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Inhibition of Amyloidogenesis by Nonsteroidal Anti-inflammatory Drugs and Their Hybrid Nitrates

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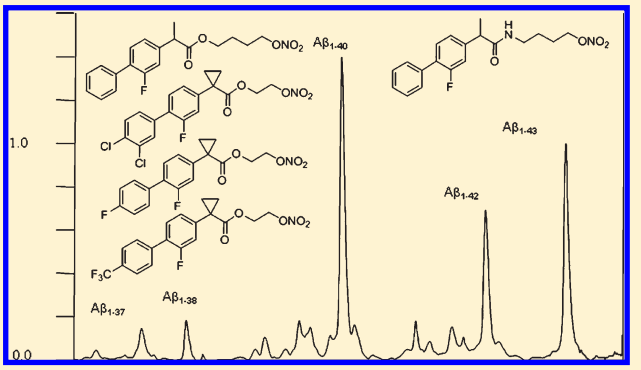
ABSTRACT: Poor blood−brain barrier penetration of nonsteroidal anti-inflammatory drugs (NSAIDs) has been blamed for the failure of the selective amyloid lowering agent (SALA) *R*-flurbiprofen in phase 3 clinical trials for Alzheimer's disease (AD). NO-donor NSAIDs (NO-NSAIDs) provide an alternative, gastric-sparing approach to NSAID SALAs, which may improve bioavailability. NSAID analogues were studied for anti-inflammatory activity and for SALA activity in N2a neuronal cells transfected with human amyloid precursor protein (APP). Flurbiprofen (**1**) analogues were obtained with enhanced anti-inflammatory and antiamyloidogenic properties compared to **1**, however, esterification led to elevated Aβ_{1-42} levels. Hybrid nitrate prodrugs possessed superior anti-inflammatory activity and reduced toxicity relative to the parent NSAIDs, including clinical candidate CHF5074. Although hybrid nitrates elevated Aβ_{1-42} at higher concentration, SALA activity was observed at low concentrations ($\leq 1\ \mu$M): both Aβ_{1-42} and the ratio of Aβ_{1-42}/Aβ_{1-40} were lowered. This biphasic SALA activity was attributed to the intact nitrate drug. For several compounds, the selective modulation of amyloidogenesis was tested using an immunoprecipitation MALDI-TOF approach. These data support the development of NO-NSAIDs as an alternative approach toward a clinically useful SALA.



INTRODUCTION

Alzheimer's disease (AD), the major cause of dementia in the elderly population, is a neurodegenerative disorder characterized by the abnormal accumulation of amyloid plaques and neurofibrillary tangles.[1,2] According to the amyloid cascade hypothesis, β-amyloid (Aβ) peptide, the main constituent of amyloid plaques, is the cause of the observed neurodegeneration in AD patients.[3] In 2001, Weggen et al. discovered that certain NSAIDs were able to selectively lower the levels of Aβ_{1-42} without affecting levels of Aβ_{1-40}; these drugs were referred to as SALAs.[4] The importance of this discovery lay in evidence that the 42 amino acid fragment, Aβ_{1-42}, aggregates more rapidly and is a much more potent neurotoxin than the more abundant Aβ_{1-40}.[3,5] Aβ_{1-42} is widely viewed as the causative molecule in AD. The enzymes responsible for the production of Aβ, namely β- and γ-secretase, remain primary drug targets for the treatment of AD.[6] Changes in the levels of Aβ elicited by NSAID SALAs in vitro and in vivo have been attributed to modulation of Aβ production,[4,7−11] via: modulation of γ-secretase activity,[4,7] down-regulation of β-secretase,[12] or Rho kinase inhibition.[13,14] In addition, NSAIDs including flurbiprofen (**1**) have been reported to inhibit amyloidosis by stabilizing transthyretin.[15] The N2a neuronal cell line stably transfected with human βAPP695 bearing the Swedish mutation (N2a.hAPP) provides a model for amyloidogenesis, producing Aβ peptides associated with human pathophysiology. Studies on this cell line demonstrated that NSAID SALAs stimulate metalloprotease degradation of Aβ_{1-40} and Aβ_{1-42}.[16] The enhanced proteolysis of Aβ is currently seen as a preferred drug discovery strategy.[17,18]

The effect of NSAID SALAs on Aβ levels was shown previously to be independent of the cyclooxygenase (COX) inhibitory activity, a dissociation of actions considered crucial for drug development, because NSAID gastrotoxicity is associated with COX inhibition.[10,11] Supported by epidemiological studies that reported a decreased risk of developing AD after chronic treatment with NSAIDs,[19−21] the COX-inactive *R*-flurbiprofen was studied in phase 3 clinical trials as a gastric-sparing therapeutic agent for AD (Scheme 1).[7] In 2008, this trial was reported as a failure due to lack of efficacy, possibly linked to poor bioavailability.[22,23] Nevertheless, CHF5074 (**11d**), a new COX-inactive analogue of flurbiprofen (**1**) appears to be on course for clinical development.[24] An alternative approach to developing a gastric-sparing NSAID has been to incorporate a gastroprotective organic nitrate moiety in a so-called NO-donating NSAID (NO-NSAID) to counteract the NSAID-induced inhibition of prostaglandin synthesis.[25] HCT-1026 (**2**),[26] an NO-NSAID prodrug of **1**, has been reported: (i) to reduce Aβ load in an amyloid transgenic mouse model,[27] (ii) to reverse cognitive deficits in mice under cholinergic blockade,[28] and (iii) to reduce inflammatory markers, including iNOS, in adult rats injected with Aβ_{1-42}.[29]

The purpose of this study was to assay libraries of indomethacin (**3**), sulindac (**4**), and flurbiprofen analogues, including novel compounds and preclinical agents (e.g., CHF5022, **11c**,[30] and

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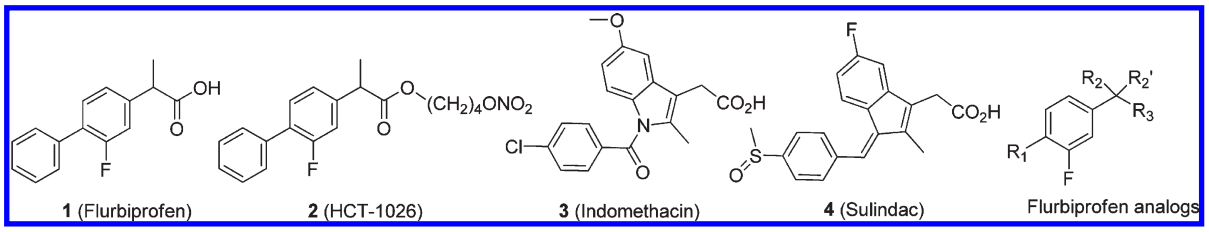
Scheme 1



11d), as a basis for development of new NSAID hybrid nitrates as SALAs. Anti-inflammatory activity and toxicity were assayed in cell culture and for selected compounds inhibition of amyloidogenesis was assayed in neuronal cell cultures. On the basis of these data, prototype hybrid nitrate analogues of **1** were synthesized and studied for SALA activity, revealing further insight into the mechanism of SALA activity associated with NO-NSAIDs.

RESULTS AND DISCUSSION

Synthesis. Synthesis was directed at the generation of a library of compounds possessing variations at the terminal phenyl ring (R_1), α-carbon (R_2 and R_2'), and isosteric replacement of the carboxylic acid (R_3) (Scheme 1).

Synthesis began with allylic bromination of the commercially available 4-bromo-3-fluorotoluene **6**, with *N*-bromosuccinimide using a catalytic amount of dibenzoyl peroxide to yield **7**, which was then reacted with potassium cyanide to give **8**. Exposure to either iodoethane or 1,2-dibromoethane yielded alkylated products **9a** and **9b** that were then oxidized to the carboxylic acid followed by Suzuki coupling to the appropriate boronic acids to give biarylcarboxylic acids **11a**−**d** in moderate yields (60−70%). Quantitative yield was attained through direct coupling of the cyano analogues **8** and **9b**, yielding the α-cyano-biaryls **12a** and **12b**, along with **13a** and **13b**. Fluorination of **13a** and **13b** was accomplished via reaction with *t*-BuLi followed by fluorination with *N*-fluorobenzenesulfonimide (NFSi) to give **14a**−**b** and **15a**−**b**. While base catalyzed oxidation of **14a**, **14b**, **15a**, and **15b** gave the corresponding biaryl fluoro-amides **16a**, **16b**, **17a**, and **17b**, the desired biaryl-fluorinated carboxylic acids were not isolated. Acid catalyzed hydrolysis of **14a** and **14b** yielded **18a** and **18b** as products that were also evaluated for inhibition of amyloidogenesis and anti-inflammatory activity. Base and acid catalyzed oxidation of the diethyl derivative **12a** was hindered by solubility issues, therefore, the corresponding acid was synthesized by first oxidizing **13a** to give the acid **19**, which was then reacted with bromoethane in aqueous NaOH and toluene to give **20**, followed by hydrolysis with LiOH to yield the desired acid **21** (Scheme 2).

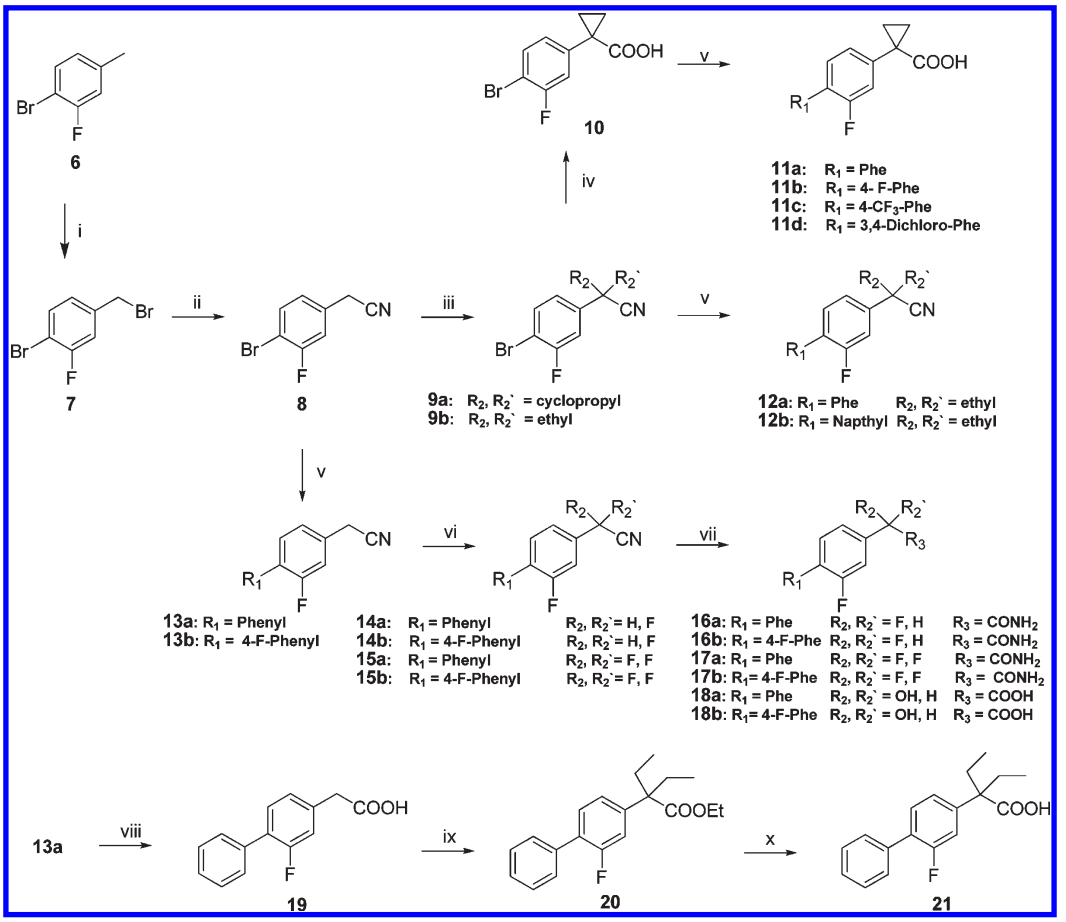
To synthesize the biaryl mono carboxylic acids **23a** and **23b**, a synthetic pathway was adopted in which **1** or **19** were reacted with *t*-butyldimethylsilyl chloride (TBSCl) and lithium bis(trimethylsilyl)amide (LiHMDS) at 0 °C to give the desired bis-ketenes **22a** and **22b**, which were then reacted with Selectfluor followed by acid−base workup to give the desired fluorinated acids, **23a** and **23b** (Scheme 3). Flurbiprofen derivatives and analogues were synthesized from **1**, **11a**, **11c**, and **11d** through alkylation with 1,2-dibromoethane for **11a**, **11c**, and **11d** to give intermediates **25a**−**c**, or via alkylation of **1** with 1,4-dibromobutane to yield **24a**. The bromides were then transformed to the corresponding nitrates with $AgNO_3$ to yield **2** and **26a**−**c**. In a similar manner, the amide **27** was synthesized by coupling of **1** with 4-aminobutanol, followed by treatment with tetrabromomethane to give **24b** and subsequent nitration with $AgNO_3$ to afford the nitrate **27** (Scheme 4).

