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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2219–2223

The geminal dimethyl analogue of Flurbiprofen as a novel $A\beta_{42}$ inhibitor and potential Alzheimer's disease modifying agent

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Received 6 January 2006; accepted 9 January 2006
Available online 7 February 2006

Abstract—The subtle modification of a selection of $A\beta_{42}$ inhibiting non-steroidal anti-inflammatory drugs (NSAIDs), through synthesis of the geminal dimethyl analogues, was anticipated to ablate their cyclooxygenase activity whilst maintaining $A\beta_{42}$ inhibition. Methylflurbiprofen **6** exhibited similar in vitro $A\beta_{42}$ inhibition to its parent NSAID Flurbiprofen and was further evaluated in the Tg2576 mouse model of Alzheimer's disease and an animal model of gastro-intestinal (GI) impairment, but proved unviable for further clinical development.

Alzheimer's disease (AD) is a progressive, adult onset, neurodegenerative disorder characterized by impairments in memory and cognition. The disease displays characteristic histological changes, including extracellular amyloid deposits and intracellular neurofibrillary tangles (NFTs).[1] The characteristic plaques present within the AD brain are primarily composed of β-amyloid (Aβ) peptides 40 and 42 amino acids in length. They are derived from the proteolytic processing of the β-amyloid precursor protein (APP) through initial cleavage by the β-secretase (BACE) and then γ-secretase enzymes. The subsequent formation of Aβ oligomers and amyloid plaques is believed to contribute to neuronal loss and ultimately failure of cognitive function. Biochemical and genetic evidence suggests that an increased production or decreased clearance of the longer $A\beta_{42}$ peptide appears to be a key contributor to AD.

In't Veld et al., in a landmark epidemiological study (Rotterdam), identified a link between prophylactic NSAID use and a significant reduction in AD risk.[2] Weggen and co-workers have identified a subset of NSAIDs that lower the level of $A\beta_{42}$ peptide at therapeutically relevant doses in vitro.[3] They further demonstrated that Ibuprofen reduces the levels of $A\beta_{42}$ in a transgenic mouse model.[4] A number of reports also indicate the potential beneficial effects of certain NSAIDs on APP processing including potential mechanisms of action through direct modulation of the γ-secretase complex.[1e,4,5] However, whilst an attractive mechanism for potential treatment of AD, prolonged NSAID usage is associated with severe gastro-intestinal (GI) complications through chronic inhibition of the COX-1 enzyme.[6] Thus, we sought to modify these agents to minimize COX activity whilst maintaining their $A\beta_{42}$ inhibitory action (Fig. 1).

Cyclooxygenase activity resides primarily in the (*S*)-isomer (**A**) of NSAIDs, the (*R*)-enantiomer (**B**) being largely COX inactive. However, in vivo racemization (**A** → **B**) is known for several NSAIDs[7] and as such might pose an issue on chronic administration of the

Keywords: Cyclooxygenase; Alzheimer's disease.
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doi:10.1016/j.bmcl.2006.01.033

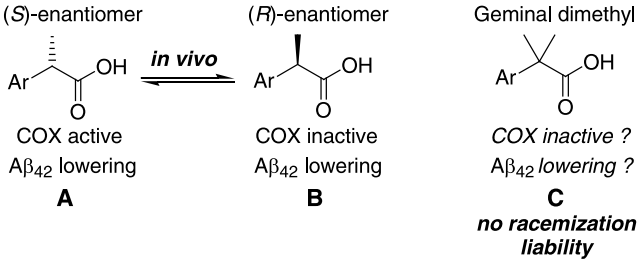


Figure 1. Hypothesis to eliminate COX activity of NSAIDs whilst maintaining $A\beta_{42}$ inhibitory activity.

(*R*)-enantiomer. For example, (*R*)-Flurbiprofen is a weak COX-1 inhibitor ($IC_{50} \sim 44$ μM in human whole blood)[8] and shows racemization in humans.[7d†] We envisaged that the dimethyl derivatives (**C**) would also be poor COX inhibitors and retain $A\beta_{42}$ inhibitory action. Furthermore, removal of the stereocenter obviously negates any complications from in vivo racemization.

