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Design, synthesis, and in vivo characterization of a novel series of tetralin amino imidazoles as γ-secretase inhibitors: Discovery of PF-3084014

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ABSTRACT

A novel series of tetralin containing amino imidazoles, derived from modification of the corresponding phenyl acetic acid derivatives is described. Replacement of the amide led to identification of a potent series of tetralin-amino imidazoles with robust central efficacy. The reduction of brain Aβ in guinea pigs in the absence of changes in B-cells suggested a potential therapeutic index with respect to APP processing compared with biomarkers of notch related toxicity. Optimization of the FTOC to plasma concentrations at the brain Aβ EC_{50} lead to the identification of compound **14f** (PF-3084014) which was selected for clinical development.

Multiple lines of genetic and pathologic evidence have implicated the Aβ peptide in the etiology of Alzheimer's disease (AD). The last step in production of amyloid-β (Aβ) peptide is a C-terminal proteolytic cleavage by γ-secretase.[1] Mutations in components of the γ-secretase complex which alter Aβ processing induce early-onset AD. Thus inhibition of γ-secretase has emerged as a clinically viable disease-modifying approach to the treatment of AD. One liability to this approach is substrate specificity; γ-secretase processes additional substrates, most notably Notch. Cleavage of Notch by γ-secretase is necessary for differentiation of certain cell types and toxic effects of γ-secretase inhibitors (GSI) have been observed within the intestine and white blood cell populations.[2] Nonetheless, substrate specificity and/or PK/PD relationships may provide a possible therapeutic window. Indeed, numerous γ-secretase inhibitors such as LY-4501391, BMS-299897, GSI-953, and BMS-708163, have advanced into human clinical trials and demonstrated the viability of this approach.[3]

We previously described a series of diamide amino imidazole γ-secretase inhibitors exemplified by compound **1** (Fig. 1) that reduce Aβ at an in vitro IC_{50} of 0.4 nM in a whole-cell assay (WCA) and an IC_{50} of 1.1 nM in a cell-free assay (CFA).[4] In addition, a single acute dose in vivo (guinea pig) showed a dose dependent reduction in brain and plasma Aβ. To evaluate the potential for notch-related toxicity over a 24 h time period, B-cell populations

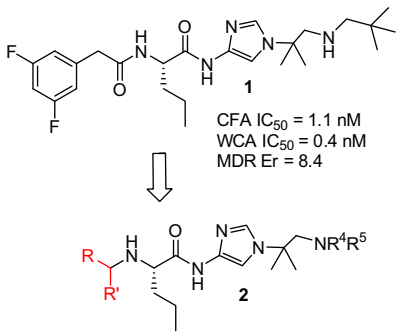

Figure 1. Replacement of the *N*-terminal amide of imidazole **1**.

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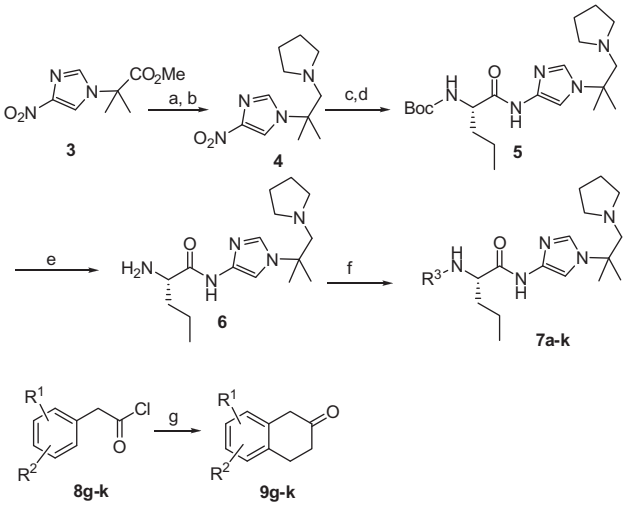
were analyzed by fluorescence activated cell sorting (FACS) in whole-blood and spleen preparations. At a 5 mg/kg dose, compound **1** significantly reduced brain $A\beta_{1-X}$ (58%) and B-cells populations (45–70%) suggesting no separation existed between Aβ lowering in the brain and notch mediated side-effects in the plasma. We speculated that the high plasma concentrations required to observe Aβ lowering effects in the brain were negatively impacting the therapeutic index. In part this could be explained by the significant P-gp mediated efflux (MDR efflux ratio = 8.4) of compound **1**. Evaluation of wide range of analogs related to compound **1** in the over expressing P-gp cell line (MDCK) indicated that efflux ratios (BA/AB) were generally greater than 4 indicating efflux was limiting central penetration.[5] Based on this observation, we began a systematic replacement of the N-terminal amide, a common P-gp recognition element. This report describes efforts to identify novel GSIs with increased therapeutic index with respect to Notch related side-effects.