Activity. Three structural approaches to analogues of **1** were developed: (1) variations at the terminal phenyl ring (R_1), (2) modification of the α-carbon (R_2 and R_2'), and (3) isosteric replacement of the carboxylic acid (R_3) (Scheme 1). Derivatization of the α-carbon is known to be a subtle modification resulting in reduction or ablation of COX activity, whereas replacement of the carboxylate group is expected to be important in enhancing blood−brain barrier penetration. For these modifications to be successful, enhanced SALA activity was desired without loss of NSAID anti-inflammatory activity. Mouse macrophage-like RAW 264.7 cells, treated with lipopolysaccharide (LPS) bacterial endotoxin, undergo induction of COX-2, inducible NO synthase (iNOS), and other inflammatory mediators.[31] Thus, the RAW 264.7 cell line is routinely used to examine the ability of agents to inhibit cellular inflammatory response. iNOS activity is readily assessed by measuring the levels of inorganic nitrite, the major product of NO oxidative metabolism, quantified using the Griess assay.[31] Flurbiprofen (**1**) itself was a modest inhibitor of iNOS induction in RAW cells treated with LPS, and no evidence of toxicity was seen at the lower concentration tested (Table 1).[28,32,33]

In comparison with **1**, several analogues gave a significantly greater anti-inflammatory response, although in the case of **14b** and **18a**, this was associated with somewhat increased toxicity. These data demonstrate that the three structural modifications can be accommodated while maintaining or enhancing anti-inflammatory activity toward iNOS induction, relative to **1**. At higher concentration, anti-inflammatory activity was increased relative to **1** again without toxicity (Table 2). Cyclopropyl analogues **11a** and **11c** gave similar responses; however, **11d** was highly toxic toward RAW cells. Comparison with other test compounds showed that toxicity was not a feature associated with one of the three structural motifs (R_1, R_2, R_3) but a property of the molecule as a whole.

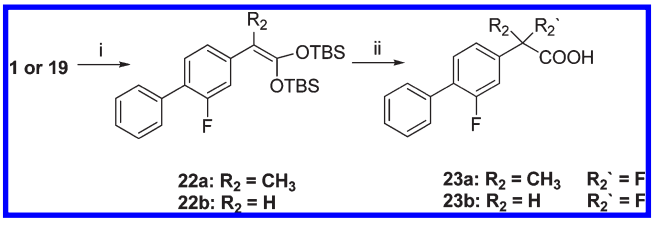
We have previously reported use of the Griess assay in measurement of the anti-inflammatory activity of nitrates toward LPS-induced RAW cells.[31] There is a potential pitfall with this approach, because metabolic denitration of nitrate drugs directly yields NO_2^-.[34] However, a complete analysis of denitration in induced and noninduced RAW cells demonstrated that simple aliphatic mononitrates, such as isosorbide mononitrate (ISMN) and **2**, release <3% of the theoretical yield of NO_2^- after 24 h.[31] We previously reported that **2** was significantly more potent than **1** in attenuating induction of iNOS activity, whereas the classical mononitrate, ISMN, showed no significant inhibition of iNOS activity, and in combination with **1** was no more efficacious than **1** alone.[28] These observations extend to the hybrid nitrate derivatives, **26a**−**c**, which were observed to be significantly more potent than their parent flurbiprofen analogue (Table 2). Interestingly, conversion of the

Scheme 2[a]



[a] Reagents and Conditions: (i) NBS, CCl_4; (ii) KCN, EtOH; (iii) 1,2 dibromoethane for **9a** or CH_3CH_2I for **9b**, NaOH, toluene−H_2O; (iv) NaOH, MeOH, 100 °C; (v) $R_1B(OH)_2$, $Pd(OAc)_2$, Na_2CO_3, TBAB, H_2O, 120 °C; (vi) NFSi, *t*-BuLi, THF, −78 °C; (vii) K_2CO_3, DMSO, H_2O_2, 0 °C yields **16a**−**b** and **17a**−**b**, or H_2SO_4 (49%), 90 °C to give **18a**−**b**; (viii) K_2CO_3, DMSO, H_2O_2, 0 °C; (ix) CH_3CH_2Br, NaOH, toluene−H_2O; (x) LiOH, THF/H_2O/MeOH, 0 °C.

Scheme 3[a]



[a] Reagents and Conditions: (i) TBSCl, LiHMDS, THF, 0 °C, 12 h; (ii) Selectfluor, CH_3CN, <50 °C.

cytotoxic **11d** to a hybrid nitrate (**26c**) completely ablated toxicity. However, neither the anti-inflammatory activity nor the ablated toxicity could be ascribed to the nitrate group because the precursor bromo-derivatives, **25a**−**c**, were the most potent anti-inflammatory agents, reducing NO_2^- production 4-fold relative to the parent carboxylic acids in the absence of any cytotoxicity.

Approaches to the design of improved analogues of **1** as antiamyloidogenic agents have been published previously. Modification of the α-carbon through the incorporation of a cyclopropyl group[11] or a dimethyl group[10] were reported to lead to increased potency for lowering Aβ while eliminating COX activity. Previous work published by Peretto et al. focused mainly on substituting the terminal phenyl ring (R_1) of the cyclopropyl analogue of **2**, leading to compound **11d**.[11] The murine N2a.hAPP cell line has previously been employed by various investigators to assay SALAs using ELISA to measure $A\beta_{1-40}$ and/or $A\beta_{1-42}$.[16,28,35] In this assay, confluent N2a/APPsw cells were treated with drug for 24 h, following which the supernatant was collected and $A\beta_{1-42}$ measured by ELISA. Compound **11d**, observed to be toxic in RAW cells at higher concentration (100 μM), was not toxic toward N2a cells at lower concentration (1 μM). However, in contrast to reports of the activity of this compound tested at higher concentrations (i.e., 50−500 μM),[30,36] at the low concentration tested herein (1 μM), **11d** did not reduce levels of $A\beta_{1-42}$ (Table 3). Carboxylate analogues possessing a phenyl R_1 substituent uniformly lowered $A\beta_{1-42}$, with the exception of the diethyl analogue **21**.

A number of derivatives (i.e., **11b**, **18a**, and **23b**) demonstrated antiamyloidogenic activity, comparable to **1**, in the absence of substantial toxicity. These compounds all contain the NSAID carboxylate group but are otherwise structurally diverse. Two compounds stood out as superior to **1**, **11a** and **14a**, both bearing an unsubstituted phenyl R_1 substituent. The activity of the nitrile **14a** was of interest because the observation of antiamyloidogenic activity shows that the carboxylate group is not an absolute requirement. This was

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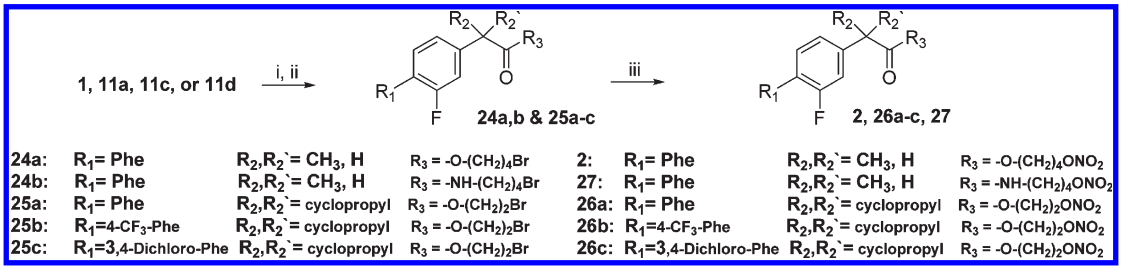
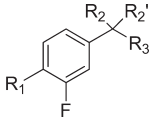
Scheme 4[a]



[a] Reagents and Conditions: (i) $NaOCH_3$, CH_3OH; (ii) $Br(CH_2)_4Br$ for **24a**, $Br(CH_2)_2Br$ for **25a**−**c**, or 4-aminobutanol followed by PPh_3 with CBr_4 for **24b**, DMF; (iii) $AgNO_3$, CH_3CN, reflux.

Table 1. Anti-inflammatory Effects of Selected 1 Analogues (10 μM)



compd	R_1	R_2, R_2'	R_3	NO_2^- as %[a]	cell viability as %[b]
DMSO				100 ± 0.8	100 ± 2.3
1	phenyl	H, CH_3	COOH	**80.7 ± 3.6**	**102 ± 2.7**
11b	4-F-phenyl	cyclopropyl	COOH	86.4 ± 3.5	102 ± 1.7
12b	napthyl	ethyl, ethyl	CN	91.3 ± 4.6	107 ± 1.1
14b	4-F-phenyl	H, F	CN	65.3 ± 9.0	84.5 ± 1.2
15b	4-F-phenyl	F, F	CN	75.7 ± 9.1	98.2 ± 3.3
16b	4-F-phenyl	H, F	$CONH_2$	74.7 ± 7.2	90.6 ± 1.1
17a	phenyl	F, F	$CONH_2$	82.2 ± 3.9	100 ± 0.4
17b	4-F-phenyl	F, F	$CONH_2$	84.1 ± 5.3	98.8 ± 1
18a	phenyl	H, OH	COOH	59 ± 3.4	89.1 ± 3.5
18b	4-F-phenyl	H, OH	COOH	76.3 ± 4.9	102 ± 1
23a	phenyl	F, CH_3	COOH	80.8 ± 3.4	99.7 ± 3.6
23b	phenyl	F, H	COOH	81.5 ± 3.9	92.7 ± 4.1

[a] Griess assay. [b] MTT assay.

the most interesting of the novel α-fluoro analogues but obviously was not suitable for further study as a hybrid nitrate.

Given the ability of at least one analogue, lacking a carboxylate group, to lower $A\beta_{1-42}$, and several such examples inhibiting iNOS activity, it was of interest to assay analogues of **3** and **4**, which contained examples with amides in place of carboxylic acids. The derivatives of **3** provided the expected anti-inflammatory activity and the expected antiamyloid activity for **3**, itself, was observed at high concentration (Table 4). Other activity of note was the amyloid raising activity of the amide derivative, **34**, which was of the same magnitude as seen for so-called selective amyloid raising agents (SARAs) such as fenofibrate (**39**). Derivatives of **4** (**28**−**33**) provided one compound with a good profile of anti-inflammatory and antiamyloidogenic activity without cytotoxicity, the desmethylated derivative **28**. Amide derivatives did not reduce $A\beta_{1-42}$ and were comparable to **4** (Table 5).

For selected agents, the dependence of antiamyloidogenesis on concentration was tested further, again using N2a.hAPP cell cultures treated with agents for 24 h, followed by ELISA assay of the supernatant. The flurbiprofen analogue **11a** was compared to **1** itself and shown to be a more potent SALA, lowering $A\beta_{1-42}$ by 40% at the highest concentration studied, with no significant toxicity (Figure 1A). In contrast, the hybrid nitrate derivative **2**, as we have reported previously,[28] showed biphasic activity. The full concentration−response data for **2** showed SALA activity more potent than **1** and similar to **11a** at low concentrations and, in contrast, SARA activity at >10 μM (Figure 1B).