We began our efforts by measuring the in vitro activity of certain NSAIDs to inhibit $A\beta_{42}$ production and confirmed the published findings (see Table 1).[3] Of note is that both enantiomers of Flurbiprofen appear to significantly reduce the amount of $A\beta_{42}$ in vitro at high, but clinically relevant, concentrations. Naproxen and Diclofenac appeared to have negligible effect on $A\beta_{42}$ production at relevant concentrations which proved surprising as these compounds constituted over 60% of the prescribed drugs in the aforementioned 'Rotterdam' epidemiological study.[2]

Next, we synthesized the geminal dimethyl analogues of a selection of these NSAIDs. We anticipated that these compounds, being structurally similar to the parent NSAID, should possess similar in vitro $A\beta_{42}$ inhibitory action but much reduced COX activity. Synthesis of these compounds is outlined (Scheme 1) along with representative examples (Table 2).[10] Fischer esterification of the aryl propionic acid (**a**) followed by alkylation under standard conditions yielded the geminal dimethyl ester (**b**). Conversion of the ester to the acid (**c**) was readily achieved by using potassium trimethylsilanolate (KOTMS).[11] However, synthesis of the geminal dimethyl analogue (**3**) of Indomethacin required an alternative synthetic procedure (Scheme 2).[12]

These compounds were then assayed for their ability to lower $A\beta_{42}$ levels (Table 2) in vitro. Only the dimethyl analogues of Carprofen **5** and Flurbiprofen (Methylflurbiprofen, **6**) showed similar inhibitory activities to their parent NSAID, the latter being the most potent (67% inhibition at 100 μM).

Despite the low level of in vivo racemization of (*R*)-Flurbiprofen, chronic usage may still lead to gastrointestinal complications, especially if the therapeutic

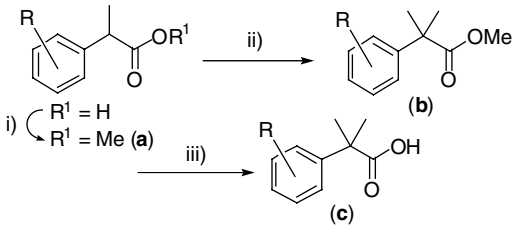
† (*R*)-Flurbiprofen has recently entered clinical trials for prostate cancer and an IND has been filed for its investigation as a treatment for Alzheimer's disease.

Table 1. In vitro potencies of common NSAIDs at $A\beta_{42}$ inhibition[a]

Compound	$A\beta_{42}$ % inhibition	
	SH-SY5Y SPA4CT	HEK APP695
Sulindac sulfide	73% at 30 μM	54% at 40 μM
Ibuprofen	41% at 600 μM	28% at 100 μM
Indomethacin	53% at 100 μM	73% at 100 μM
Naproxen	3% at 300 μM	ND
Sulindac sulfone	9% at 30 μM	0% at 40 μM
Diclofenac	17% at 100 μM	ND
R-Flurbiprofen	23% at 100 μM	51% at 100 μM
S-Flurbiprofen	37% at 100 μM	65% at 100 μM

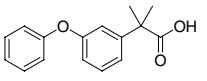
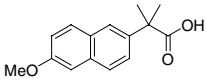
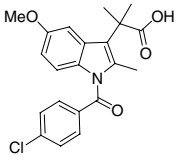
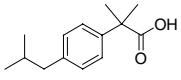
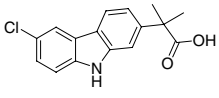
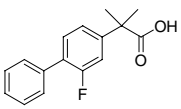
ND, no significant inhibition of $A\beta_{40}$ was observed.

[a] Inhibitory action measured in SH-SY5Y and HEK cell lines overexpressing either the direct substrate SPA4CT or the precursor APP695, respectively (Ref. 9).



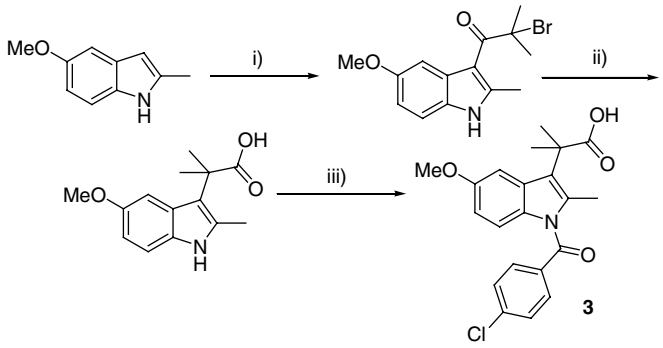
Scheme 1. Reagents and conditions: (i) MeOH, H_2SO_4 (cat), reflux; (ii) LDA, THF, DMPU, −78 °C then MeI; (iii) KOTMS, THF, 45 °C.

Table 2. Inhibitory potencies against $A\beta_{42}$ for geminal dimethyl NSAID derivatives[a]

Parent NSAID	Geminal dimethyl derivative	Compound	% inhibition at 100 μM (HEK cells)
Fenoprofen		**1**	0
Naproxen		**2**	0
Indomethacin		**3**	2
Ibuprofen		**4**	3
Carprofen		**5**	40
Flurbiprofen		**6**	67

'Methylflurbiprofen'

[a] $A\beta_{42}$ inhibitory activity measured at 100 μM in a HEK cell line overexpressing APP695 (Ref. 9). No inhibition of $A\beta_{40}$ was observed.