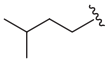
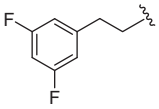
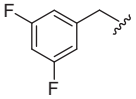
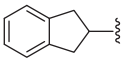
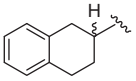
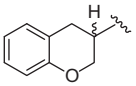
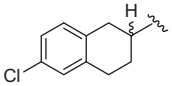
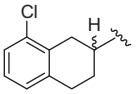
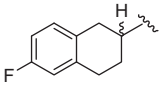
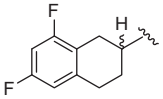
A general synthetic strategy for the preparation of substituted amino imidazoles is illustrated in Scheme 1. Starting with nitroimidazole **3**, reduction of the ester group to its corresponding aldehyde followed by reduction amination gave nitroimidazole **4** in good overall yield.[4] Hydrogenation of the nitroimidazole over Pd/C provided an intermediate aminoimidazole that was directly acylated with the activated acid of Boc-norvaline to furnish compound **5**. Acid catalyzed deprotection and subsequent reductive amination with an appropriate aldehyde or ketone gave analogs **7a–k**. The synthesis of the corresponding tetralin ketones (**9g–k**) was readily accomplished by Friedel-Crafts acylation of the phenyl acetyl chloride derivatives (**8g–k**) using ethylene.

Compounds **7a–d** were identified from this initial SAR studies and the cell free and whole cell potency was evaluated (Table 1).[6] When comparing isopentyl **7a** to 3-pentyl analog **7b** there was 10-fold potency improvement in the whole cell assay. Furthermore, 3,5-difluorobenzyl (**7d**) and 3,5-difluro phenethyl (**7c**) analogs gave modest potency improvements, but were significantly less active than compound **1**. Despite the loss in potency, we were gratified that analogs **7b–d** showed a reduced liability toward P-gp mediated efflux as measured by MDR efflux ratios (Er).[5] Next, we postulated that incorporating α-branching would restrict the conformation of **7c** or **7d**, reduce the number of rotatable bonds, and lock the aryl group in an active binding



Scheme 1. Reagents and conditions: (a) DIBAL, CH_2Cl_2, −30 °C; (b) pyrollidine, 4A°MS, CH_2Cl_2, then $Na(OAc)_3BH$, 60% over 2 steps; (c) Pd/C (10%), H_2 (40 psi), MeOH, rt; (d) $BocNorCO_2H$, TPTU, iPr_2EtN, DMF, 80% for 2 steps; (e) TFA, CH_2Cl_2; (f) R^3CHO or **9g–k**, CH_2Cl_2, $Na(OAc)_3BH$ then separate, 30–45%, for 2 steps; (g) C_2H_4, $AlCl_3$, CH_2Cl_2.

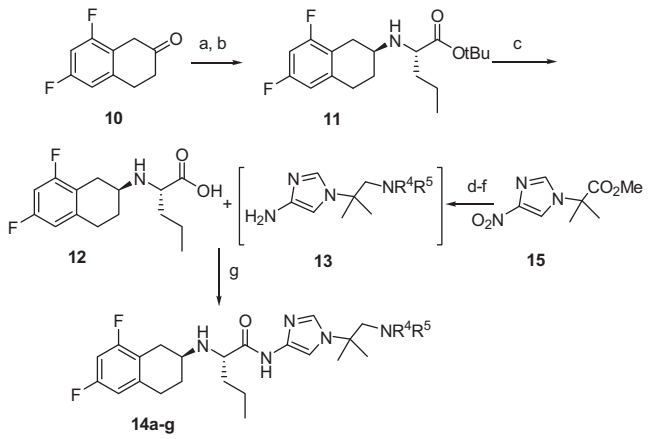
Table 1
Whole cell assay (WCA), cell free assay (CFA), and MDR efflux ratios (MDR Er) for representative gamma secretase inhibitors **7a–k**

