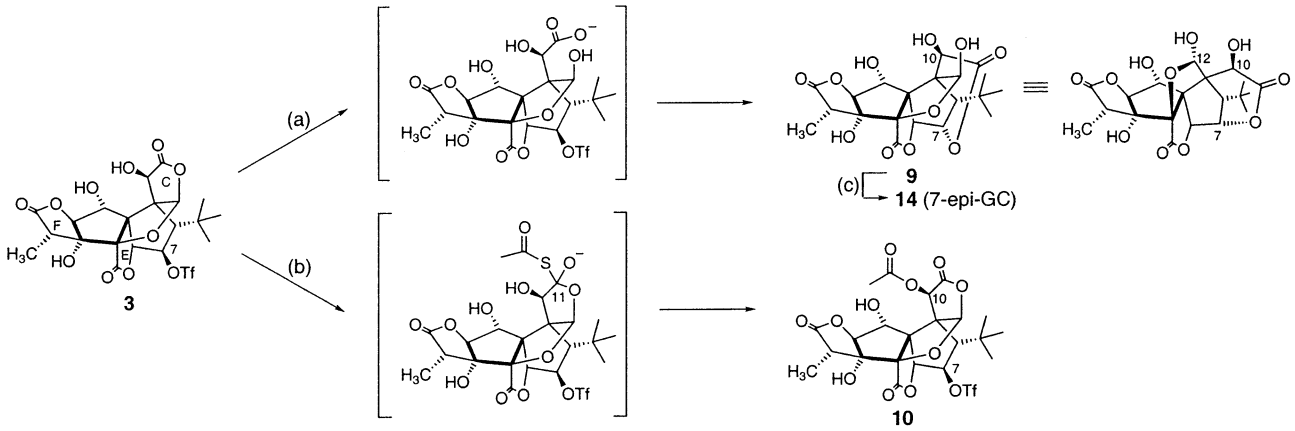
Scheme 1[a]



[a] Reagents: (a) Tf_2O, pyridine, CH_2Cl_2; (b) $NaOCOCH_3$, DMSO; (c) $NaOCOCH_2C_6H_5$, DMSO; (d) NaN_3, DMSO; (e) TBAF, CH_3CN; (f) TBACl, CH_3CN; (g) 2 N NaOH.

Scheme 2[a]



[a] Reagents: (a) MeOH, 2,6-lutidine; (b) $KSCOCH_3$, DMF; (c) 1 M NaOH, acidic workup.

and a 7-*O*-dansyl-GB derivative was also less potent than the parent compound.[22]

Preliminary studies showed that 7α-F-GB was equipotent to GB and 15-fold more potent as a PAFR antagonist as compared to GC (**2**),[24] despite the fact that fluorine is sterically equivalent to OH and more polar than hydrogen.[25] Since configuration of the fluorine atom is α, whereas that of the 7-hydroxyl in GC (**2**) is *β*, it is not clear whether this difference in activity is due to changes in stereochemistry, steric effects, or electronic effects. In the following, we describe a series of ginkgolide derivatives with variation at the critical 7-position and the assessment of these derivatives for their ability to displace radioligand binding to cloned PAFR.

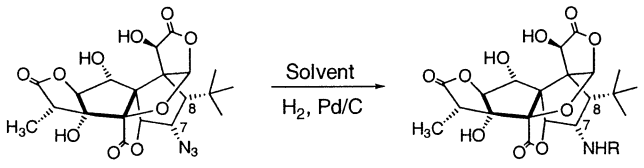
Results

Synthesis. For the synthesis of derivatives with variation at C-7, a crucial intermediate was 7*β*-OTf-GB (**3**). GC (**2**) reacted with remarkable selectivity at 7-OH with trifluoromethanesulfonic (Tf) anhydride giving **3** in very high yield, with no reactions occurring at other hydroxyl groups.[26] This selectivity is noteworthy, as 10-OH, and in some cases 1-OH, of GC (**2**) is generally the more reactive hydroxyl group,[19,21] although we recently observed higher reactivity of 7-OH when acetylation was performed under strong acidic conditions.[27]

7*β*-OTf-GB (**3**) was reacted with various nucleophiles as depicted in Scheme 1 to give derivatives **4**−**8**. The inverted configuration at C-7 was reflected by considerable changes in coupling constants in ^{1}H NMR spectra, i.e., $^3J_{7,8}$ and $^3J_{6,7}$ are 12 and 4 Hz in GC (**2**), whereas they are 3−5 Hz and ca. 0 Hz, respectively, when the configuration at C-7 is inverted. The reactions shown in Scheme 1 generally proceeded in good yield, but in several other cases the nucleophilic substitution did not proceed as expected. When reacting with a soft nucleophile such as NaSCN, only starting material was recovered. Increase in the basicity of the nucleophiles, as in NaCN and aliphatic amines, resulted in a complex mixture of products, probably due to reaction at C-11, as previously described.[28] In addition to the presence of multiple electrophilic sites in **3** it is believed that the steric hindrance of the bulky *tert*-butyl group, which is in close proximity to the reaction site, is responsible for lack of reaction. This assumption is corroborated by reaction of **3** with halogens; incorporation of fluorine (7α-F-GB, **7**) proceeded in high yield and chlorine (7α-Cl-GB, **8**) in slightly lower yield, whereas the larger bromine was introduced in trace amounts only, while no iodine product could be detected. These results were not affected by changing the solvent or the halide counterion.