It was important to determine whether this biphasic activity was peculiar to compound **2** or if, indeed, this was common to related NSAID hybrid nitrates. Therefore, nitrate **26a** was studied over a range of concentrations, revealing a similar response, albeit with less efficacy as a SALA than **2** (Figure 2A). To determine if the nitrate group was relevant to the observed biphasic activity of these NSAID hybrid nitrates, the antiamyloidogenic activity of the nitrate **2** was compared with that of the simple ethyl ester **38** in N2a.hAPP cell culture incubations (Figure 2B). The ester behaved as a typical SARA agent increasing $A\beta_{1-42}$ 2.5-fold at 10 μM, indicating the importance of the nitrate for SALA activity at low concentrations. However, this SALA activity of hybrid nitrate NSAIDs toward $A\beta_{1-42}$ modulation is unlikely to be associated with NO bioactivity delivered by the nitrate functionality since we have previously noted that the classical nitrate ISMN is without activity in this assay.[28] The alkyl ester linker of the hybrid nitrates, **2** and **26a**, is expected to be labile toward nonspecific esterase activity; therefore, it was important to test a hybrid nitrate with a stable linker. The amide linked nitrate, **27**, was synthesized expressly to test if the more labile ester linker of **2** and **26a** was essential for the observed antiamyloidogenic activity. The antiamyloidogenic activity at low concentration (0.5 μM) was retained compared to **26a**, indicating that the intact nitrate is required for SALA activity (Figure 2C). This appears to be only the second example where biological activity can be attributed to the intact NO-NSAID, rather than to the NO-NSAID acting as a prodrug releasing NSAID, aliphatic nitrate, and/or NO.[37]

The NO-flurbiprofens, NCX-2216 and HCT-1026 (**2**), have been reported to lower Aβ in mouse models, however, NCX-2216 was not detected in brain tissue,[38] and both plasma and brain levels of HCT-1026 were reported to be below detection limits.[39] Hydrolysis of HCT-1026 yields 4-hydroxybutyl nitrate, and it is possible that this metabolite has brain bioavailaibility because animal studies have shown that the level of inorganic nitrite in the brain increases after oral administration of HCT-1026.[29,40] It was of interest both to confirm the increased stability of the amide linked hybrid nitrate **27** relative to the ester linked homologue, **2**, and to determine if unlike **2**, the NO-flurbiprofen, **27**, was detectable in the brain. Therefore, **27** (10 mg/kg) was delivered by ip injection to male C75/BL6 mice (25 g) and brain and plasma levels detected by LC-MS/MS at 30 and 120 min after injection. The brain/plasma ratio of **27** was 37% and 13% at 30 and 120 min, respectively (Figure 3). Most importantly, **27** was present in detectable amounts both in the brain and plasma 2 h after administration, in contrast to reports on **2**.

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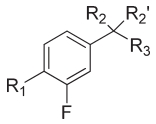
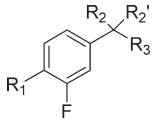
Table 2. Anti-inflammatory Effects of 1 and Analogues (100 μM)



compd	R_1	R_2, R_2'	R_3	NO_2^- as %[a]	cell viability as %[b]
DMSO				100 ± 0.8	100 ± 2.3
1	phenyl	H, CH_3	COOH	**69.1 ± 3.2**	**109 ± 2.7**
2	phenyl	H, CH_3	$COO(CH_2)_4ONO_2$	49.8 ± 2.5	108 ± 3.4
11a	phenyl	cyclopropyl	COOH	60.7 ± 1.3	101 ± 3.1
11c	4-CF_3-phenyl	cyclopropyl	COOH	62.4 ± 3.4	102 ± 4.6
11d	3,4 dichlorophenyl	cyclopropyl	COOH		19.1 ± 2.8
25a	phenyl	cyclopropyl	$COO(CH_2)_2Br$	18.9 ± 1.0	109 ± 4.0
25b	4-CF_3-phenyl	cyclopropyl	$COO(CH_2)_2Br$	14.7 ± 3.2	113 ± 2.1
25c	3,4 dichlorophenyl	cyclopropyl	$COO(CH_2)_2Br$	27.8 ± 2.0	114 ± 3.7
26a	phenyl	cyclopropyl	$COO(CH_2)_2ONO_2$	47.3 ± 1.5	113 ± 6.5
26b	4-CF_3-phenyl	cyclopropyl	$COO(CH_2)_2ONO_2$	42.8 ± 2.1	128 ± 1.8
26c	3,4 dichlorophenyl	cyclopropyl	$COO(CH_2)_2ONO_2$	40 ± 2.4	135 ± 2.9

[a] Griess assay. [b] MTT assay.

Table 3. Effects of 1 and Analogues (1 μM) on A$\beta_{1\text{-}42}$ Levels

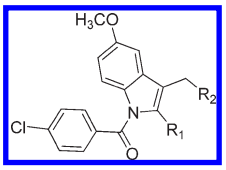


compd	R_1	R_2, R_2'	R_3	$A\beta_{1-42}$ as %	cell viability as %[a]
DMSO				100 ± 3.2	100 ± 0.9
1	phenyl	H, CH_3	COOH	**86.6 ± 0.9**	**101 ± 0.9**
11a	phenyl	cyclopropyl	COOH	69.5 ± 2.0	103 ± 1.9
11b	4-F-phenyl	cyclopropyl	COOH	90.4 ± 1.3	102 ± 1.3
11c	4-CF_3-phenyl	cyclopropyl	COOH	99.3 ± 10.7	97.2 ± 3.1
11d	3,4 dichlorophenyl	cyclopropyl	COOH	125 ± 5.0	92.9 ± 0.6
12a	phenyl	ethyl, ethyl	CN	125 ± 5.1	97.3 ± 0.7
12b	naphthyl	ethyl, ethyl	CN	105 ± 3.3	91.6 ± 1.4
14a	phenyl	F, H	CN	65.6 ± 1.5	93.1 ± 1.0
14b	4-F-phenyl	F, H	CN		69.4 ± 0.3
15b	4-F-phenyl	F, F	CN	125 ± 5	98.2 ± 0.6
16a	phenyl	F, H	$CONH_2$	101 ± 0.9	102.8 ± 1.0
16b	4-F- phenyl	F, H	$CONH_2$	-	69.1 ± 3.0
17a	phenyl	F, F	$CONH_2$	115 ± 3.5	102 ± 1.3
17b	4-F-phenyl	F, F	$CONH_2$	109 ± 5.8	95.9 ± 0.4
18a	phenyl	OH, H	COOH	82.5 ± 1.6	101 ± 0.3
18b	4-F-phenyl	OH, H	COOH	106 ± 1.3	99.7 ± 1.4
21	phenyl	ethyl, ethyl	COOH	124 ± 7.1	95.5 ± 0.3
23a	phenyl	F, CH_3	COOH	98.8 ± 2.1	101 ± 1.8
23b	phenyl	F, H	COOH	91.8 ± 1.3	98.7 ± 1.3

[a] MTT assay.

The SARA activity of flurbiprofen derivatives **2**, **26a**, and **38** at higher concentrations has precedent in SARA agents, including NSAIDs such as celecoxib, and other drugs, such as **39**, that have been reported to elevate $A\beta_{1-42}$ levels while lowering the level of other fragments including $A\beta_{1-38}$.[41−43] To study the effect of agents on amyloidogenesis in more detail, we determined the profile of Aβ fragment production from N2a.hAPP cells for selected compounds using an immunoprecipitation MALDI-TOF technique introduced in 2009.[16] This technique quantifies the amyloid fragments $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$, using $A\beta_{1-43}$ as an internal standard, because this fragment is not a product of amyloidogenesis. In the N2a.hAPP amyloidogenesis assay, we observed that the known SARA **39** (200 μM) increased $A\beta_{1-42}$ levels 3-fold but had modest effects on $A\beta_{1-37}$ and $A\beta_{1-38}$ although lowering the level of the latter fragment (Figure 4). In contrast, fibric acid, the free acid form of **39**, was reported to lower the levels of $A\beta_{1-42}$.[16] A similar observation on $A\beta_{1-42}$ modulation was made herein with **1** and its ester, **38**. Whereas, the ester acted as a SARA, the free acid, **1**, at 100 μM, selectively lowered $A\beta_{1-42}$ and elevated $A\beta_{1-37}$ relative to the vehicle control (Figure 4). Indeed, the amyloid peptide product profiles for the three carboxylate esters, **2**, **25c**, and **39** are very similar at high concentrations; all act as SARAs, significantly and selectively increasing $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$. In contrast, the hybrid nitrate ester, **2**, behaves quite differently; at low concentrations, **2** lowers total Aβ generation

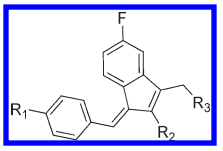
Table 4. Anti-amyloidogenic Inhibitory Activity and Anti-Inflammatory Activity of 3 Analogues



compd	R_1	R_2	$A\beta_{1-42}$ as %[a]	NO_2^- as %[b]	cell viability as %[c]
DMSO			100 ± 3	100 ± 7.4	100 ± 7.4
3	CH_3	COOH	108 ± 6	70.9 ± 7.4	98.7 ± 1
3 (100 μM)	CH_3	COOH	76.7 ± 2.9	39.8 ± 10.5	92.0 ± 7.3
34	CH_3	CONH-4-chlorophenyl	295 ± 27.2	68.6 ± 6.3	92.2 ± 5.3
35	H	COOH	102 ± 5.3	65.9 ± 0.4	97.9 ± 1.1
36	CH_3	$NHCH_2CH_2OH$	118 ± 9.4	52.5 ± 2.3	100 ± 1.6
37	CH_3	$CONHCH_2CH_2$-3-pyridine	135 ± 8.7	100 ± 7.4	95.6 ± 2.6

[a] 1 μM. [b] Griess assay; 10 μM unless otherwise stated. [c] MTT assay in RAW cells; 10 μM unless otherwise stated.

Table 5. Anti-amyloidogenic Activity and Anti-inflammatory Activity of 4 Analogues



compd	R_1	R_2	R_3	$A\beta_{1-42}$ as %[a]	NO_2^- as %[b]	cell viability as %[c]
DMSO				100 ± 3.8	100 ± 11.4	100 ± 1.6
4	$SOCH_3$	CH_3	COOH	100 ± 0.1	85.7 ± 9.3	104 ± 1.2
5	SCH_3	CH_3	COOH	82.6 ± 2.2		
28	SCH_3	H	COOH	80.8 ± 4.3	47.5 ± 7.2	100 ± 1.6
29	$SOCH_3$	H	COOH	95 ± 5	91.6 ± 11	104 ± 1.2
30	SCH_3	H	$CONHCH_2Ph$	94.5 ± 1.8	58.4 ± 12.5	100 ± 1.6
31	SCH_3	H	$CONHCH_2CH_3$	95.7 ± 1.8	80.4 ± 11.1	104 ± 1.2
32	CF_3	H	COOH	91 ± 1.5	29.2 ± 8	100 ± 1.6
33	SCF_3	H	COOH	99.4 ± 0.4	1.7 ± 6.3	104 ± 1.2

[a] 1 μM. [b] Griess assay at 10 μM. [c] MTT assay in RAW cells at 10 μM.

with similar efficacy to the flurbiprofen analogue, **11a**, although with less selectivity for $A\beta_{1-42}$.

Sulindac (**4**) is known to act as an NSAID SALA. The expected selective lowering of $A\beta_{1-42}$ and raising of both $A\beta_{1-37}$ and $A\beta_{1-38}$ levels was observed (Figure 4).[44] Flurbiprofen derivative **11a** was a more potent SALA than both **1** and **4** (Figures 1, 4), moreover, the profile of Aβ modulation by **11a** was very different from that of **4**. The concept that NSAID SALAs act as γ-secretase modulators was initially rationalized by a shift in secretase cleavage from producing $A\beta_{1-42}$ to production of $A\beta_{1-37}$, however, current opinion is that SALAs do not operate universally via this synchronized shift from $A\beta_{1-42}$ production to $A\beta_{1-38}$ production.[43] As we have seen, the well-established SALA and SARA activity of several drugs is recapitulated in the N2a.hAPP model system. However, a simple "control experiment" has shown that in this system, the SALA and SARA activity is not related to γ-secretase modulation. In this experiment, wild-type N2a cells were treated with drug or vehicle for 24 h, at which time, conditioned media (CM) was removed from the culture dish and added to media containing a freshly made solution of $A\beta_{1-42}$ and $A\beta_{1-40}$. CM delivered the same modulation of $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ observed when drugs were incubated in N2a.hAPP cell cultures. Further experiments showed that the modulation of $A\beta_{1-42}$ and $A\beta_{1-40}$ levels by SARA and SALA drugs was mediated by expression of

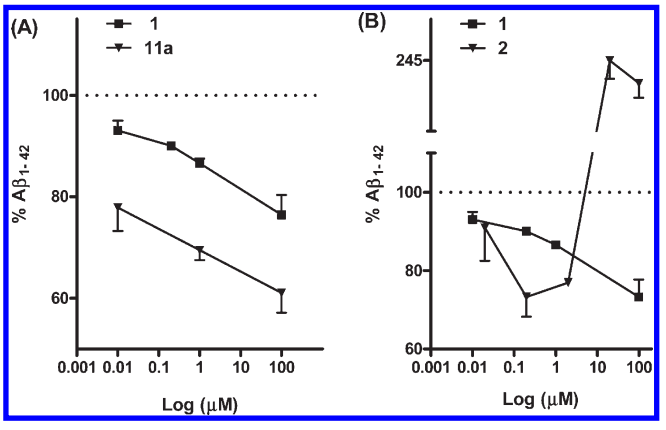


Figure 1. Flurbiprofen and derivatives showed varied SALA and SARA activity in neuronal cell incubations. Levels of $A\beta_{1-42}$ from N2a/APPsw cell supernatants were measured by ELISA and normalized to DMSO vehicle treated control after incubation with different concentrations of compounds **1** and **11a** (A) or **1** and **2** (B). The average level of $A\beta_{1-42}$ in the DMSO treated groups was ~500 pg/mL. The data show mean and SEM from at least four separate experiments performed using separate cell passages.