Compound	R^3	WCA IC_{50}[a,b] (nm)	CFA IC_{50}[b,c] (nm)	MDR Er[d]
7a		3100	>10,000	4.4
7b		310	6120	2.3
7c		754	250	1.9
7d		480	499	1.8
7e		272	148	1.8
7f		53.9	85.7	2.2
7g		155	93.3	2.9
7h		12.4	8.9	1.3
7i		46.8	31.0	3.3
7j		19.5	19.3	1.8
7k		5.4	4.1	1.5

[a] IC_{50} values in the whole cell assay (WCA) were obtained from H4 APP_{Sw} cells by measuring $A\beta_{1-X}$.[6]
[b] Values are geometric mean of at least two experiments; compounds were typically dosed at log intervals from 0.1 nM to 10 μM.
[c] IC_{50} values in the cell free assay (CFA) were obtained from human HeLa cells by measuring $A\beta_{1-40}$ by DELFIA-based immunoassay.[6]
[d] MDR1-MDCK assay utilizes MDCK cells transfected with the gene that encodes for human P-glycoprotein.[5]

conformation. Thus, analogs **7e** and **7f** were prepared to validate this design concept. Previous SAR in the diamide imidazole series demonstrated that incorporation of mono or di-fluorines on the aryl ring of the phenyl acetic acid derivative improved potency.[4] Toward this end, substituted tetralins **7h–k** were prepared as racemic mixtures. We were gratified to find that cell free and whole cell potency was similar for **7k** compared to the diamide **1** while reducing P-gp mediated efflux.

To facilitate analog synthesis, a new route was established that incorporated the difluorotetralin in the early stages of the synthesis, avoiding the need for a late stage chiral separation (Scheme 2). Reductive amination of **10** with (*S*)-Boc Norvaline *t*-butyl ester provided a mixture of diastereomers which was separated using chiral HPLC to provide **11**. Acid catalyzed hydrolysis of the *t*-butyl ester **11** provided acid **12**, whose stereochemistry was unequivocally confirmed by single-crystal X-ray analysis. With carboxylic acid **12** in hand, we sought coupling conditions with the intermediate



Scheme 2. Reagents and conditions: (a) $Na(OAc)_3BH$, NH_2NorCO_2tBu, CH_2Cl_2; (b) chiral separation, 20–45% over 2 steps; (c) HCl, 90%; (d) DIBAL, CH_2Cl_2, −30 °C; (e) HNR^4R^5, 4A°MS, CH_2Cl_2, then $Na(OAc)_3BH$, 30–73% over 2 steps; (f) Pd/C (10%), H_2 (40 psi), MeOH, rt; (g) TPTU, iPr_2EtN, DMF, 60–80% for 2 steps.

aminoimidazole **13** that avoided protection of the secondary amine of **12**. Coupling attempts with CDI or EDC/HBTU failed to provide the desired amide but TPTU activation followed by treatment with the amino-imidazole derived from **15** cleanly provided the desired analogs **14a–g**.