Steric hindrance may also be the prerequisite for two remarkable products arising from reaction of triflate **3** with MeOH and $NaSCOCH_3$, respectively (Scheme 2). In the former case **3** was dissolved in MeOH and 2,6-lutidine and reacted for 3 days at 70 °C expecting to provide 7α-OMe-GB; instead a new product with a molecular weight similar to GC (**2**), but with a different ^{1}H NMR spectrum, was obtained. Extensive NMR studies revealed a new relactonized structure, neo-

Table 1. Reduction of 7α-N_3-GC (**6**) in Different Solvents



6

compound	solvent	R	yield (%)[a]
11	MeOH	CH_3	95
12	EtOH	CH_2CH_3	68
13	THF	H	98

[a] Isolated yield (after flash chromatography).

ginkgolide C (**9**) (Scheme 2), arising from opening of lactone C, followed by displacement of the triflate group. The structure of this compound **9** with a new rearranged ginkgolide skeleton not encountered earlier was determined by high resolution mass spectrometry, rotating frame nuclear Overhauser and exchange spectroscopy (ROESY), correlation spectroscopy (COSY), and heteronuclear single-quantum coherence (HSQC) NMR experiments (see Supporting Information). Moreover, treatment of neoginkgolide C (**9**) with 1 M NaOH followed by acidic workup resulted in a clean conversion to the thermodynamically more favorable 7-epi-GC (**14**). The reaction between triflate **3** and $NaSCOCH_3$ did not give the expected 7α-$SCOCH_3$-GB, but instead the 10-acetate, while the 7-triflate group remained intact to give **10** (Scheme 2). This product might arise from a reaction by thioacetate at C-11, followed by a transfer of the acetate to 10-OH, tautomerization to thionic acid, and relactonization to give the final product (Scheme 2).

Another interesting feature was the reduction of azide **6** using Pd/C in MeOH under hydrogen. The reaction did not provide the expected amine, but instead gave *N*-methylamine **11** in quantitative yield (Table 1). To investigate this further, the reaction was carried out in EtOH, which gave *N*-ethylamine **12** as the major product. The desired primary amine **13** was obtained when THF was used as solvent (Table 1). This intriguing reaction might provide a convenient way to convert azides directly into various alkylamines, an aspect which is under further investigation.

For the synthesis of 7-epi-GC (**14**) (Scheme 1) various approaches were attempted; GC (**2**) and 4-nitrobenzoic acid were treated with diethyl azodicarboxylate (DEAD) and Ph_3P in a Mitsunobu reaction, but no reaction was observed. Instead, 7β-OTf-GB (**3**) was reacted with KNO_2 and 18-crown-6 ether in a reaction that could potentially lead to 7-epi-GC (**14**) directly from **3**,[29] but only starting material was recovered. Instead, the inversion of 7-OH of GC (**2**) was accomplished using acetate as the nucleophile, followed by basic hydrolysis of the acetate. Acetylation of 7β-OTf-GB (**3**) was achieved by reaction with NaOAc; attempts to use the more reactive CsOAc led to decomposition of **3**, while using $CsOCCF_3$ did not lead to any reaction. The hydrolysis was accomplished by treating **4** with 2 N NaOH to give 7-epi-GC (**14**) in 95% yield (Scheme 1).

Since the importance of sterochemistry at 1- and 10-OH of ginkgolides for PAFR activity is not known, we were interested in preparing 1- and 10-OTf derivatives of GB. However, reaction of GB (**1**) with Tf anhydride failed to give any desired product, and instead several elimination products were obtained, originating from elimination of 3-OH, as well as 1-OH. Similarly, reaction of GB (**2**) with *p*-toluenesulfonyl (tosyl) chloride gave two main products, both with a tosyl group at 10-OH, and elimination of either 3-OH or both 3- and 1-OH, respectively.

To further investigate the importance of stereochemistry at C-7, we planned a series of corresponding 7β-substituted derivatives. Thus 7-epi-GC (**14**) was reacted with Tf anhydride, but only starting material was recovered. This lack of reaction is most likely due to a change in steric environment of 7α-OH, relative to 7β-OH, due to the *tert*-butyl group.

Finally, the observation that an aromatic substituent at 10-OH of GB (**1**) and GC (**2**) increases the antagonistic effect at PAFR[19,21,22] led us to investigate whether a similar increase would be observed for 7α-GB derivatives. Benzylated derivatives **15**−**18** (Table 3) were therefore prepared, following previously described procedures.[19,21]

Pharmacology. Derivatives **4**−**9** and **11**−**18** were tested for their ability to displace [^{3}H]-WEB 2086 binding to cloned PAFR (Tables 2 and 3) using membrane fractions from hearts and skeletal muscles of PAFR transgenic mice, as previously described.[30] In these fractions, GB (**1**) had a K_i value of 0.88 μM, thus being similar to the previously determined K_i value of 0.56 μM.[22]

Derivatives with 7α-substituents were all more potent than GC (**2**) (Table 2), but within this group of compounds there were marked differences; 7α-OAc, 7α-OCOBn, 7α-OH, and 7α-NH_2 ginkgolide B derivatives all had K_i values between 2.4 and 7.8 μM, thus being slightly more potent than GC (**2**), but still significantly less potent than GB (**1**). Compounds **6**, **7**, **11**, and **12** with 7α-N_3, 7α-F, 7α-NHMe, and 7α-NHEt substituents, respectively, were equipotent to GB (**2**) with K_i values in the range of 0.55−1.62 μM. Finally, 7α-chloro ginkgolide B (**8**) was the most potent compound in this series with a K_i value of 0.11 μM, thus being the most potent nonaromatic ginkgolide derivative described.