metalloprotease activity induced by drug treatment of cells.[16] Levels of $A\beta_{1-42}$ and $A\beta_{1-40}$ were measured by the MALDI-TOF

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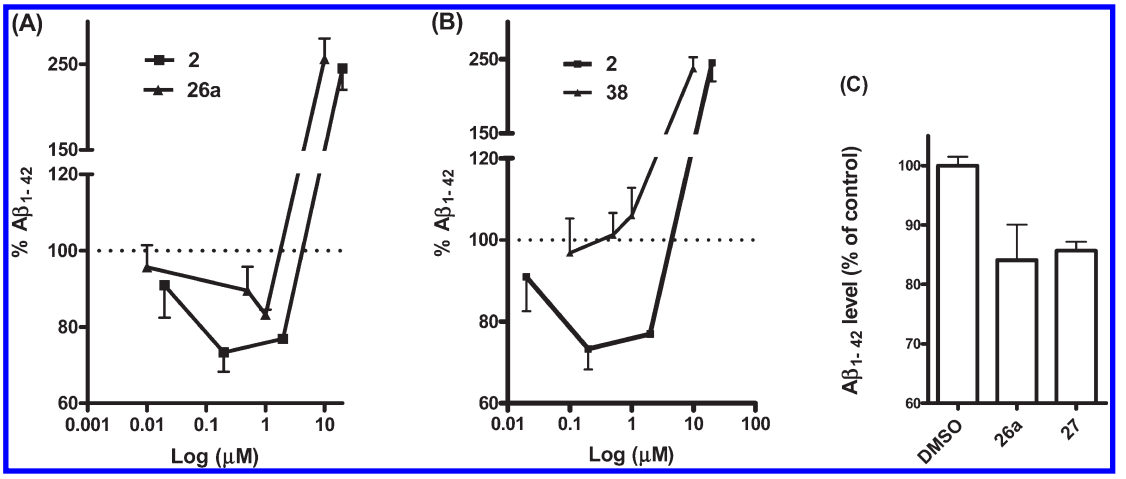

Figure 2. Hybrid nitrate flurbiprofen derivatives caused biphasic regulation of $A\beta_{1-42}$ in neuronal cell incubations, acting as SALAs at lower concentration. Levels of $A\beta_{1-42}$ from N2a/APPsw cell supernatants were measured by ELISA and normalized to DMSO vehicle treated control after incubation with different concentrations of compounds **2** and **26a** (A), **2** and **38** (B), or **26a** (0.5 μM) and **27** (0.5 μM) (C). The average level of $A\beta_{1-42}$ in DMSO treated group was ~500 pg/mL. The data show mean and sem from at least four separate experiments performed using separate cell passages.

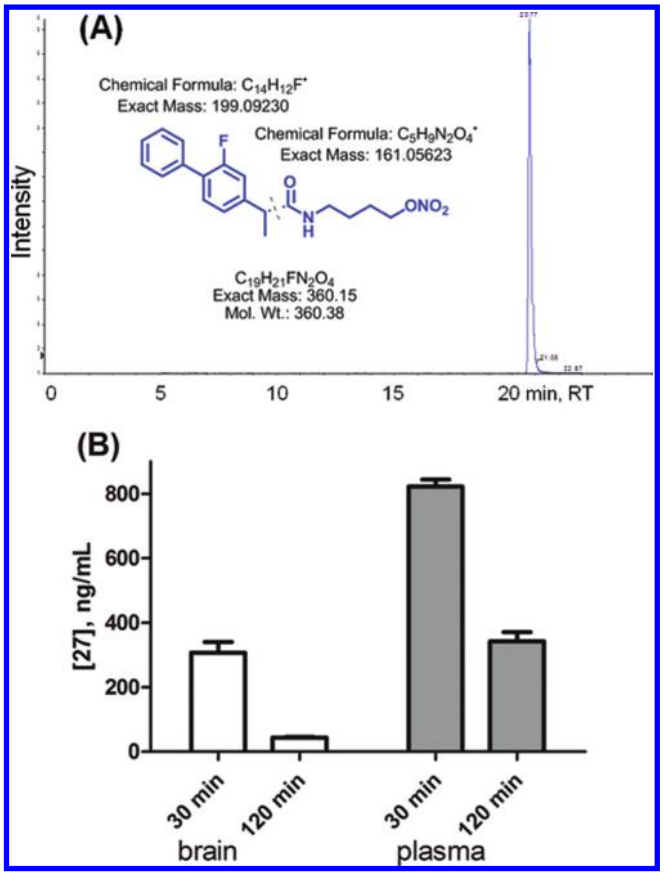

Figure 3. Hybrid nitrate **27** has brain bioavailaibility and in vivo stability after administration to mice (10 mg/kg ip) 30 or 120 min prior to sacrifice. Quantitation of **27** by LC-MS/MS used MRM analysis of the m/z transition 361 → 200. (A) Representative MRM chromatogram of **27** from mouse brain sample 120 min after administration, showing fragment observed as protonated cation (m/z = 200) in MRM. (B) Quantitative analysis of brain and plasma levels at 30 and 120 min using LC-MS/MS detection. Data (N = 4) show mean and SEM.

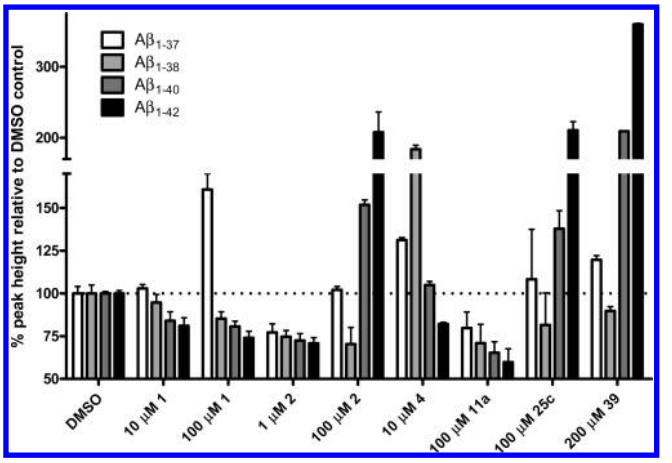

Figure 4. Quantitative analysis of $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ in neuronal cell supernatant after drug treatment. For each individual experiment, peak heights from IP-MALDI-TOF spectra were normalized to the peak height of the $A\beta_{1-43}$ standard after incubation of drugs with N2a/APPsw cells. These normalized values were then expressed relative to the DMSO vehicle control in each set of experiments. See ref 16 for full details. The data show mean and sem from at least four separate experiments.

method after treatment of N2a cells with drugs for 24 h and transfer of the CM to a solution of $A\beta_{1-42}$ and $A\beta_{1-40}$ (Figure 5). The results of this experiment confirm that **1**, **2**, and **28** are able to act as SALAs by increasing degradation of $A\beta_{1-42}$ and $A\beta_{1-40}$ and decreasing the ratio of $A\beta_{1-42}/A\beta_{1-40}$.

CONCLUSIONS

The poor blood−brain barrier penetration common to most NSAIDs has been blamed for the failure of the SALA *R*-flurbiprofen in phase 3 clinical trial for AD. Hybrid nitrate NO-NSAIDs provide an alternative, gastric-sparing approach to NSAID SALAs of use in AD. Because these compounds contain an ester linkage in place of the carboxylate anion, improved CNS bioavailability is

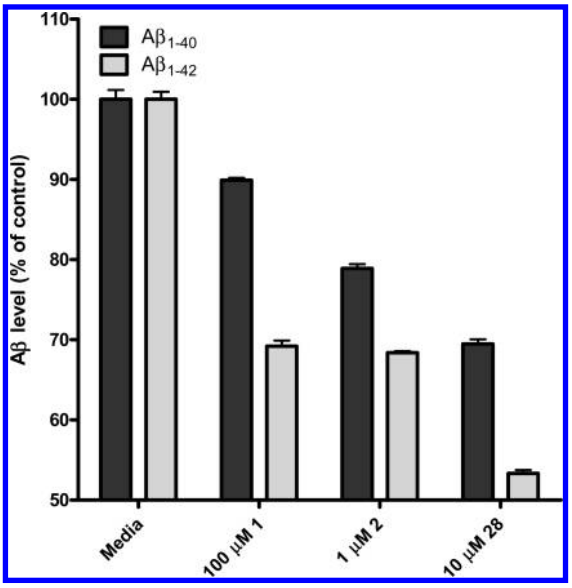

Figure 5. SALA activity was observed in mixtures of $A\beta_{1-40} + A\beta_{1-42}$ treated with conditioned media (CM) from drug treated N2a/WT cells. Cells were treated with **1** (100 μM), **2** (1 μM), and **28** (10 μM) for 24 h before the collection of the CM. CM was added to a mixture of $A\beta_{1-40} + A\beta_{1-42}$ and incubated for 8 h. CM from cell free wells (media) was used as the control. Aβ was quantified by IP-MALDI-TOF and normalized to "media" control. Data show mean and sd from two separate cell cultures.

expected. In this work, analogues of indomethacin and sulindac did not show a significantly improved profile of anti-inflammatory activity nor $A\beta_{1-42}$ lowering. However, analogues of flurbiprofen, **1**, were prepared with superior anti-inflammatory and antiamyloidogenic activity to the parent NSAID. A number of examples were converted to hybrid nitrate ester prodrugs, including a prodrug of the AD drug candidate, **11d**.[24] The hybrid nitrates possessed superior anti-inflammatory activity to the parent NSAID. Esterification of **1** led to elevated $A\beta_{1-42}$, however, this SARA activity was observed only at high concentrations for the hybrid nitrates, whereas at low concentrations (<1 μM), $A\beta_{1-42}$ and the ratio of $A\beta_{1-42}/A\beta_{1-40}$ were lowered. Interestingly, the intact hybrid nitrate was found to be necessary for the SALA activity of these NO-NSAIDs. An amide-linked NO-NSAID SALA, **27**, was shown to have brain bioavailability and to be detectable in brain and plasma 120 min after administration. These data support the optimization of hybrid nitrate SALAs, based upon modified flurbiprofen scaffolds that retain anti-inflammatory activity, and provide enhanced brain bioavailability.

EXPERIMENTAL SECTION

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Murine biotinylated antihuman Aβ 4G8 and 6E10 antibodies produced by Signet were purchased from Covance Research Products, Inc. (Dedham, MA). All cell culture supplies, human Aβ ELISA kit, and streptavidin Dynabeads T1 were purchased from Invitrogen (Carlsbad, CA).

Cell Culture and Treatments. The murine neuroblastoma N2a cells obtained from American Type Culture Collection (Manassas, VA) were cultured in 1:1 Dulbecco's Modified Eagle's Medium (DMEM) and OPTI-MEM supplemented with 5% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin. N2a cells stably transfected with the Swedish mutant of human APP (N2a/APPsw were kind gift of Dr. Gopal Thinakaran, University of Chicago) were additionally supplemented with 200 μg/mL G418 but omitted during all experiments. RAW 264.7 mouse macrophage-like cells, provided by Dr. J. Cook (University of Illinois at Chicago, Chicago, IL), were maintained in DMEM, supplemented with 1% penicillin−streptomycin, and 10% fetal bovine serum. Cells were maintained at 37 °C and 5% CO_2.

Griess Assay. RAW cells were plated at a concentration of 25×10^4 cells/well in a 24-well plate and incubated at 37 °C for 24 h. The medium was changed, and the cells were drug treated, followed 30 min later by the addition of lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 (LPS; Sigma-Aldrich). Twenty-four h later, 100 μL of the supernatant was removed and incubated with the Griess reagent (100 μL; 0.5% sulfanilamide, 0.05% (*N*-1-naphthyl)ethylenediamine dihydrochloride, 2.5% H_3PO_4, and 97% H_2O by weight) for 30 min at rt in the dark. The absorbance was measured at 530 nm on a Dynex MRX II microplate spectrophotometer and calibrated using a standard curve constructed with sodium nitrite to yield nitrite concentration.