With an improved synthetic route to the single diastereomer of racemic tetralin **7k**, we studied compound **14a** (Table 2) in Aβ efficacy studies. Guinea pigs were dosed acutely at doses ranging from 3.2 to 32 mg/kg, sc, and tissues were collected at 3 h for Aβ measurement in brain, CSF, and plasma by DELFIA (Fig. 2A).[7] A clear relationship existed between inhibition of Aβ in the brain, CSF, and plasma. At the 3.2 mg/kg dose, plasma exposure was 100 ng/mL (211 nM) and brain exposure was 587 ng/mL (1239 nM), which produced a significant reduction (33% brain, 31% CSF, 30% plasma) in $Aβ_{1-X}$. For a more comprehensive analysis of the time course of Aβ changes in brain, CSF, and plasma, guinea pigs were dosed acutely at 10 mg/kg, sc and tissues were taken at time intervals from 3 to 24 h (Fig. 2B). Significant reductions in $Aβ_{1-X}$ were detected from 3.2 h to 10 h with maximal response occurring from 3 to 10 h. At 24 h, the Aβ levels in plasma and CSF had returned to baseline while brain Aβ showed a longer duration of action. To understand the effects of Notch processing, analog **14a** was evaluated in fetal thymic organ culture (FTOC) for B- and T-cell populations (Table 2).[8] Compound **14a** had a mean EC_{50} of 3.26 μM which represents >500-fold separation from the APP whole-cell IC_{50}. To translate this selectivity in vivo, compound **14a** was dosed at 7 and 32 mg/kg three times over a 24 h period (time 0, 12, and 24 h) and tissues were collected at 3 h after the final dose (Fig. 2C). Under this dosing paradigm, brain and plasma Aβ levels were significantly reduced. At the 7 mg/kg dose, there was not a significant reduction in blood or marginal zone B cell populations as measured by FACS. This suggested a potential therapeutic index between the doses needed for a 50% reduction in brain Aβ and doses that impacted B-cell reductions. These results indicate more potent inhibition of APP versus early and sensitive biomarkers of notch cleavage, as the latter event is a prerequisite for B cell maturation.

Previous SAR in the diamide imidazoles suggested that replacement of the pyrrolidine group in the imidazole side chain led to a potency improvement in vitro and in vivo. Modification using a range of amines (**14b–g**) yielded several analogs with comparable whole cell potency and in vivo efficacy in guinea pig compared to **14a** (Table 2). To directly compare analogs, an ED_{50} in brain was generated at a 3 h time point. Despite the comparable whole cell potency for all analogs, the in vivo efficacy was superior for morpholine **14e** and neopentyl **14f** compared to the other analogs. In order to fully understand the impact of the FTOC assay compared with the Aβ lowering effects, we decided to normalize the FTOC EC_{50} to the plasma concentration (C_p) observed at the brain ED_{50}

Table 2
Whole cell potency, FTOC selectivity, and in vivo Aβ lowering data for representative γ-secretase inhibitors

Compound	NR^4R^5	WCA IC_{50} (nM)[a]	ED50 for $Aβ_{1-X}$ in brain[b]	FTOC EC_{50} (μm)[c]	FTOC/Cp @brain ED_{50}[e]
14a		2.9	9.9 mg/kg, sc	3.26	9.8
14b		3.3	4.1 mg/kg, sc	2.56	46.1
14c		19.3	41.2 mg/kg, sc	6.1	3.5
14d		4.6	16.1 mg/kg, sc	3.01	ND[d]
14e		6.9	2.9 mg/kg, sc	1.09	16.3
14f		1.2	2.3 mg/kg, sc	1.83	228.8
14g		4.9	14.6 mg/kg, sc	5.1	37.7

[a] IC_{50} values in the whole cell assay (WCA) were obtained from H4 APP_{Sw} cells by measuring $Aβ_{1-X}$.[6] Compounds were dosed at concentrations ranging from 0.01 to 313 nM. Values are geometric mean of at least two experiments.

[b] In vivo activity was determined by measuring $Aβ_{1-X}$, $Aβ_{1-40}$, and $Aβ_{1-42}$ in guinea pig brain, CSF, and plasma by Delfia ELISA.[7] Significant differences between groups were detected by one-way ANOVA followed by Dunnett's post-hoc in GraphPad Prism v5. Treatment effects were considered statistically significant following p <0.05 at the level of the ANOVA and post-hoc versus vehicle.

[c] Fetal thymic organ cultures (FTOC) were prepared for assessment of compound effects on Notch processing.[8]

[d] ND: not determined.

[e] Plasma concentration values were determined using a linear least squares regression model with dose versus measured plasma concentrations.