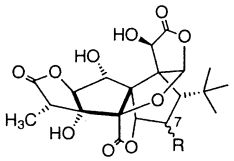
The relactonized compound **9** (Scheme 2) was also tested for binding to PAFR and was found to be essentially inactive with a K_i value > 40 μM. Benzyl derivatives were investigated as well (Table 3), and as expected a 10-*O*-benzyl group significantly improved the affinity for PAFR. Compounds **15** and **17** were the most potent with K_i values of 0.12 and 0.10 μM, respectively, while 10-*O*-benzyl-GC (**16**) and 10-*O*-benzyl-7-epi-GC (**18**) were slightly less potent with K_i values of 1.67 and 0.60 μM, respectively.

Discussion

Herein the effect of modification of the C-7 position of ginkgolides has been investigated by synthesis of 15 analogues (**4**−**18**), which have been prepared from native ginkgolides GB (**1**) and GC (**2**) and evaluated with the cloned PAFR.

The derivatives with 7α-substituents were prepared by nucleophilic substitution of 7β-OTf-GB (**3**), but in

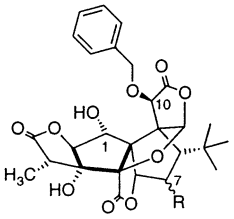
Table 2. K_i Values of Ginkgolide B (**1**) and C (**2**), and 7-Epi Derivatives



compound	R	K_i (μM)[a]	compound	R	K_i (μM)[a]
GB (1)	H	0.88	7α-F-GB (**7**)	α-F	0.99
GC (2)	β-OH	12.6[b]	7α-Cl-GB (**8**)	α-Cl	0.11
7α-OAc-GB (**4**)	α-OAc	7.84	7α-NHMe-GB (**11**)	α-NHMe	0.61
7-epi-GC (**14**)	α-OH	4.26	7α-NHEt-GB (**12**)	α-NHEt	1.62
7α-$OCOCH_2Ph$-GB (**5**)	α-$OCOCH_2Ph$	2.40	7α-NH_2-GB (**13**)	α-NH_2	8.64
7α-N_3-GB (**6**)	α-N_3	0.55			

[a] Inhibition of [^{3}H]-WEB 2086 binding. Values are means of two independent experiments performed in triplicate. [b] Data from previous studies.[22]

Table 3. K_i Values of Benzylated Derivatives



compound	R	K_i (μM)[a]
10-OBn-GB (**15**)	H	0.12
10-OBn-GC (**16**)	β-OH	1.67
10-OBn-7α-F-GB (**17**)	α-F	0.10
10-OBn-epi-GC (**18**)	α-OH	0.60

[a] Inhibition of [^{3}H]-WEB 2086 binding. Values are means of two independent experiments performed in triplicate.

several cases these reactions did not proceed as expected. Attempts to introduce larger halogens such as bromine and iodine, as well as other nucleophiles, failed. In the reaction between **3** and $NaSCOCH_3$, the 7-OTf group remained intact; instead the reaction presumably took place at C-11 of lactone C to give **10** (Scheme 2). Reaction of **3** with MeOH and 2,6-lutidine gave rise to neoginkgolide C (**9**) with a novel rearranged skeleton (Scheme 2). This compound had a K_i value > 40 μM, which is in agreement with previous studies which showed that modification of lactone C significantly reduced PAFR binding (Figure 1).[28]

During the reduction of azide **6** interesting observations were made; when carried out in MeOH this reaction did not give the expected primary amine **13**, but gave *N*-methylamine **11** instead and when carried out in EtOH the reduction gave *N*-ethylamine **12**. Besides being a potential novel procedure for a direct conversion of azides into alkylated amines, it also raises mechanistic considerations. Treatment of **13** with Pd/C in MeOH gave **11**, albeit in lower yield than starting from azide **6**, and with several side products. This implies that in the preparation of **11** and **12** the azide **6** is initially reduced to amine **13**, which then reacts instantly with the oxidized solvent to form an imine that is reduced to yield the products. Further studies are required to confirm this pathway, as well as the generality of this reaction. These studies are ongoing in our laboratory.

The prepared derivatives were tested for binding to cloned PAFR (Tables 2 and 3). It was observed that 7α-derivatives were slightly more potent than the 7β-derivatives, as GC (**2**) had a K_i value of 12.6 μM, while for 7-epi-GC (**14**), the K_i is 4.26 μM. Likewise 7α-OAc-GB (**4**) had a K_i of 7.84 μM, while 7β-OAc-GB (i.e., 7-OAc-GC) had been shown to have low potency comparable to that of GC (**2**).[27] Furthermore, the 7α derivative **5** is a reasonable potent PAFR antagonist with a K_i value of 2.40 μM (Table 2) in contrast to 7β-*O*-(4-methylphenyl)-GB, which is devoid of PAFR activity.[23] However as these differences are relatively small, the C-7 configuration seems to play only a minor role for PAFR antagonistic activity.

The nature of the 7α-substituent, on the other hand, had a major impact on the binding to PAFR. Introduction of azide and fluorine groups yielded compounds that were equipotent to GB (**1**) (Table 2), while introduction of a chlorine as in 7α-Cl-GB (**8**) leads to a dramatic increase in binding affinity. Thus **8** with $K_i = 0.11$ μM, was 115-fold more potent than GC (**2**) and 8-times more potent than GB (**1**), thereby being the most potent nonaromatic ginkgolide derivative described to date. It appears that polar groups that can form hydrogen bonds decrease activity, as seen in 7-epi-GC (**14**) and 7α-NH_2-GB (**13**), with a hydroxyl and a primary amino group, respectively, at C-7, both having binding affinities lower than GB. On the other hand, alkylation of **13** to give 7α-NHMe-GB (**11**) and 7α-NHEt-GB (**12**) led to significant increases in binding affinities, with K_i values of 0.61 μM and 1.62 μM, respectively. Rationalization of these trends requires further molecular mechanistic studies of the ginkgolide-PAFR interaction, which are ongoing in our laboratory.