Amyloid β Measurement from N2a/APPsw Cells Supernatant. N2a/APPsw cells were plated 24 h before the experiment at a density of 25×10^4 cells/well in a 24-well plate. Cells were washed with phosphate buffered saline (PBS; 50 mM, pH 7.4) before the addition of 500 μL of DMEM supplemented with 0.2% fetal bovine serum (FBS) followed by drug treatment. Cells were incubated for a period of 24 h. CM was collected, followed by the addition of NaN_3 (0.01% final concentration) and a mixture of protease inhibitors (10 mM phenanthroline and P2714 protease inhibitor cocktail from Sigma containing 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, aprotinin, bestatin, E-64, leupeptin, and ethylenediaminetetraacetate (EDTA)), and centrifuged for 2 min at a speed of 10 000*g*. $A\beta_{1-42}$ levels were determined by sandwich ELISA using human $A\beta_{1-42}$ ELISA kit and following the supplied protocol. For immunoprecipitation, cells were treated as described above but plated in a 6-well plate at a density of 100×10^4 cells/well in 2 mL media. Twenty-four h after treatment, 1 mL of the CM is collected, followed by the addition of NaN_3 and protease inhibitors and spiked with $A\beta_{1-43}$ at approximately 1 ng/mL final concentration as an internal standard before performing immunoprecipitation. A stock of 200 ng/mL $A\beta_{1-43}$ was prepared in sodium bicarbonate (50 mM, pH 9.0) and stored in aliquots in 1.5 mL low binding eppendorf tubes at −80 °C; once defrosted, any remaining $A\beta_{1-43}$ stock was discarded. Following immunoprecipitation the samples were analyzed in a MALDI-TOF instrument as described below for the purpose of measuring the relative abundance of Aβ peptides. In all experiments, Aβ levels were assayed relative to vehicle controls by treating all the groups with the identical sample of $A\beta_{1-43}$ internal standard.

Aβ Degradation Associated with N2a/WT Cells. N2a/WT cells were plated 24 h before the experiments at a density of 100×10^4 cells/well in 6-well plates. Cells were washed with PBS before the addition of 2 mL of DMEM supplemented with 0.2% FBS followed by drug treatment. Eighteen h after drug treatment, CM was collected and treated with NaN_3 and centrifuged at 10 000*g*; the mixture of $A\beta_{1-40}$ and $A\beta_{1-42}$ was added and further incubated for 8 h at 37 °C. The CM was then treated with the protease inhibitor mixture and spiked with $A\beta_{1-43}$ as an internal standard followed by immunoprecipitation and MALDI-TOF analysis.

Immunoprecipitation and MALDI/TOF Analysis. Immunoprecipitation of Aβ was performed by the addition of a mixture of 0.25 μL of biotinylated 4G8 antibody and 0.25 μL of biotinylated 6E10 antibody, samples were then rotated at room temperature for 4 h followed by the addition of 4 μL T1 streptavidin coated magnabeads and rotated for an additional 2 h. Beads were washed three times with NH_4CO_3 (10 mM, pH 8.0), and the Aβ peptides were eluted using 4 μL of a mixture of 75% acetonitrile and 0.5% TFA in H_2O, followed by the addition of 3 μL of a saturated matrix solution (α-cyano-4-hydroxycinnamic acid in 1:1 acetonitrile and H_2O). One μL of this mixture was

spotted onto a MALDI plate and analyzed using a MALDI-TOF instrument (Applied Biosystems). Mass spectra were acquired automatically in a linear positive mode at 1950 shot per spectrum. For each individual experiment, peak heights were normalized to the peak height of the $A\beta_{1-43}$ standard; these normalized values were then expressed relative to the DMSO vehicle control in each set of experiments.

MTT Assay. The MTT assay was performed by incubating the cells in a culture media containing 0.5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h, the cells were then washed with PBS and the formed purple formazan crystals were solubilized in DMSO. The plate was shaken on a plate rocker for 30 min, and then the absorbance was measured at 570 nm using 630 nm as a reference wavelength on a Dynex MRX II microplate spectrophotometer.

Bioavailability. Use of animals was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago. Nitrate **27** was dissolved in DMSO/H_2O (1:1 v/v) and administrated at 10 mg/kg via ip injection to male C57BL/6 mice (25 g). Animals were sacrificed at 30 and 120 min, and both blood and whole brain were collected for analysis. Two equal portions of each brain or blood sample were processed for analysis. Brain tissue was homogenized in methanol manually and then centrifuged at 4 °C, 13000 rpm for 10 min. The remaining pellet was washed with methanol (0.3 mL) and supernatants combined. After concentration under N_2, reconstitution in MeOH/H_2O (1:1, v/v), and centrifugation at 4 °C, 13000 rpm for 15 min, samples were analyzed by LC-MS/MS. Plasma was extracted in a similar way prior to analysis by LC-MS/MS, except acetonitrile was used instead of methanol. LC separation was achieved using a Waters Xbridge C18 3.0 mm × 100 mm, 3.5 μm column, and a linear gradient mobile phase from 55% to 95% MeOH in 0.1% formic acid over 21 min at a flow rate of 350 μL/min. MS/MS analysis was performed on a Sciex API 3000 triple-quad instrument monitoring the transition m/z = 361 → m/z = 200 in positive ion mode. Quantitative extraction from plasma was estimated by spiking untreated samples. Concentrations were interpolated from a standard curve constructed from analyte in MeOH/H_2O (4−500 ng/mL).

General Methods. Unless stated otherwise, all reactions were carried out under an atmosphere of dry argon in oven-dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 °C. CH_2Cl_2 was distilled over CaH_2, and THF was distilled over Na(s)/benzophenone. All other solvents were of anhydrous quality purchased from Aldrich Chemical Co. and used as received. Commercially available starting materials and reagents were purchased from Aldrich, TCI, and Fisher Scientific and were used as received unless specified otherwise. Indomethacin and sulindac libraries were provided by Prof. Larry Marnett of Vanderbilt University. Analytical thin layer chromatography (TLC) was performed with Sorbent Silica glass backed TLC plates (20 cm × 20 cm, 60 Å, 250 μm) with UV detection at 254 nm. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker Avance 400 MHz spectrometer or a Bruker DPX 400 MHz spectrometer. Chemical shifts are reported in ppm with the solvent resonance as internal standard ([$CDCl_3$ 7.27 ppm, 77.23 ppm] [DMSO-d_6 2.5 ppm, 39.51 ppm] and [MeOD-d_4 4.78, 49.0] for ^{1}H, ^{13}C, respectively). Data are reported as follows: chemical shift, number of protons, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, bs = broad singlet, m = multiplet), and coupling constants. Compounds submitted for biological testing were found to be >95% pure via analytical HPLC analysis using Shimadzu HPLC. High resolution mass spectral data was collected in-house using Shimadzu LCMS IT-TOF.

*1-Bromo-4-bromomethyl-2-fluorobenzene (**7**).*[45] *N*-Bromosuccinimide (11.39 g, 640 mmol) was added to a solution of 1-bromo-2-fluoro-4-methylbenzene **6** (5.36 mL, 8.01 g, 42 mmol) in CCl_4 (100 mL). The mixture was heated to 80 °C, and then dibenzoyl peroxide (0.27 g, 32 mmol) was added. The mixture was stirred at this temperature for 1 h and then cooled to rt and washed in sequence with H_2O (2 × 80 mL) and brine (2 × 50 mL), dried over Na_2SO_4, and concentrated to afford red oil (∼14 g), which was purified by column chromatography (hexane/ethyl acetate [10:1]) to give **7** as a yellow crystalline solid (12.2 g, yield 75.6%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.53 (t, 1H), 7.19−7.17 (dd, 1H), 7.08−7.06 (dd, 1H), 4.42 (s, 2H).

*4-Bromo-3-fluorophenyl)acetonitrile (**8**).* A solution of **7** (12.0 g, 41 mmol) and KCN (2 g, 0.04 mol) in ethanol (100 mL) was heated to 80 °C for 2 h and then cooled to rt and concentrated under vacuo. The residue was suspended in H_2O (200 mL) and extracted with ethyl acetate (3 × 150 mL). Combined organic extracts were washed with brine (2 × 50 mL), dried over Na_2SO_4, and concentrated to afford a brown oil. Column chromatography (hexane/ethyl acetate [7:1]) yielded **8** as a light-yellow solid (5.0 g, yield 52%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57 (dd, 1H), 7.13 (dd, 1H), 7.02 (dd, 1H), 3.72 (s, 2H).

*1-(4-Bromo-3-fluorophenyl)cyclopropanecarbonitrile (**9a**).* To a solution of **8** (5.0 g, 23 mmol) and 1,2-dibromoethane (3 mL, 35 mmol) in toluene (20 mL), 50% NaOH (20 mL), and TBAB (1.6 g, 5 mmol) were added. The mixture was vigorously stirred at rt for 4 h and then diluted with H_2O (20 mL) and extracted with ethyl acetate (3 × 150 mL). The organic layer was washed with 1 M HCl (25 mL) and then with brine (2 × 15 mL), dried over Na_2SO_4, and concentrated to afford a brown solid (5.7 g). Column chromatography (hexane/ethyl acetate [5:1]) afforded **9a** as yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54−7.50 (t, 1H), 7.05−7.03 (dd, 1H, J = 7.48 Hz), 7.00−6.98 (dd, 1H, J = 7.48 Hz), 1.80−1.77 (q, 2H), 1.43−1.40 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 160.42 (d, J = 240 Hz), 138.01 (d, J = 6.9 Hz), 133.94, 122.54 (d, J = 3.5 Hz), 121.49, 114.07 (d, J = 8.4 Hz), 108.23 (d, 20.8 Hz), 18.71, 13.50.

*1-(4-Bromo-3-fluorophenyl)diethylcarbonitrile (**9b**).* To a solution of **8** (235 mg, 1.1 mmol) and iodoethane (240 mg, 2.2 mmol) in toluene (20 mL), 50% NaOH (20 mL) and TBAB (420 mg, 2.8 mmol) were added. The mixture was vigorously stirred at rt for 4 h and then diluted with H_2O (20 mL) and extracted with ethyl acetate (3 × 100 mL). The organic solution was washed with 1 M HCl and then with brine, dried over Na_2SO_4, and concentrated to afford a brown solid (950 mg). Column chromatography (hexane/ethyl acetate [5:1]) afforded **9b** as white solid (278 mg, yield 93.5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73−7.70 (t, 1H), 7.18−7.15 (dd, 1H, J = 7.48 Hz), 7.12−7.09 (dd, 1H, J = 7.48 Hz), 2.10−2.01 (m, 2H), 1.92−1.83 (m, 2H), 0.95−0.91 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 160.11 (d, J = 240 Hz), 139.82 (d, J = 6.9 Hz), 133.46, 122.83 (d, J = 3.5 Hz), 120.97, 114.28 (d, J = 2.4 Hz), 107.96 (d, 21.1 Hz), 49.19, 33.33, 9.82.

*1-(4-Bromo-3-fluorophenyl)cyclopropanecarboxylic Acid (**10**).* To a solution of **9a** (5.1 g, 21 mmol) in methanol (10 mL), 35% NaOH (40 mL) was added and the mixture was heated to 100 °C for 8 h. After cooling to rt, the mixture was acidified (pH 2) with 2 M HCl, and the precipitate was filtered off, washed with H_2O, and redissolved in 5% $NaHCO_3$. Insoluble materials were filtered off and the solution acidified with 2 M HCl. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to afford **10** as a white solid (4.0 g, 72.5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.82 (bs, 1H), 7.51−7.47 (t, 1H), 7.14−7.12 (dd, 1H, J = 9.39 Hz), 7.05−7.03 (dd, 1H, J = 9.39 Hz), 1.73−1.70 (q, 2H), 1.29−1.22 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 180.50, 159.83 (d, J = 246.1 Hz), 140.25 (d, J = 7.0 Hz), 133.15, 127.28 (d, J = 3.4 Hz), 118.78 (d, J = 22.2 Hz), 107.20 (d, J = 20.7 Hz), 28.30, 17.54.

General Procedure for Biaryl Suzuki Coupling. A suspension of the appropriate aryl bromide (1 equiv), arylboronic acid (2 equiv), TBAB (1.1 equiv), and $Pd(OAc)_2$ (catalytic amount) in 2 M Na_2CO_3 (5−10 mL) was heated to 120 °C in a sealed tube for a period of 1−4 h. After cooling to rt, 2 M HCl (3 mL) and ethyl acetate (10 mL) were added and the mixture filtered through a Celite pad. The organic layer was separated, washed with brine (2 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/ethyl acetate [1:1]) to afford the corresponding biaryl compounds.

*1-(2-Fluorobiphenyl-4-yl)cyclopropanecarboxylic Acid (**11a**).* The general procedure using **10** (259 mg, 1 mmol), phenylboronic acid (244 mg, 2 mmol), TBAB (340 mg, 1 mmol), and $Pd(OAc)_2$ (30 mg) afforded the title compound as a white solid (yield 73.7%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63−7.55 (t, 4H), 7.50−7.36 (m, 2H), 7.24−7.16 (q, 2H), 1.76−1.73 (q, 2H), 1.35−1.33 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 178.68, 160.63 (J = 243.2 Hz), 140.60 (d, J = 8.0 Hz), 135.70, 130.56, 129.15, 129.13, 128.61, 128.21, 128.08, 127.84, 126.56 (d, J = 3.0 Hz), 118.45 (d, J = 23.0 Hz), 28.01, 17.90. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{16}H_{13}FO_2$, 255.0826; observed, 255.0832.