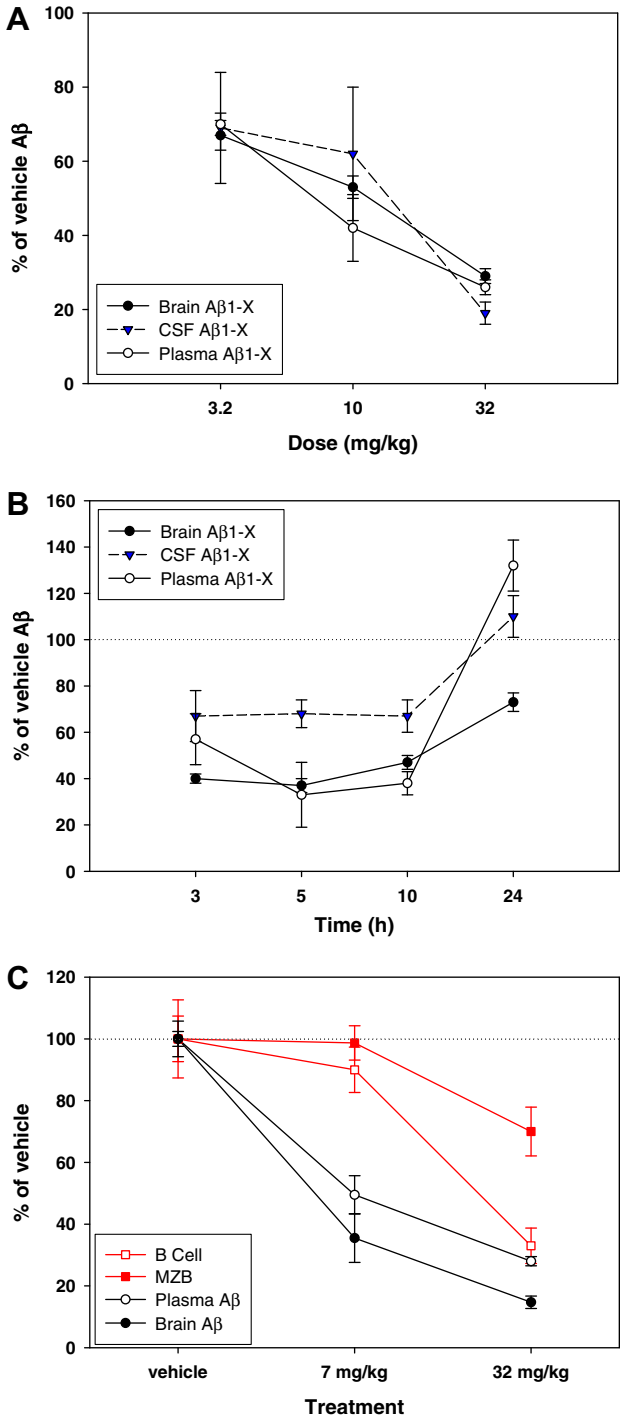


Figure 2. In vivo characterization of GSI **14a**; dose responsive of brain, CSF and plasma Aβ2A; time course of brain, plasma, and CSF Aβ (2B); reduction of marginal and blood B-cell populations (2C). (A and B) In vivo activity was determined by measuring $A\beta_{1-X}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ were measured in guinea pig brain and plasma by Delfia ELISA. Extracts were analyzed for changes in $A\beta_{1-X}$ using an IGEN assay.[7] Mean ± S.E.M. exposure or percentage of vehicle Aβ are represented. (C) Spleen and whole blood B-cells (relative numbers or percentage) were evaluated by flow cytometry.[8]

obtained from guinea pigs. This would serve as a means to rank order compounds with respect to in vivo efficacy and early biomarkers related to Notch processing. Compound **14f** (PF-3084014) showed the largest ratio of the tetralin amino-imidazoles and was therefore selected for additional efficacy studies and long term safety evaluation.[8]

In conclusion, a series of tetralin amino imidazoles were designed and synthesized based on SAR from a diamide series that suffered from significant P-gp mediated efflux. To improve brain penetration, variation of the C-terminal phenyl acetic acid analogs with non-peptidic groups resulted in the tetralin variations. Incorporation of fluorines on the aryl ring resulted in a significant improvement in whole cell potency. Further in vivo profiling demonstrated that compound **14a** reduced brain, CSF, and plasma Aβ in a dose responsive manner over time with a separation between Aβ and Notch related side-effects. Optimization of the FTOC/plasma concentrations obtained at the brain ED_{50} led to the discovery of PF-3084014 (**14f**) which was selected for clinical development.

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