Introduction of benzyl groups in the 10-OH position of ginkgolides is known to improve affinity for PAFR,[19,21,22] but whether this is true for 7α-substituted derivatives was not known. The affinities of 10-*O*-benzyl-7α-F-GB (**17**) and 10-*O*-benzyl-7-epi-GC (**18**) (Table 3) shows 10- and 7-fold improved affinity compared to their nonbenzylated derivatives. Thus 10-benzylation of 7α-substituted derivatives improves binding affinity as previously shown for other ginkgolide derivatives.

In conclusion we have synthesized several ginkgolide derivatives with modifications at C-7. These syntheses have led to several unexpected products such as **9** and **10**, as well as a potential novel procedure for a direct conversion of azides into alkylamines. Moreover, contrary to previous convictions, we have shown that

introducing lipophilic groups in the C-7 position, in particular chlorine, significantly improves PAFR affinity compared to GB (**1**). This gives rise to new possibilities for improving affinity of ginkgolides to PAFR, as well as providing material for future SAR studies of ginkgolides and PAFR.

Experimental Section

Chemistry. General Procedures. GB (**1**) and GC (**2**) was obtained by extraction of leaves from *G. biloba*, purification by column chromatography and recrystallization as previously described.[31,32] The purity was >98% as estimated by ^{1}H NMR. Unless otherwise noted, materials were obtained from a commercial supplier and were used without further purification. Solvents were dried by eluting through alumina columns. Flash column chromatography was performed using ICN silica gel (32−63 mesh). Thin-layer chromatography was carried out using precoated silica gel 60 F_{254} plates with thickness of 0.25 mm. Plates were heated and spots were detected by monitoring at 254 nm. ^{1}H and ^{13}C NMR spectra were obtained on Bruker DMX 300 MHz or Bruker DMX 400 MHz spectrometers and are reported in parts per million (ppm) relative to internal solvent signal, with coupling constants (*J*) in hertz (Hz). HSQC, COSY, and ROESY spectra were obtained on a Bruker DMX 400 MHz or Bruker DMX 500 MHz spectrometer. Analytical and preparative high performance liquid chromatography (HPLC) were performed on a HP 1100 LC instrument with detection by UV at 219 and 254 nm. Preparative HPLC was performed using a 10 μm C18 reversed-phase VYDAC column (250 × 22 mm) with a flow of 4 mL/min and eluting with either eluent A or B. A: water/CH_3CN/TFA (60:40:0.1), raising to (40:60:1) after 20 min. B: water/CH_3CN/TFA (65:35:0.1), raising to (40:60:1) after 20 min. Analytical HPLC were performed using a 5 μm C18 reversed-phase Phenomenex Luna column (150 × 4.60 mm), with a flow of 1 mL/min eluting with water/CH_3CN/TFA 70:30:0.1. Compounds **9** and **14** were eluted with water/CH_3CN/TFA 80:20:0.10 and compounds **11**−**13** with water/CH_3CN 90:10. Accurate mass determinations were performed on a JEOL JMS−HX110/100A HF mass spectrometer using a 3-nitrobenzyl alcohol (NBA) matrix and Xe ionizing gas and are within ±10 ppm of theoretical values. All were crystalline compounds that decompose above 200 °C

7-Trifluoromethanesulfonyloxy Ginkgolide B (3). In a mixture of dry CH_2Cl_2 (1.0 mL) and dry pyridine (1.5 mL) was dissolved GC (**2**) (184 mg, 0.42 mmol). The solution was cooled to −20 °C under argon, and trifluoromethane sulfonic anhydride (78 μL) was added dropwise. The reaction was stirred at −20 °C for 2 h and allowed to warm to room temperature over 1 h. The solvent was removed in vacuo, the residue was dissolved in EtOAc (30 mL) and washed with 1 N HCl (3 × 20 mL) and brine (10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/CH_3OH/EtOAc (30:1:1 and 20:1:1) to obtain **3** as white crystals (232 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (s, *tert*-butyl), 1.13 (d, J = 9.6, CH_3), 2.22 (d, J = 16.5, 8-H), 2.82 (q, J = 9.6, 14-H), 4.15 (dd, J = 8.0, 5.9, 1-H), 4.73 (d, J = 8.0, 2-H), 5.08 (d, J = 7.4, 10-H), 5.24 (dd, J = 16.5, 5.6, 7-H) 5.41 (d, J = 5.6, 6-H), 5.54 (d, J = 5.9, 1-OH), 6.20 (s, 12-H), 6.62 (s, 3-OH), 7.63 (d, J = 7.4, 10-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.1, 29.2 (3C), 32.6, 42.1, 49.0, 64.2, 68.1, 69.4, 74.4, 75.2, 84.0, 86.3, 93.2, 99.9, 109.4, 118.6 (q, $^1J_{CF}$ = 316.6, CF_3), 173.9, 176.9, 179.2. HRMS: $C_{21}H_{23}F_3O_{13}S$ requires M + 1 at *m*/*z* 573.0890; found, 573.0872.