*1-(2,4′-Difluorobiphenyl-4-yl)cyclopropanecarboxylic Acid (**11b**).* The general procedure using **10** (150 mg, 0.58 mmol), 4-F-phenylboronic acid (162 mg, 1.16 mmol), TBAB (206 mg, 0.64 mmol), and $Pd(OAc)_2$ (20 mg) afforded the title compound as a white solid (yield 75.5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.70−7.33 (m, 5H), 7.24−7.17 (q, 2H), 1.76−1.70 (q, 2H), 1.34−1.32 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 179.96, 140.99, 132.57, 131.95, 131.32, 130.69, 130.08 (d, J = 3.6 Hz), 128.90, 128.21 (d, J = 3.4 Hz) 127.16, 126.54, 127.61, 118.51 (d, J = 22.8 Hz), 28.35, 17.54. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{16}H_{12}F_2O_2$, 273.0733; observed, 273.0746.

*1-(2-Fluoro-4′-(trifluoromethyl)biphenyl-4-yl)cyclopropanecarboxylic Acid (**11c**).* The general procedure using **10** (259 mg, 1.0 mmol), 4-CF_3-phenylboronic acid (228 mg, 1.2 mmol), TBAB (322 mg, 1.0 mmol), and $Pd(OAc)_2$ (40 mg) afforded the title compound as a white solid (yield 67.8%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.91 (bs, 1H), 7.72−7.65 (q, 4H), 7.42−7.38 (t, 1H), 7.26−7.19 (q, 2H), 1.76 (s, 2H), 1.35 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 180.29, 160.06, 157.58, 140.68 (d, J = 3.2 Hz), 138.65, 129.95, 129.55, 129.22, 128.89 (d, J = 1.3 Hz), 126.47 (d, J = 13.3 Hz), 126.21 (d, 2.9 Hz), 125.02 (d, J = 3.8 Hz), 118.13, 117.90, 28.04, 17.20. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{17}H_{12}F_4O_2$, 323.0701; observed, 323.0710.

*1-(3′,4′-Dichloro-2-fluorobiphenyl-4-yl)cyclopropanecarboxylic Acid (**11d**).* The general procedure using **10** (259 mg, 1.0 mmol), 3,4-dichlorophenylboronic acid (190 mg, 1.2 mmol), TBAB (322 mg, 1.0 mmol), and $Pd(OAc)_2$ (25 mg) afforded the title compound as a white solid (yield 72.9%) ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52−7.49 (t, 2H), 7.37−7.33 (t, 1H), 7.23−7.01 (m, 4H), 1.75−1.72 (q, 2H), 1.35−1.32 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 179.96, 160.10 (d, J = 246.5 Hz), 140.99 (d, 9.2 Hz), 132.57, 130.69, 130.06, 129.55, 129.22, 128.90, 128.19, 127.61, 126.54, 118.45 (d, J = 22.8 Hz), 28.35, 17.54. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{16}H_{11}Cl_2FO_2$, 323.0047; observed, 323.0050.

*2-Ethyl-2-(2-fluorobiphenyl-4-yl)butanenitrile (**12a**).* The general procedure using **9b** (150 mg, 0.56 mmol), phenylboronic acid (177.3 mg, 0.83 mmol), TBAB (206 mg, 0.64 mmol), and $Pd(OAc)_2$ (20 mg) afforded the title compound as a white solid (140 mg, yield 94.3%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56−7.54 (d, 2H), 7.47−7.43 (m, 3H), 7.40−7.36 (t, 1H), 7.27−7.25 (d, 1H), 7.25−7.19 (d, 1H), 2.12−2.03 (m, 2H), 1.97−1.88 (m, 2H), 0.98−0.90 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 160.05 (d, J = 242.8 Hz), 139.36 (d, J = 7.0 Hz), 135.42, 131.44, 129.35 (d, J = 4.0 Hz), 129.59, 128.94, 127.52, 122.50, 121.91, 118.5 (d, J = 22.8 Hz) 113.79, 113.55, 51.17, 33.38, 10.51.

*2-Ethyl-2-(3-fluoro-4-(naphthalen-1-yl)phenyl)butanenitrile (**12b**).* The general procedure using **9b** (51 mg, 0.19 mmol), 2-napthylboronic acid (120 mg, 0.70 mmol), TBAB (125 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg) afforded the title compound as a yellow solid (45 mg, yield 75.1%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (s, 1H), 7.93−7.88 (m, 3H), 7.58−7.50 (m, 4H), 7.31−7.24 (q, 2H), 2.15−2.05 (m, 2H), 2.03−1.88 (m, 2H), 0.98−0.89 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 162.09 (d, J = 246.2 Hz), 140.29 (d, J = 9.8 Hz), 133.75, 133.17, 133.87, 131.69 (d, J = 5.5 Hz), 128.80, 128.65, 128.50, 128.07, 127.18 (d, J = 4.1 Hz), 126.80 (d, J = 3.7 Hz), 124.22, 122.81 (d, J = 4.7 Hz), 122.21, 121.15, 115.60, 50.01, 34.21, 10.16.

*2-(2-Fluorobiphenyl-4-yl)acetonitrile (**13a**).* The general procedure using **8** (428 mg, 2.0 mmol), phenylboronic acid (490 mg, 4.0 mmol), TBAB (710 mg, 2.2 mmol), and $Pd(OAc)_2$ (67 mg) afforded the title compound as a yellow solid (350 mg, yield 82.8%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.42 (m, 6H), 7.18−7.10 (m, 2H), 3.75 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 159.40 (d, J = 249.7 Hz), 134.52, 131.10 (d, J = 3.8 Hz), 130.61 (d, J = 8.0 Hz), 129.26, 128.58, 128.55, 128.21, 127.93, 127.67, 123.56 (d, J = 3.4 Hz), 116.93, 115.53 (d, J = 24.5 Hz), 22.76.

*2-(2,4′-Difluorobiphenyl-4-yl)acetonitrile (**13b**).* The general procedure using **8** (500 mg, 2.1 mmol), 4-F-phenylboronic acid (560 mg, 4.0 mmol), TBAB (710 mg, 2.2 mmol), and $Pd(OAc)_2$ (45 mg) afforded the title compound as a yellow solid (480 mg, yield 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51−7.39 (m, 3H), 7.19−7.11 (m, 4H), 3.81 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 162.21 (d, J = 247.6 Hz), 158.04, 130.91 (d, J = 3.6 Hz), 130.70 (d, J = 8.0 Hz), 130.44, 130.29 (d, J = 2.9 Hz), 130.20 (d, J = 2.9 Hz), 127.59 (d, J = 13.5 Hz), 124.80, 123.61 (d, J = 3.5 Hz), 116.84, 115.57 (d, J = 24.6 Hz), 115.18 (d, J = 21.7 Hz), 22.75.

*Preparation of Fluoromethylnitriles (**14a**, **14b**, **15a**, **15b**).*[46] To a solution of the appropriate benzylic nitrile in anhydrous THF (approximately 5−10 mL of THF/1 mmol of nitrile) at −78 °C, *t*-BuLi (2.2 equiv for difluorination, 1.1 equiv for monofluorination) was added over a period of 2 min. The resulting orange to dark-red solution was stirred for 1 h at −78 °C. A solution of NFSi (2.5 equiv for difluorination, 1.3 equiv for monofluorination) in anhydrous THF (approximately 2−4 mL of THF/mmol of NFSi) was added over 2 min. After this addition, the solution was stirred for 3 h at −78 °C. The reaction was quenched with 0.01 M HCl, and the resulting solution was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo to give a yellow residue. Purification was achieved using silica gel flash chromatography (hexane/ethyl acetate [5:1]).

*2-Fluoro-2-(2-fluorobiphenyl-4-yl)acetonitrile (**14a**).* The general procedure using **13a** (192 mg, 0.91 mmol), *t*-BuLi (1.2 mL, 2.0 mmol), and NFSi (710 mg, 2.4 mmol), yielding after purification a mixture of **14a** (yellow solid, 42 mg, yield 20.1%) and **15a** (yellow solid, 111 mg, yield 49.4%). **14a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59−7.38 (m, 8H), 6.11−6.09 (d, 1H, J = 11.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 159.73 (d, J = 250.5 Hz), 134.34, 132.15 (d, J = 6.7 Hz), 132.11, 128.98, 128.79, 128.65, 128.49, 127.92, 123.25 (d, J = 3.9 Hz), 115.39 (d, J = 25.2 Hz), 114.77 (d, J = 33.3 Hz), 79.18 (d, J = 182.8 Hz), 60.37. APCI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_9F_2N$, 228.0630; observed, 228.0625.

*2,2-Difluoro-2-(2-fluorobiphenyl-4-yl)acetonitrile (**15a**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.32 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz): 159.58 (d, J = 251.0 Hz), 133.79, 133.74 (d, J = 13.6 Hz), 131.93 (d, J = 3.8 Hz), 131.76 (d, J = 7.8 Hz), 129.00 (d, J = 3.0 Hz), 128.78, 128.34, 124.93, 121.33 (q, J = 5.0 Hz), 116.82, 113.58 (d, J = 26.6 Hz), 112.19 (t, J = 47.9 Hz), 107.92 (t, J = 245.0 Hz). APCI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_8F_3N$, 246.0536; observed, 246.0515.

*2-(2,4′-Difluorobiphenyl-4-yl)-2-fluoroacetonitrile (**14b**).* Synthesized via the general procedure using **13b** (115 mg, 0.5 mmol), *t*-BuLi (0.65 mL, 1.1 mmol), and NFSi (394 mg, 1.5 mmol), yielding after purification a mixture of **14b** (yellow solid, 56 mg, yield 42.1%) and **15b** (yellow solid, 32 mg, yield 32%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54−7.30 (m, 5H), 7.15 (t, 2H, J = 8.4 Hz), 6.08 (d, 1H, J = 46.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 162.50 (d, J = 248.6 Hz), 159.25 (d, J = 250.9 Hz), 131.82 (dd, J = 21.0 Hz, 7.7 Hz), 131.29, 130.74 (dd, J = 13.5 Hz, 2.9 Hz), 130.43 (d, J = 2.7 Hz), 130.34 (d, J = 2.9 Hz), 128.84, 122.96 (t, J = 3.8 Hz), 115.45, 115.24, 115.06 (dd, J = 25.4 Hz, 4.4 Hz), 114.41 (d, J = 33.2 Hz), 78.88 (d, J = 183.0 Hz). APCI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_8F_3N$, 246.0536; observed, 246.0511.

*2-(2,4′-Difluorobiphenyl-4-yl)-2,2-difluoroacetonitrile (**15b**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52−7.37 (m, 5H), 7.09 (t, 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 163.04 (d, J = 249.2 Hz), 159.45 (d, J = 251.7 Hz), 132.68 (d, J = 13.2 Hz), 131.86 (d, J = 8.0 Hz), 131.73 (d,

J = 3.6 Hz), 130.83 (d, J = 3.0 Hz), 130.75 (d, J = 3.0 Hz), 129.92, 129.88, 121.40 (q, J = 4.9 Hz), 115.83 (d, J = 21.6 Hz), 113.64 (dt, J = 26.6 Hz, 5.1 Hz), 112.12 (d, J = 48.3 Hz), 107.79 (t, J = 244.3 Hz). APCI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_7F_4N$, 264.0442; observed, 264.0460.

*General Procedure for Fluoromethylnitrile (**16a**,**b** and **15a**,**b**) to Fluoromethylamide (**16a**,**b** and **17a**,**b**).* To a stirred solution of fluoronitrile in DMSO (30 mg/1.5 mL) was added K_2CO_3 and the mixture was cooled to 0 °C. To this mixture was added dropwise H_2O_2 at the same temperature, and the reaction mixture was stirred for 45 min at rt under N_2. The reaction was quenched by adding cold H_2O, and the mixture was extracted three times with ethyl acetate (3 × 75 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH [95:5]) to give corresponding fluoroamides.

*2-Fluoro-2-(2-fluorobiphenyl-4-yl)acetamide (**16a**).* White solid, yield 75.4%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59−7.38 (m, 8H), 6.11−6.09 (d, 1H, J = 11.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.10, 159.73 (d, J = 250.5 Hz), 136.52, 134.34, 132.15 (d, J = 6.7 Hz), 132.11, 128.98, 128.79, 128.65, 128.49, 123.25 (d, J = 3.9 Hz), 115.39 (d, J = 25.2 Hz), 114.77 (d, J = 33.3 Hz), 79.18 (d, J = 182.8 Hz). ESI-HRMS (m/z): $[M + Na]^+$ calcd for $C_{14}H_{11}F_2NO$, 270.0701; observed, 270.0709.