Scheme 2. Reagents: (i) $ZnCl_2$, BuLi then 2-bromoisobutyryl bromide; (ii) 2.4 M NaOH, EtOH; (iii) KHMDS (2.5 equiv), THF, 4-chlorobenzoyl chloride.

Table 3. Pharmacokinetic parameters for Methylflurbiprofen (**6**)[a]

	Rat	Mouse	Dog	Monkey
IV				
Cl (mL/min/kg)	1.3	0.7	0.28	3.8
V_{dss} (L/kg)	0.15	0.22	0.11	0.3
$T_{1/2}$	3.4	4.6	11.3	8.0
PO				
C_{max} (μM)	15.2	24.4	76.4	27.3
T_{max} (h)	2.0	0.25	0.33	0.25
F%	79	81	87	90

[a] Methylflurbiprofen dosed at 2 mg/kg po and iv in four species.

dose is high. The degree of racemization is also species specific, possibly presenting problems with chronic dosing paradigms in pre-clinical animal studies.[7a,b] Based on this information, we envisaged Methylflurbiprofen would be superior due to its similar in vitro activity at $A\beta_{42}$ inhibition, lack of activity against COX, and inherent achirality. Encouragingly, Methylflurbiprofen was inactive in a human whole blood assay against COX-1 ($IC_{50} > 100$ μM). (For comparison (*S*)-Flurbiprofen has an $IC_{50} \sim 0.2$ μM against COX-1 in human whole blood.) Furthermore, Methylflurbiprofen showed no inhibitory activity against the Notch receptor cleavage at 200 μM, a potential benefit compared to direct γ-secretase inhibition[13] and the pharmacokinetic parameters are excellent (see Table 3), showing good bioavailability (79–90%), half-life (3.4–11.3 h), and low clearance (0.3–3.8 mL/min/kg) in four species. Ancillary pharmacology also showed excellent dose proportionality, no adverse toxicology, no adverse effects in a canine cardiovascular model, and no significant activity when screened against a panel of other enzyme targets (data not shown).

Utilizing Ibuprofen as a positive control, Methylflurbiprofen was then tested in vivo using the Tg2576 transgenic mouse[14] model to ascertain its ability to inhibit the production of $A\beta_{42}$ peptide. Subchronic 3 day dosing at 21 mg/kg/day (qid) revealed no significant efficacy in lowering levels of $A\beta_{42}$. In vitro data indicated Methylflurbiprofen as a more potent compound than Ibuprofen but surprisingly no difference in activity is observed between them in this assay (Fig. 2). No significant effects on $A\beta_{40}$ levels were observed.

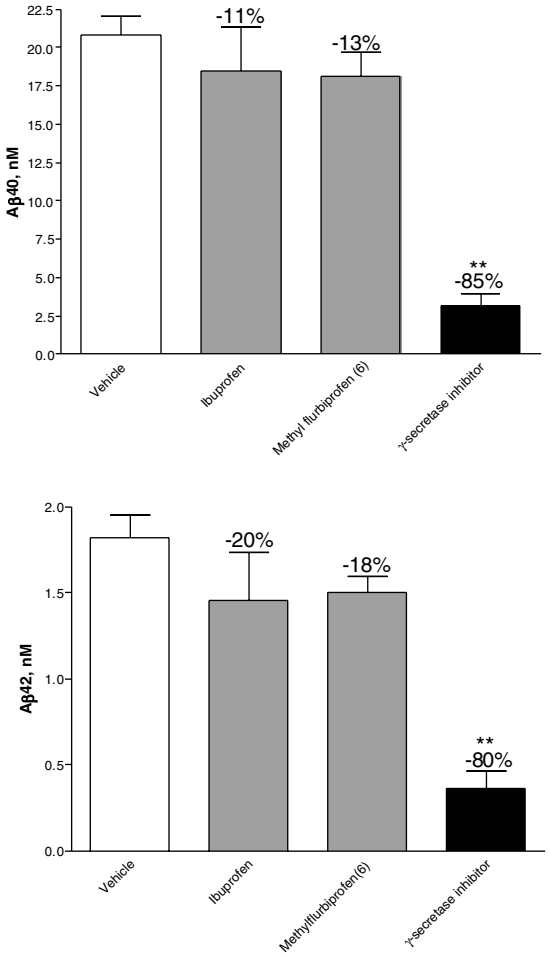


Figure 2. Brain levels of $A\beta_{40}$ (top) and $A\beta_{42}$ (bottom). Ibuprofen and Methylflurbiprofen dosed at 21 mg/kg (qid) for 3 days in Tg2576 mice ($n = 8$). A γ-secretase inhibitor (positive control) was given as a single 10 mg/kg dose on the final day ($n = 4$). Soluble $A\beta_{40}$ and $A\beta_{42}$ peptide was extracted from brain homogenate using DEA, see Refs. 4 (dosing) and 15 (extraction) for assay details.