7α-*O*-Acetate Ginkgolide B (4). Sodium acetate (163 mg, 1.99 mmol) and **3** (228 mg, 0.39 mmol) were dissolved in DMSO (3 mL), and the solution was stirred at 65 °C for 17 h. The solvent was removed in vacuo, and the residue was partitioned between 1 N HCl (20 mL) and EtOAc (25 mL). The aqueous phase was extracted with EtOAc (3 × 25 mL), the combined organic phases were washed with brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/EtOAc/MeOH (10:1:1) to obtain **4** as white crystals (144 mg, 75%). A portion (20 mg) of this was recrystallized (MeOH/H_2O) for pharmacological evaluation (9 mg). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.1, CH_3), 1.10 (s, *tert*-butyl), 1.91 (d, J = 3.3, 8-H), 2.06 (s, $COCH_3$), 2.84 (q, J = 7.1, 14-H), 4.03 (dd, J = 7.7, 3.6, 1-H), 4.66 (d, J = 7.7, 2-H), 4.86 (d, J = 3.6, 1-OH), 5.01 (s, 6-H), 5.15 (d, J = 6.8, 10-H), 5.25 (d, J = 3.3, 7-H), 6.16 (s, 12-H), 6.52 (s, 3-OH), 7.42 (d, J = 6.8, 10-OH). ^{13}C NMR (100 MHz, CD_3OD): δ 8.0, 21.0, 30.7 (3C), 33.8, 43.4, 53.2, 70.1, 70.3, 72.5, 75.3, 79.6, 80.9, 84.6, 92.6, 99.8, 112.7, 171.3, 171.5, 175.0, 178.1. HPLC−UV: 96%. HRMS: $C_{22}H_{27}O_{12}$ requires M + 1 at *m*/*z* 483.1503; found, 483.1525.

7α-*O*-Phenylacetate Ginkgolide B (5). Sodium phenylacetate (44 mg, 0.28 mmol) and **3** (32 mg, 0.07 mmol) were dissolved in DMSO (0.8 mL) and heated at 65 °C for 5 h, the solvent was removed in vacuo, the residue was partitioned between 1 N HCl (10 mL) and EtOAc (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with 1 N HCl (2 × 10 mL), water (5 × 10 mL), and brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash column chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1), recrystallized (MeOH), and further purified by preparative HPLC (eluent A) to give **5** (10 mg, 26%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (s, *tert*-butyl), 1.12 (d, J = 6.9, CH_3), 1.93 (d, J = 2.9, 8-H), 2.84 (q, J = 6.9, 14-H), 3.70 (s, CH_2), 4.04 (dd, J = 3.6, 7.7, 1-H), 4.62 (d, J = 7.7, 2-H), 4.72 (d, J = 3.6, 1-OH), 4.99 (s, 6-H), 5.16 (d, J = 6.6, 10-H), 5.26 (d, J = 2.9, 7-H), 6.16 (s, 12-H), 6.48 (s, 3-OH), 7.25−7.35 (m, aromatic, 5H) 7.46 (d, J = 6.6, 10-OH). ^{13}C NMR (100 MHz, CD_3OD): δ 8.0, 30.8 (3C), 33.8, 42.2, 43.4, 53.3, 70.1, 70.3, 72.5, 75.2, 80.2, 81.0, 84.6, 92.6, 99.9, 112.7, 128.4, 129.7, 130.5, 134.7, 171.4, 172.2, 175.0, 178.1. HPLC−UV: 98%. HRMS: $C_{28}H_{31}O_{12}$ requires M + H at *m*/*z* 559.1816; found, 559.1826.

7α-Azido Ginkgolide B (6). Sodium azide (87 mg, 1.34 mmol) and **3** (153 mg, 0.27 mmol) were dissolved in DMSO (2.5 mL), and the solution was heated at 65 °C for 26 h. The solvent was removed in vacuo. The solid was partitioned between saturated aqueous NH_4Cl (20 mL) and EtOAc (20 mL), and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give the **6** as white crystals (109 mg, 88%). A portion (23 mg) of this was recrystallized (MeOH/H_2O) for pharmacological evaluation (12 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.12 (d, J = 7.1, CH_3), 1.13 (s, *tert*-butyl), 1.80 (d, J = 4.0, 8-H), 2.73 (q, J = 7.1, 14-H), 4.05 (dd, J = 7.6, 3.6, 1-H), 4.70 (d, J = 7.6, 2-H), 4.74 (d, J = 4.0, 7-H), 4.97 (d, J = 3.6, 1-OH), 5.06 (d, J = 6.0, 10-H), 5.25 (s, 6-H), 6.10 (s, 12-H), 6.52 (s, 3-OH), 7.05 (d, J = 6.0, 10-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.8, 30.1 (3C), 32.7, 41.6, 51.6, 67.2, 68.4, 68.5, 71.0, 73.7, 79.6, 82.9, 92.9, 98.2, 110.3, 169.5, 173.4, 176.2. HPLC−UV: 99%. HRMS: $C_{20}H_{24}O_{10}N_3$ requires M + 1 at *m*/*z* 466.1462; found, 466.1445.

7α-Fluoro Ginkgolide B (7). Tetrabutylammonium fluoride hydrate (37 mg, 0.14 mmol) and **3** (62 mg, 0.11 mmol) were dissolved in CH_3CN (1 mL) and heated at 80 °C for 1.5 h. The solvent was removed in vacuo, the residue was partitioned between 1 N HCl (10 mL) and EtOAc (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (2 × 15 mL) and brine (2 × 15 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash column chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) followed by preparative HPLC (eluent B) to give **7** as white crystals (34 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.25 (s, *tert*-butyl), 1.26 (d, J = 7.1, CH_3), 1.94 (dd, $^2J_{HF}$ = 45.5, J = 2.3, 8-H), 3.05 (q, J = 7.1, 14-H), 4.24 (d, J = 8.0, 1-H), 4.59 (d, J = 8.0, 2-H), 5.18 (s, 10-H), 5.35 (d, $^2J_{HF}$ = 10.9, 6-H), 5.38 (dd, $^1J_{HF}$ = 48.8, J = 2.3, 7-H), 6.14 (s, 12-H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.0, 29.5 (3C), 32.9, 42.4, 53.7

($^2J_{CF}$ = 20.4 Hz), 68.8, 69.1, 71.5, 74.5, 79.5 ($^2J_{CF}$ = 36.0 Hz), 83.7, 91.7, 96.9 ($^1J_{CF}$ = 184.2 Hz), 98.8, 111.6, 171.2, 173.9, 177.1. HRMS: $C_{20}H_{23}FO_{10}$ requires M + 1 at *m/z* 443.1354; found, 443.1370.