*2-(2,4′-Difluorobiphenyl-4-yl)-2-fluoroacetamide (**16b**).* White solid, yield 82.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54−7.30 (m, 5H), 7.15−7.13 (t, 2H, J = 8.4 Hz), 6.08−6.02 (d, 1H, J = 46.8 Hz,). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.52, 162.50 (d, J = 248.6 Hz), 159.25 (d, J = 250.9 Hz), 131.82 (dd, J = 21.0 Hz, 7.7 Hz), 131.29, 130.74 (dd, J = 13.5 Hz, 2.9 Hz), 130.43 (d, J = 2.7 Hz), 130.34 (d, J = 2.9 Hz), 128.84, 122.96 (t, J = 3.8 Hz), 115.45, 115.06 (dd, J = 25.4 Hz, 4.4 Hz), 114.41 (d, J = 33.2 Hz), 78.88 (d, J = 183.0 Hz). ESI-HRMS (m/z): $[M + Na]^+$ calcd for $C_{14}H_{10}F_3NO$, 288.0607; observed, 288.0609.

*2,2-Difluoro-2-(2-fluorobiphenyl-4-yl)acetamide (**17a**).* White solid, yield 81.0%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.43 (s, 1H), 8.11 (s, 1H), 7.71−7.69 (t, 1H, J = 8.0 Hz), 7.76−7.46 (m, 7H). ^{13}C NMR (DMSO-d_6, 400 MHz): 171.60, 160.63 (d, J = 242.5 Hz), 134.58, 131.22 (d, 3.8 Hz), 128.99 (d, J = 2.9 Hz), 128.58, 128.32, 127.90, 127.63, 125.63, 121.55 (d, J = 3.9 Hz), 117.59, 114.09 (d, J = 10.06 Hz), 113.96 (d, J = 19.5 Hz). ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{10}F_3NO$, 266.0787; observed, 266.0797.

*2-(2,4′-Difluorobiphenyl-4-yl)-2,2-difluoroacetamide (**17b**).* White solid, yield 87.9%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.42 (s, 1H), 8.10 (s, 1H), 7.71−7.62 (m, 3H), 7.53−7.49 (m, 2H), 7.34 (t, 2H, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): 164.77 (t, J = 30.7 Hz), 162.20 (d, J = 245.8 Hz), 158.61 (d, = 247.4 Hz), 134.62 (td, J = 25.7 Hz, 8.1 Hz), 131.50 (d, J = 3.2 Hz), 131.12 (d, J = 2.7 Hz), 131.03 (d, J = 3.0 Hz), 130.36, 129.78 (d, J = 13.0 Hz), 127.64, 121.78, 115.71 (d, J = 21.5 Hz), 113.81 (t, J = 254.0 Hz), 113.82 (dt, J = 25.8 Hz, 6.4 Hz). ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_9F_4NO$, 282.0547; observed, 282.0544.

*General Procedure for Acid Hydrolysis of Fluoromethylnitriles (**16a**, **16b**) to Fluoromethylacetic Acids (**18a**, **18b**).* A vigorously stirred emulsion of the fluoromethylnitrile in a 49% aqueous solution of H_2SO_4 (5 mL per 100 mg of substrate) was heated at 80 °C for 3 h. After being cooled to rt, the aqueous phase was extracted with diethyl ether (3 × 50 mL). Acids were washed out from the combined organic phases with satd $NaHCO_3$. The aqueous phase was acidified to pH 2 and extracted with Et_2O (3 × 50 mL). The combined extracts were dried over Na_2SO_4 and concentrated. The residue was recrystallized from chloroform: petroleum ether.

*2-(2-Fluorobiphenyl-4-yl)-2-hydroxyacetic Acid (**18a**).* White solid, yield 56.0%. ^{1}H NMR (MeOD-d_4, 400 MHz): δ 7.54−7.33 (m, 8H), 5.21 (s, 1H). ^{13}C NMR (MeOD-d_4, 100 MHz): 175.72, 165.27, 162.83, 159.73, 143.03 (d, J = 7.8 Hz), 142.95, 133.13, 132.12, 131.84 (d, J = 3.3 Hz), 129.08 (d, J = 13.6 Hz), 122.20 (d, J = 3.1 Hz), 116.54 (d, J = 21.8 Hz), 115.58 (d, J = 24.3 Hz), 73.42. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_{11}FO_3$, 245.0609; observed, 245.0639.

*2-(2,4′-Difluorobiphenyl-4-yl)-2-hydroxyacetic Acid (**18b**).* White solid, yield 41.5%. ^{1}H NMR (MeOD-d_4, 400 MHz): δ 7.56−7.54 (t, J = 6.8 Hz, 1H), 7.46−7.44 (t, 1H, J = 8.0 Hz), 7.39−7.31 (m, 3H), 7.18−7.16 (t, 2H, J = 8.8 Hz), 5.21 (s, 1H). ^{13}C NMR (MeOD-d_4, 100 MHz): 175.72, 164.05 (d, J = 245.7 Hz), 160.95, 160.18, 142.99 (d, J = 7.8 Hz), 133.13, 132.11 (d, J = 3.2 Hz), 132.04, 131.82 (d, J = 3.3 Hz), 129.01 (d, J = 13.7 Hz), 124.17 (d, J = 3.1 Hz), 116.43 (d, J = 21.8 Hz), 115.46 (d, J = 24.3 Hz), 73.42. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_{10}F_2O_3$, 263.0525; observed, 263.0575.

*2-(2-Fluorobiphenyl-4-yl)acetonitrile (**19**).* The acid catalyzed hydrolysis procedure used in the preparation of **18a**, starting with **13a** (510 mg, 2.41 mmol) in the presence of H_2SO_4 (100 mL) afforded the product as a white solid (480 mg, yield 86.3%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.37 (m, 6H), 7.21−7.15 (m, 2H), 3.80 (s, 2H).

*Preparation of the Biaryl-Biethyl Ester (**20**).* To a solution of **19** (350 mg, 1.5 mmol) and bromoethane (500 mg, 5 mmol) in toluene (20 mL), 50% NaOH (20 mL) and TBAB (700 mg, 5 mmol) were added. The mixture was vigorously stirred at rt for 8 h and then diluted with H_2O (25 mL) and extracted with ethyl acetate (3 × 50 mL). The organic solution was washed with 1 M HCl (1 × 50 mL) and then with brine, dried over Na_2SO_4, and concentrated to afford a brown solid. Purification was achieved using silica gel flash chromatography to give the product as a white solid (300 mg, 70.3%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50−7.36 (m, 7H), 7.05−7.01 (m, 1H), 4.34−4.32 (q, 2H), 2.12−2.02 (m, 2H), 1.99−1.90 (m, 2H), 1.01−0.98 (t, 6H), 0.71−0.70 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 174.30, 165.40, 140.05, 130.69, 130.28, 129.61, 128.93, 128.40, 127.58, 125.48, 122.69 (d, J = 4.82 Hz), 117.42, 113.56, 61.33, 52.09, 26.67, 14.30, 8.43.

*Preparation of Biaryl-Diethyl Acid (**21**).* The ester **20** (200 mg, 0.5 mmol) was dissolved in CH_3CN (15 mL) with a few drops of H_2O. TEA (0.2 mL, 1.5 mmol) was then added, followed by LiBr (430 mg, 5 mmol), and the reaction was vigorously stirred at reflux for 6 h. The reaction was cooled to rt and extracted with diethyl ether (3 × 50 mL). The aqueous layer was then acidified to pH ∼ 2 with 1 M HCl and extracted with ethyl acetate (3 × 50 mL). Combined organic extracts were washed with brine (2 × 30 mL) and H_2O (1 × 25 mL), dried over Na_2SO_4, and concentrated to give the product as a white solid (56 mg, 39% conversion). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59−7.41 (m, 6H) 7.29−7.19 (m, 2H), 2.14−2.07 (m, 2H), 1.99−1.92 (m, 2H), 1.01−0.94 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 178.25, 160.67 (d, J = 245.6 Hz), 139.39 (d, J = 7.4 Hz), 134.62, 130.65 (d, J = 3.9 Hz), 129.52, 128.55 (d, J = 2.8 Hz), 127.99, 127.54, 121.92 (d, J = 3.3 Hz), 121.42, 117.20, 113.82 (d, J = 24.8 Hz), 49.19, 33.38, 9.35. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{18}H_{19}FO_2$, 285.1296; observed, 285.1273.

*Preparation of Bis-TBS Ketene Acetals (**22a** and **22b**).* The appropriate biaryl acid (1 equiv) and TBDSCl (2.3 equiv) were dissolved in THF at rt. LiHMDS (2.2 equiv) was introduced dropwise at 0 °C for 15 min. The resulting reaction mixture was allowed to warm to rt and stirred overnight before being concentrated under vacuum. The residual oil was redissolved in hexanes, filtered, and concentrated. After confirmation of the olefinic proton and TBDS protection by ^{1}H NMR, the product was used in the next step without further purification.

General Procedure for Fluorination of the α-Carbon Using Selectfluor.[47] The bis-TBS ketene acetal (1.0 equiv) in MeCN (10 mL) was added dropwise to Selectfluor (1.5 equiv) in MeCN for 15 min while maintaining reaction <50 °C. The reaction was allowed to warm to rt and stirred for an additional 15 min. The reaction was then diluted with 1 M HCl (25 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was extracted with 1 M NaOH (3 × 50 mL). The aqueous layer was washed with ethyl acetate (3 × 50 mL), followed by acidification of the aqueous layer to pH ∼1 with 3 M HCl. The acidic aqueous layer was extracted with ethyl acetate (3 × 75 mL), and combined organic extracts were dried over Na_2SO_4 and concentrated.

*2-Fluoro-2-(2-fluorobiphenyl-4-yl)propanoic Acid (**23a**).* Synthesized using fluorination general procedure using the following values: **22a** (232 mg, 0.491 mmol), Selectfluor (226 mg, 0.638 mmol), yielding **23a** as a white solid (42 mg, yield 32.6%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.15 (bs, 1H), 7.44−7.27 (m, 6H), 7.14−7.05 (m, 2H), 1.52 (d, 3H, J = 7.18 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.90, 161.52 (d, J = 242.2 Hz), 141.98 (d, J = 7.4 Hz), 134.80, 132.21, 129.84, 129.60, 128.97, 128.94, 128.46, 127.98, 123.60, 115.42 (d, J = 24.8 Hz), 81.81, 19.02. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{15}H_{12}F_2O_2$, 261.0733; observed, 261.0706.

*2-Fluoro-2-(2-fluorobiphenyl-4-yl)acetic Acid (**23b**).* Synthesized using fluorination general procedure using the following values: **22b** (58 mg, 0.13 mmol), Selectfluor (40 mg, 0.10 mmol), yielding **23b** as a white solid (12 mg, yield 38.2%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.52 (m, 2H), 7.43−7.32 (m, 4H), 7.14−7.09 (m, 2H), 5.40−4.28 (d, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 174.9, 161.0 (d, J = 242.2 Hz), 141.9 (d, J = 7.4 Hz), 135.50, 132.05, 130.9 (d, J = 3.2 Hz), 129.52, 129.03 (d, J = 13.7 Hz), 128.51, 128.0, 127.83, 123.62, 115.40 (d, J = 24.8 Hz), 72.60. ESI-HRMS (m/z): $[M - H]^+$ calcd for $C_{14}H_{10}F_2O_2$, 247.0576; observed, 247.0601.

*4-Bromobutyl R-(−)-2-Fluoro-a-methyl-4-diphenylacetate (**24a**).* Flurbiprofen (1.0 g, 4.09 mmol) was dissolved in methanol (10 mL), and then 0.5 M sodium methoxide in methanol (8.18 mL, 4.09 mmol) was added. The reaction mixture was stirred at rt for 10 min, and then the solvent was evaporated under reduced pressure, yielding the sodium salt (1.08 g, 4.09 mmol), which was suspended in dimethylformide (10 mL) and stirred for 10 min, followed by dropwise addition of 1,4-dibromobutane (1.5 mL, 2.74 g, 12.6 mmol). The reaction mixture was stirred at rt for an additional 48 h. The reaction mixture was poured into H_2O (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were washed with H_2O (3 × 30 mL) and brine (3 × 50 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified by column chromatography (hexane/CH_2Cl_2 [1/1]) to give the product as yellow oil (1.35 g, 87.5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53−7.50 (m, 2H), 7.45−7.34 (m, 4H), 7.14−7.07 (m, 2H), 4.11−4.09 (t, 2H, J = 6.15 Hz), 3.73−3.71 (q, 1H, J = 7.15 Hz), 3.34−3.32 (t, 2H, J = 6.28 Hz), 1.86−1.73 (m, 4H), 1.52−1.50 (d, 3H, J = 7.17 Hz).