Presumably, to achieve in vivo efficacy these compounds have to enter the brain, and as such we measured the brain and plasma exposure levels of Methylflurbiprofen **6** and various NSAIDs upon oral administration (Table 4).

Brain penetration for these compounds in mice proved low, with an average brain to plasma ratio of 3%

Table 4. Brain and plasma levels of common NSAIDs and Methylflurbiprofen[a]

	Brain (μM)	Plasma (μM)	Brain/plasma ratio
Ibuprofen	18	592	0.03
R-Flurbiprofen	41	1188	0.04
Indomethacin	3	266	0.01
Naproxen	27	887	0.03
Sulindac sulfide	1.7	296	0.006
Mefanamic acid	2.7	87	0.03
Fenoprofen	41	1358	0.03
Acetaminophen	43	64	0.68
Methylflurbiprofen (**6**)	13	849	0.02

[a] 50 mg/kg po dosing in mice ($n = 3$). Drug levels measured at T_{max}.

(excluding acetaminophen). This indicated that the brain levels achieved within our transgenic study did not achieve the in vitro IC_{50} of $A\beta_{42}$ inhibition. This highlighted an apparent disconnect between brain levels, IC_{50}, and in vivo efficacy, and appears contrary to published data.[4] Similar findings have also been observed by Lanz and co-workers.[16]

Epidemiological data suggest compounds of this nature only reveal efficacy at reducing plaque within the AD brain after chronic usage. Therefore, GI tract liabilities from prolonged administration of Methylflurbiprofen **6** were analyzed in a radioactive Cr leakage assay (Fig. 3). Human whole blood COX-1 analysis of Methylflurbiprofen indicated an $IC_{50} > 100$ μM. However, a 5 day, 100 mg/kg (bid) dosing regimen in rat showed significant leakage within the ^{51}Cr assay, the effects being comparable to a 1 day, 30 mg/kg (bid) dose of Ibuprofen. This dose was chosen to analyze the consequence of elevated plasma levels potentially achieved upon chronic dosing. Whilst the COX-1 activity of **6** is markedly reduced, plasma levels in this assay (average of 430 μM, 1 h post-final dose) clearly exceed those needed for COX-1 inhibition, thus producing gastro-intestinal lesions.

In summary, epidemiological data available show a tantalizing link between chronic NSAID usage and a reduction in the risk of contracting Alzheimer's disease.

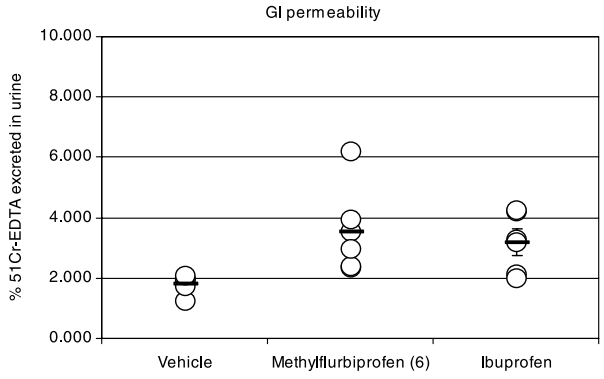


Figure 3. GI permeability as measured using a ^{51}Cr-EDTA excretion assay. Methylflurbiprofen was dosed at 100 mg/kg (bid) for 5 days and Ibuprofen at 30 mg/kg (bid) for 1 day ($n = 6$). Plasma levels for Methylflurbiprofen at 1 h post-final dose averaged 430 μM, Assay details are described in Ref. 17.

Continued use of racemic Flurbiprofen poses obvious gastro-intestinal complications and use of (*R*)-Flurbiprofen as a clinical agent for the treatment of AD might also encounter similar complications due to in vivo racemization. Methylflurbiprofen has reduced activity toward the COX enzyme and an inherent lack of racemization liability. However, despite being as potent in vitro at $A\beta_{42}$ inhibition as (*R*)-Flurbiprofen and more potent than Ibuprofen, it appears only marginally efficacious in vivo at lowering $A\beta_{42}$ levels in the Tg2576 mouse model. Coupled with the observation that high doses of **6** cause significant GI leakage, this compound proved unviable for further development.

Acknowledgments

The authors thank Merryl Cramer, Janice Brunner, and Dan Cui for assistance with PK studies.

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