7α-Chloro Ginkgolide B (8). Tetrabutylammonium chloride (86 mg, 0.31 mmol) and **3** (36 mg, 0.06 mmol) were dissolved in CH_3CN (1.4 mL) and heated at 80 °C for 12 h. The solvent was removed in vacuo, and the residue partitioned between 1 N HCl (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with water (4 × 10 mL) and brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by preparative HPLC (eluent B) and recrystallized ($CH_3CN/CHCl_3$) to give **8** as white crystals (9 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.0, CH_3), 1.17 (s, *tert*-butyl), 2.19 (d, J = 4.2, 8-H), 2.85 (q, J = 7.0, 14-H), 4.03 (dd, J = 7.7, 3.3, 1-H), 4.63 (d, J = 3.3, 1-OH), 4.68 (d, J = 7.8, 2-H), 4.84 (d, J = 4.2, 7-H), 5.13 (d, J = 6.4, 10-H), 5.26 (s, 6-H), 6.17 (s, 12-H), 6.53 (s, 3-OH), 7.57 (d, J = 6.4, 10-OH). ^{13}C NMR (100 MHz, CD3OD): δ 8.7, 31.2 (3C), 34.5, 42.5, 54.2, 65.1, 69.0, 70.0, 72.2, 74.4, 83.7, 84.2, 90.7, 99.4, 111.3, 169.9, 174.4, 177.1. HPLC−UV: 98%. HRMS: $C_{20}H_{24}O_{10}Cl$ requires M + 1 at *m/z* 459.1058; found, 459.1052.

Neoginkgolide C (9). Triflate **3** (27 mg, 0.05 mmol) was dissolved in dry MeOH (470 μL) and 2,6-lutidine (150 μL) was added, and the reaction mixture was heated at 65 °C for 3 days. The solvent was removed in vacuo and the residue purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) to give the crude product, which was further purified by preparative HPLC (eluent A) to give **9** as white crystals (6 mg, 29%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.20 (m, CH_3 and *tert*-butyl), 1.64 (dd, J = 1.4, 1.2, 8-H), 3.72 (q, J = 7.1, 14-H), 4.46 (d, J = 8.0, 2-H), 4.59 (d, J = 1.2, 10-H), 4.72 (d, J = 8.0, 1-H), 5.00 (dd, J = 1.4, 1.3, 7-H), 5.14 (d, J = 1.3, 6-H), 5.96 (s, 12-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.6, 30.2 (3C), 32.7, 41.3, 47.8, 60.2, 66.9, 67.8, 73.6, 75.6, 82.5, 83.4, 92.7, 93.7, 104.9, 170.2, 171.7, 177.6. HPLC−UV: 98%. HRMS: $C_{20}H_{24}O_{11}$ requires M + Na at *m/z* 463.1216; found, 463.1245.

10-*O*-Acetate-7-trifluoromethanesulfonyloxy Ginkgolide B (10). Potassium thioacetate (4 mg, 0.035 mmol) and **3** (3 mg, 0.006 mmol) were dissolved in dry DMF (35 μL) and heated at 40 °C for 3 h. The solvent was removed in vacuo, and residue was partitioned between water (10 mL) and EtOAc (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (5 × 10 mL) and brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) to give **10** (1.3 mg, 18%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (s, *tert*-butyl), 1.13 (d, J = 7.1, CH_3), 2.21 (s, $COCH_3$), 2.31 (d, J = 12.6, 8-H), 2.85 (q, J = 7.1, 14-H), 4.08 (dd, J = 5.9, 5.8, 1-H), 4.75 (d, J = 5.9, 2-H), 5.09 (dd, J = 12.6, 4.2, 7-H), 5.48 (d, J = 4.2, 6-H), 6.13 (s, 10-H), 6.33 (s, 12-H), 6.53 (s, 3-OH), 6.71 (d, J = 5.8, 1-OH). HRMS: $C_{23}H_{26}O_{14}F_3S$ requires M + 1 at *m/z* 615.0995; found, 615.1016.

7α-*N*-Methylamino Ginkgolide B (11). Azide **6** (38 mg, 0.08 mmol) was dissolved in dry MeOH (1.2 mL), and Pd/C (10%, 12 mg) was added. The suspension was stirred under an atmosphere of H_2 for 48 h. The solvent was removed in vacuo, EtOAc (10 mL) was added, and the solution was filtered through Celite. The solvent was removed in vacuo, and the crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give white crystals, which were recrystallized (MeOH) to give **11** (23 mg, 65%) as white crystals. ^{1}H NMR (400 MHz, CD_3OD): δ 1.22 (m, *tert*-butyl and CH_3), 1.89 (d, J = 4.4, 8-H), 2.47 (s, CH_3), 3.06 (q, J = 7.0, 14-H), 3.46 (d, J = 4.4, 7-H), 4.24 (d, J = 7.2, 1-H), 4.53 (d, J = 7.2, 2-H), 5.05 (s, 6-H), 5.31 (s, 10-H), 6.17 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.2, 31.3 (3C), 33.4, 34.3, 43.3, 53.5, 69.1, 69.6, 70.8, 72.6, 75.4, 79.3, 84.4, 94.5, 100.6, 112.2, 172.6, 174.8, 178.4. HPLC−UV: 97%. HRMS: $C_{21}H_{28}O_{10}N$ requires M + 1 at *m/z* 454.1713; found, 454.1719.