*N-(4-Bromobutyl)-2-(2-fluorobiphenyl-4-yl)propanamide (**24b**).* A solution of (*R*)-2-(2-fluorobiphenyl-4-yl)propanoic acid (0.60 g, 2.4 mmol) in $SOCl_2$ (4.4 g, 36.9 mmol) was heated at reflux. After 2 h, the reaction mixture was allowed to cool to rt and the excess of $SOCl_2$ removed under vacuum. Both a solution of the residual material in CH_2Cl_2 (2 mL) and a solution of NaOH (98.3 mg, 2.40 mmol) in H_2O (0.32 mL) were simultaneously added to a cooled (−20 °C) solution of 4-amino-1-butanol (438.2 mg, 4.92 mmol) in CH_2Cl_2 (2 mL) under vigorous stirring. The reaction was kept at −20 °C for 2 h and allowed to warm to rt overnight. The organic layer was washed with H_2O, $NaHCO_3$, and brine, and concentrated to afford the intermediate alcohol (50% yield) as an oil, which was used in the subsequent procedure without further purification. The intermediate alcohol (390 mg, 1.24 mmol) and PPh_3 (356.3 mg, 1.36 mmol) in CH_2Cl_2 (8 mL) were cooled to 5 °C, and a solution of tetrabromomethane (493 mg, 1.48 mmol) in CH_2Cl_2 (4 mL) was added dropwise over 10 min. The resulting clear solution was stirred at rt for 4 h, before removal of solvent under vacuum. The concentrate was purified by flash chromatography (hexane/ethyl acetate [20:80]) to yield pure **24b** (64% yield) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.56−7.53 (m, 2H), 7.47−7.35 (m, 4H), 7.16−7.11 (m, 2H), 3.57−3.54 (q, 1H), 3.39−3.37 (t, 2H), 3.31−3.24 (m, 2H), 1.87−1.78 (m, 2H), 1.67−1.60 (m, 2H), 1.55−1.53 (d, 3H, J = 6.9 Hz).

*General Procedure for Alkylation of Biaryl Cyclopropyl Acids (**11a**, **11c**, **11d**).* To a solution of the appropriate carboxylic acid (1 equiv) in CH_2Cl_2 was added oxalyl chloride (5 eq, 2.0 M in CH_2Cl_2), and the mixture was stirred at rt for 5 h. The mixture was concentrated and redissolved in CH_2Cl_2 containing TEA (2.5 equiv), followed by the dropwise addition of 2-bromoethanol (1.0−1.5 equiv). After stirring overnight, the solution was concentrated and purified by column chromatography.

*2-Bromoethyl-1-(2-fluorobiphenyl-4-yl)cyclopropanecarboxylate (**25a**).* Synthesized using general procedure with the following values: **11a** (130 mg, 0.50 mmol), oxalyl chloride (1.25 mL, 2.5 mmol), 2-bromoethanol (0.1 mL, 0.8 mmol), and TEA (0.5 mL) afforded **25a** as colorless oil (160 mg, yield 86.8%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59−7.39 (m, 6H), 7.25−7.21 (qs, 2H), 4.41−4.38 (t, 2H), 3.50−3.46 (t, 2H), 1.75−1.71 (q, 2H), 1.33−1.29 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.68, 159.8 (d, J = 246.6 Hz), 140.57 (d, J = 8.0 Hz), 135.70, 130.56, 129.15, 129.13, 128.61, 128.21, 128.08, 127.84, 126.56, 126.53, 118.32 (d, J = 23.1 Hz), 64.39, 28.87, 17.19. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{18}H_{16}BrFO_2$, 363.0390; observed, 363.0396.

*2-Bromoethyl-1-(2-fluoro-4′-(trifluoromethyl)biphenyl-4-yl)cyclopropanecarboxylate (**25b**).* Synthesized using general procedure with the following values: **11c** (270 mg, 0.83 mmol), oxalyl chloride (2.1 mL, 4.15 mmol), 2-bromoethanol (0.3 mL, 2.4 mmol), and TEA (1.0 mL) afforded **25b** as colorless oil (280 mg, yield 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73−7.67 (q, 4H), 7.43−7.39 (t, 1H), 7.28−7.21 (qs, 2H), 4.42−4.39 (t, 2H), 3.50−3.47 (t, 2H), 1.80−1.73 (q, 2H), 1.33−1.26 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.31, 160.44 (d, 246.6 Hz), 141.55, 139.11, 130.25, 130.21, 129.86, 129.27, 129.24, 126.62, 126.59, 125.34, 125.30, 122.80, 118.46, 64.26, 28.60, 16.99. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{15}BrF_4O_2$, 431.0264; observed, 431.0264.

*2-Bromoethyl-1-(3′,4′-dichloro-2-fluorobiphenyl-4-yl)cyclopropanecarboxylate (**25c**).* Synthesized using general procedure with the following values: **11d** (260 mg, 0.80 mmol), oxalyl chloride (2.0 mL, 4.0 mmol), 2-bromoethanol (0.1 mL, 0.8 mmol), and TEA (0.5 mL) afforded **25c** as a white solid (300 mg, yield 87.8%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.69 (s, 1H), 7.65−7.59 (d, 1H), 7.54−7.18 (m, 4H), 4.40−4.37 (t, 2H), 4.14−4.12 (t, 2H), 1.73−1.70 (q, 2H), 1.29−1.24 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.92, 159.92 (d, J = 242.0 Hz), 141.14 (d, J = 8.0 Hz), 135.04, 132.17, 131.50, 130.35, 129.72, 129.61, 129.57, 127.84, 126.25, 125.12, 118.12 (d, J = 23.0 Hz), 63.89, 28.32, 16.66. ESI-HRMS (m/z): $[M]^+$ calcd for $C_{18}H_{14}BrCl_2FO_2$, 430.9538; observed, 430.9611.

*General Procedure for Nitration of the Ethyl Bromide Esters (**25a**−**c**).* To a solution of the appropriate bromide (1 equiv) in anhydrous MeCN was added $AgNO_3$ (2−5 equiv), and the reaction was stirred at reflux for 2 h. The mixture was filtered through Celite, the filtrate concentrated, and the resulting crude oil purified by column chromatography (hexane/ethyl acetate [3:1]) to give the desired nitrates characterized below.

*2-(Nitrooxy)ethyl 1-(2-Fluorobiphenyl-4-yl)cyclopropanecarboxylate (**26a**).* Synthesized using general nitration procedure with the following values: **25a** (120 mg, 0.33 mmol) and $AgNO_3$ (112 mg, 0.66 mmol) afforded **26a** as yellow oil (85 mg, yield 74.5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.58−7.38 (m, 6H), 7.27−7.14 (m, 2H), 4.64−4.63 (t, 2H), 4.36−4.35 (t, 2H), 1.70−1.68 (m, 2H), 1.32−1.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.35, 159.90 (d, J = 242.0 Hz), 141.12(d, J = 8.0 Hz), 139.83, 135.11, 130.07, 129.53, 128.59, 128.06, 127.31, 125.93, 117.79, 117.56, 69.67, 60.75, 28.13, 16.76. ESI-HRMS (m/z): $[M + Na]^+$ calcd for $C_{18}H_{16}FNO_5$, 368.0905; observed, 368.0911.

*2-(Nitrooxy)ethyl 1-(2-Fluoro-4′-(trifluoromethyl)biphenyl-4-yl)cyclopropanecarboxylate (**26b**).* Synthesized using general nitration procedure with the following values: **25b** (220 mg, 0.49 mmol) and $AgNO_3$ (415 mg, 2.44 mmol) afforded **26b** as colorless oil (190 mg, yield 90.1%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73−7.67 (q, 4H), 7.43−7.39 (t, 1H), 7.25−7.18 (qs, 2H), 4.67−4.64 (t, 2H), 4.38−4.36 (t, 2H), 1.73−1.70 (q, 2H), 1.33−1.31 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.54, 160.46 (d, J = 240.2 Hz), 141.33(d, J = 8.0 Hz), 139.10, 130.36 (d, J = 3.6 Hz), 129.85, 129.29 (d, J = 3.1 Hz), 128.23, 126.58 (d, J = 3.3 Hz), 125.38 (q, J = 11.2 Hz), 124.10, 122.83, 118.35, 118.12, 70.00, 61.20, 28.54, 17.09. ESI-HRMS (m/z): $[M\text{-}NO_3]^+$ calcd for $C_{18}H_{16}FNO_5$,: 351.1008; observed, 351.1029.

*2-(Nitrooxy)ethyl 1-(3′,4′-Dichloro-2-fluorobiphenyl-4-yl)cyclopropanecarboxylate (**26c**).* Synthesized using general nitration procedure with the following values: **25c** (300 mg, 0.69 mmol) and $AgNO_3$ (589 mg, 3.47 mmol) afforded **26c** as colorless oil (210 mg, yield 73.0%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65−7.16 (m, 6H), 4.66−4.64 (t, 2H), 4.38−4.35 (t, 2H), 1.75−1.68 (q, 2H), 1.35−1.28 (q, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.92, 159.92 (d, J = 242.0 Hz), 141.14 (d, J = 8.0 Hz), 135.04, 132.17, 131.50, 130.35 (d, J = 1.8 Hz), 130.01, 129.72, 129.61, 127.88 (d, J = 4.0 Hz), 126.28 (d, J = 3.8 Hz), 125.26, 118.12 (d, J = 23.0 Hz), 69.59, 60.84, 28.16, 16.76. ESI-HRMS (m/z): $[M - NO_3]^+$ calcd for $C_{18}H_{14}Cl_2FNO_5$, 351.0349; observed 351.0536.

*4-(Nitrooxy)butyl 2-(2-fluorobiphenyl-4-yl)propanoate (**2**).* The bromide **24a** (1.34 g, 3.58 mmol) was dissolved in anhydrous MeCN (20 mL), and then $AgNO_3$ (1.84 g, 10.74 mmol) was added. The suspension was stirred at rt for 1 h and then at reflux 14 h. The solid was filtered through Celite, and the solvent was removed under vacuum. Flash column chromatography of the residue (hexane/ethyl acetate [7:3]) afforded the desired product as yellow oil (1.11 g, 85.9%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53−7.50 (m, 2H), 7.43−7.32 (m, 4H), 7.14−7.09 (m, 2H), 4.37 (t, 2H, J = 6.28 Hz), 4.11 (t, 2H, J = 6.15 Hz), 3.73 (q, 1H, J = 7.15 Hz), 1.86−1.73 (m, 4H), 1.52 (d, 3H, J = 7.17 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.9, 161.0, 158.5, 141.9, 135.5, 132.18, 130.9, 129.0, 128.5, 127.83, 123.6, 115.4, 115.1, 72.6, 64.1, 45.1, 25.0, 23.6, 18.3. ESI-HRMS (m/z): $[M + Na]^+$ calcd for $C_{19}H_{20}FNO_5$, 384.1218; observed, 384.1227.

*4-[[2-(2-Fluorobiphenyl-4-yl)propanoyl]amino]butyl Nitrate (**27**).* The bromide **24b** (0.24 mmol) was dissolved in CH_3CN, and $AgNO_3$ (82.39 mg, 0.48 mmol) was added. The suspension was stirred at rt for 14 h and then at reflux for 2 h. The solid was filtered through Celite pad, and the solvent was removed under vacuum. The resulting residue was purified by column chromatography (hexane/ethyl acetate [60:40]) to afford the product as yellow oil (63% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55−7.52 (m, 2H), 7.47−7.34 (m, 4H), 7.15−7.09 (m, 2H), 4.43 (t, 2H), 3.56 (q, 1H), 3.30−3.24 (m, 2H), 1.77−1.53 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.85, 161.00, 158.53, 124.85, 135.33, 131.02, 128.90 128.50, 127.90, 127.76, 123.55, 115.32, 115.09, 73.98, 47.58, 40.37, 26.05, 24.13, 18.49. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{21}FN_2O_4$, 361.1558; observed, 361.1568.

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ABBREVIATIONS USED

SALA, selective amyloid lowering agent; AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, β-amyloid peptide; NSAID, nonsteroidal anti-inflammatory drug; LPS, lipopolysaccharide; iNOS, inducible NO synthase; ISMN, isosorbide mononitrate; SARA, selective amyloid raising agent; CM, conditioned media; DMEM, Dulbecco'S Modified Eagle'S Medium; PBS, phosphate buffered saline; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NFSI, *N*-fluorobenzenesulfonimide; TBSCl, *tert*-butyldimethylsilyl chloride; LiHMDS, lithium bis(trimethylsilyl)-amide.

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