7α-*N*-Ethylamino Ginkgolide B (12). Azide **6** (48 mg, 0.10 mmol) was dissolved in dry EtOH (1.0 mL), and Pd/C (10%, 15 mg) was added. The suspension was stirred under an atmosphere of H_2 for 48 h. The solvent was removed in vacuo, EtOAc (10 mL) was added, and the solution was filtered through Celite. The solvent was removed in vacuo and the residue purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give white crystals, which were recrystallized (MeOH) to give **12** (22 mg, 47%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.11 (t, J = 7.1, CH_3), 1.23 (m, *tert*-butyl and CH_3), 1.89 (d, J = 4.5, 8-H), 2.57 (dq, J = 7.1, 12.0, CH_2, 1H), 2.94 (dq, J = 7.1, 12.0, CH_2, 1H), 3.06 (q, J = 7.1, 14-H), 3.56 (d, J = 4.5, 7-H), 4.22 (d, J = 7.3, 1-H), 4.53 (d, J = 7.3, 2-H), 5.08 (s, 6-H), 5.27 (s, 10-H), 6.16 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.2, 15.5, 31.3 (3C), 34.4, 41.6, 43.3, 53.5, 67.5, 69.2, 70.8, 72.6, 75.3, 80.0, 84.4, 94.4, 100.6, 112.3, 172.6, 174.9, 178.4. HPLC−UV: 98%. HRMS: $C_{22}H_{29}O_{10}N$ requires M + 1 at *m/z* 468.1870; found, 468.1867.

7α-Amino Ginkgolide B (13). Azide **6** (10 mg, 0.02 mmol) was dissolved in dry THF (0.4 mL), and Pd/C (10%, 8 mg) was added. The suspension was stirred under an atmosphere of H_2 for 14 h. EtOAc (10 mL) was added and the solution filtered through Celite. The solvent was removed in vacuo to give white crystals which were recrystallized (MeOH) to give **13** as white crystals (5 mg, 49%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.20 (s, *tert*-butyl), 1.23 (d, J = 7.1, CH_3), 1.90 (d, J = 3.2, 8-H), 3.08 (q, J = 7.1, 14-H), 3.83 (d, J = 3.2, 7-H), 4.26 (d, J = 7.0, 1-H), 4.51 (d, J = 7.0, 2-H), 5.01 (s, 6-H), 5.04 (s, 10-H), 6.18 (s, 12-H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.3, 31.0 (3C), 34.1, 43.2, 54.7, 60.3, 68.9, 70.9, 72.6, 74.9, 83.9, 84.4, 95.0, 100.3, 111.8, 172.3, 174.4, 178.4. HPLC−UV: 97%. HRMS: $C_{42}H_{26}O_{10}N$ requires M + H at *m/z* 440.1557; found, 440.1594.

7-Epi-ginkgolide C (14). Acetate **4** (42 mg, 0.087 mmol) was dissolved in a mixture of MeOH and 2 N NaOH (2:1, 1.8 mL) and stirred for 5 h. HCl (1 N) was added, and the aqueous phase was extracted with EtOAc (3 ×20 mL). The combined organic phases were washed with brine (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to give **14** (36 mg, 95%) as white crystals. A portion (15 mg) of this was recrystallized ($MeOH/H_2O$) for pharmacological evaluation (6 mg). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 7.0, CH_3), 1.12 (s, *tert*-butyl), 1.64 (d, J = 2.7, 8-H), 2.89 (q, J = 7.0, 14-H), 4.11 (dd, J = 4.5, 6.8, 1-H), 4.37 (dd, J = 2.7, 6.3, 7-H), 4.59 (d, J = 6.8, 2-H), 4.98 (s, 6-H), 5.04 (d, J = 2.7, 10-H), 5.52 (d, J = 6.3, 7-OH), 5.64 (d, J = 4.5, 1-OH), 6.13 (s, 12-H), 6.45 (s, 3-OH), 6.73 (d, J = 2.7, 10-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.0, 30.2 (3C), 32.6, 41.4, 52.3, 68.8, 69.6, 71.5, 73.4, 76.2, 80.8, 82.8, 92.9, 98.6, 109.7, 170.0, 172.7, 176.3. HPLC−UV: 98%. HRMS: $C_{20}H_{25}O_{11}$ requires M + 1 at *m/z* 441.1397; found, 441.1395.

10-*O*-Benzyl Ginkgolide B (15). Synthesis and analytical data as previously described.[19,21]

10-*O*-Benzyl Ginkgolide C (16). K_2CO_3 (31 mg, 0.22 mmol) was added to a solution of **2** (12 mg, 0.02 mmol) dissolved in DMF (0.2 mL) followed by addition of benzyl chloride (30 μL, 0.26 mmol). The suspension was stirred for 2.5 h at 60 °C. The solvent was removed in vacuo, the residue was partitioned between 1 N HCl (10 mL) and EtOAc (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (2 × 10 mL) and brine NaCl (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) and further by preparative HPLC (solvent system A) to give **16** (9 mg, 77%) as white crystals. ^{1}H NMR (400 MHz, CD_3OD): δ 1.21 (s, *tert*-butyl), 1.23 (d, J = 7.1, CH_3), 1.76 (d, J = 12.5, 8-H), 3.01 (q, J = 7.1, 14-H), 4.13 (dd, J = 12.3, 4.3, 7-H), 4.19 (d, J = 7.4, 1-H), 4.49 (d, J = 7.4, 2-H), 4.76 (d, J = 10,2, CH_2, 1H), 5.04 (s, 6-H), 5.02 (d, J = 4.3, 6-H), 5.25 (s, 10-H), 5.46 (d, J = 10.2, CH_2, 1H), 6.14 (s, 12-H), 7.37−7.44 (m, aromatic, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.2, 29.1 (3C), 32.2, 41.6, 50.5, 64.1, 67.1, 73.8, 74.3, 75.6,

77.2, 79.3, 83.5, 90.6, 98.5, 110.1, 128.9 (2C), 129.5 (2C), 129.8, 134.2, 170.8, 170.8, 175.5. HPLC−UV: 98%. HRMS: $C_{27}H_{30}O_{11}$ requires M + Na at *m*/*z* 553.1686; found, 553.1684.

10-*O*-Benzyl-7α-fluoro Ginkgolide B (17). Synthesized as described for **16** using K_2CO_3 (107 mg, 0.77 mmol), **7** (34 mg, 0.08 mmol), and benzyl chloride (89 μL, 0.77 mmol) in DMF (1.8 mL). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (30:1:1) to give **17** (16 mg, 39%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (s, *tert*-butyl), 1.30 (d, J = 7.0, CH3), 1.88 (dd, $^2J_{HF}$ = 44.5, J = 2.3, 8-H), 2.94 (d, J = 3.1, 1-OH), 3.06 (q, J = 7.0, 14-H), 4.28 (dd, J = 8.1, 3.1, 1-H), 4.50 (d, J = 8.1, 2-H), 4.68 (d, J = 9.4, CH_2, 1H), 4.95 (s, 10-H), 5.29 (d, $^2J_{HF}$ = 10.2, 6-H), 5.30 (dd, $^1J_{HF}$ = 50.1, J = 1.5, 7-H), 5.41 (d, J = 9.4, CH_2, 1H), 6.04 (s, 12-H), 7.36 (m, aromatic, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.2, 30.3 (3C), 32.9, 41.7, 53.3 ($^2J_{CF}$ = 20.4), 68.2, 71.6, 74.1, 74.4, 75.1, 79.5 ($^2J_{CF}$ = 35.6), 83.6, 90.2, 96.0 ($^1J_{CF}$ = 184.2), 98.2, 110.9, 128.7 (2C), 129.1 (2C), 129.3, 134.8, 170.2, 170.8, 175.1. HPLC−UV: 98%. HRMS: $C_{27}H_{30}O_{10}F$ requires M + 1 at *m*/*z* 533.1823; found, 533.1784.

10-*O*-Benzyl-7-epi-ginkgolide C (18). Synthesized as described for **16** using K_2CO_3 (50 mg, 0.36 mmol), **14** (16 mg, 0.04 mmol), and benzyl chloride (42 μL, 0.36 mmol) in DMF (0.3 mL). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH/EtOAc (20:1:1) and further by preparative HPLC (eluent A) to give **18** (11 mg, 56%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (s, *tert*-butyl), 1.29 (d, J = 7.0, CH_3), 1.83 (d, J = 3.1, 8-H), 2.60 (d, J = 3.6, 1-OH), 2.66 (d, J = 11.0, 7-OH), 3.06 (q, J = 7.0, 14-H), 3.40 (bs, 3-OH), 4.28 (dd, J = 3.6, 7.8, 1-H), 4.48 (m, 2-H, 7-H), 4.72 (d, J = 9.2, CH_2, 1H), 4.96 (s, 6-H), 5.51 (s, 10-H), 5.50 (d, J = 9.2, CH_2, 1H), 6.09 (s, 12-H), 7.39−7.44 (m, aromatic, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.3, 30.6 (3C), 33.1, 41.6, 52.9, 68.4, 71.3, 74.0, 74.4, 74.7, 77.6, 82.3, 83.2, 90.7, 98.5, 110.5, 129.2 (2C), 129.6 (2C), 130.1, 133.8, 170.3, 170.5, 175.4. HPLC−UV: 99%. HRMS: $C_{27}H_{31}O_{11}$ requires M + 1 at *m*/*z* 531.1866; found, 531.1895.

Radioligand Binding Assay. The radioligand binding assays were performed as previously described.[30] In brief, membrane fractions from hearts and skeletal muscles of PAFR-Transgenic mice (50 μL suspension containing 158 fmol of PAFR) were mixed with 2 pmol of [^{3}H]-WEB 2086 in 50 μL of buffer [25 mM HEPES/NaOH (pH 7.4), 0.25 M sucrose, 10 mM $MgCl_2$, 0.1% BSA] and the compound to be tested in 100 μL of buffer in a 96-well microplate in triplicate for each concentration. These mixtures were incubated at 25 °C for 90 min, upon which the receptor-bound [^{3}H]-WEB 2086 was filtered and washed with cold buffer. The filters were then dried at 50 °C for at least 90 min, 25 μL of MicroScint-0 scintillation cocktail was added, and filters were placed in a TopCount microplate scintillation counter. Binding data were analyzed with the nonlinear curve-fitting program Microplate Manager III (Bio-Rad, Hercules, CA). Nonspecific binding was determined using methods as previously described.[30]

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Supporting Information Available: COSY, HSQC, and ROESY spectra of compound **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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