ACS Medicinal Chemistry Letters

Letter

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An Orally Available BACE1 Inhibitor That Affords Robust CNS Aβ Reduction without Cardiovascular Liabilities

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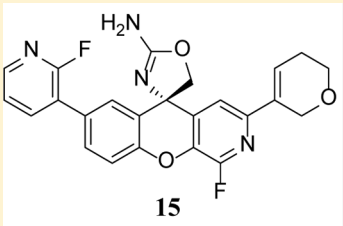
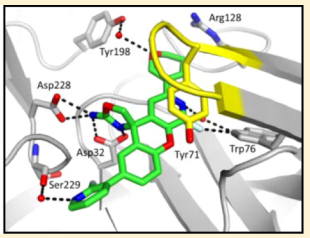
Supporting Information

ABSTRACT: BACE1 inhibition to prevent Aβ peptide formation is considered to be a potential route to a disease-modifying treatment for Alzheimer's disease. Previous efforts in our laboratory using a combined structure- and property-based approach have resulted in the identification of aminooxazoline xanthenes as potent BACE1 inhibitors. Herein, we report further optimization leading to the discovery of inhibitor **15** as an orally available and highly efficacious BACE1 inhibitor that robustly reduces CSF and brain Aβ levels in both rats and nonhuman primates. In addition, compound **15** exhibited low activity on the hERG ion channel and was well tolerated in an integrated cardiovascular safety model.



15



KEYWORDS: *β-site amyloid precursor protein cleaving enzyme 1 (BACE1), Alzheimer's disease (AD), Aβ, aminooxazoline, xanthene*

Alzheimer's disease (AD) is the most common neurodegenerative disorder and accounts for 50−80% of dementia cases identified each year. Current therapeutics for AD only provide temporary symptomatic relief and do not address the underlying neuropathology. A therapeutic treatment that directly modifies the progression of the disease remains one of the largest unmet medical needs in neurobiology.

The most widely held hypothesis for the underlying pathogenesis of AD posits that aggregation of β-amyloid peptides (Aβ) and deposition into amyloid plaques in the neural parenchyma play an essential role. Aβ is produced by sequential endoproteolytic cleavage of amyloid precursor protein (APP) by the aspartyl protease β-site APP cleaving enzyme-1 (BACE1) and γ-secretase.[1,2] Inhibition of BACE1 decreases the production of all major forms of Aβ including the most pathogenic species Aβ42,[3,4] indicating that BACE1 is a prime therapeutic target for the development of disease-modifying therapies for AD.[5−7] Genetic evidence published recently has provided additional support for the amyloid hypothesis by demonstrating that even modest reductions in the β-cleavage of APP (resulting from a mutation in the APP gene) could significantly protect against the development of AD.[8]

Despite extensive research efforts for over a decade, the identification of brain penetrant and efficacious BACE1 inhibitors remains a major challenge. Early work focused on peptidic transition state mimetics that met with limited success.[9] In recent years, a major advance in the design of BACE1 inhibitors was the discovery of 2-amino heterocycles that engage both active site aspartyl moieties of BACE1.[10−15] These ligands possessed more rigid structural characteristics and lower molecular weight, offering a promising opportunity to develop potent, brain penetrant inhibitors. This approach was supported by encouraging results in a recent report of a nonpeptidic BACE1 inhibitor that exhibited a significant reduction in central Aβ levels in preclinical species and healthy volunteers in early stage clinical trials.[16]

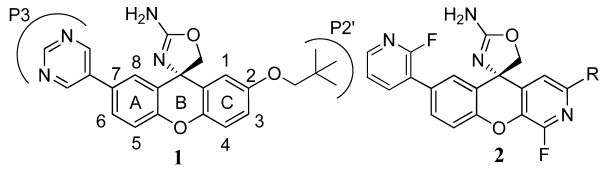
Previously, we reported the discovery of potent aminooxazoline xanthene BACE1 inhibitors rationally designed using a structure- and property-based approach.[12] In the course of lead optimization, compound **1** was identified as a potent BACE1 inhibitor.[17] Compound **1** acutely lowered CSF and brain Aβ40 by approximately 80% and 61%, respectively, 4 h postdose

Received: November 6, 2014
Accepted: December 29, 2014
Published: December 29, 2014

DOI: 10.1021/ml500458t
ACS Med. Chem. Lett. 2015, 6, 210−215

when administered orally to rats at 30 mg/kg. However, further advancement of this compound was hampered due to off-target activity at the hERG cardiac ion channel, which translated into an induced prolongation of the QTc interval in an integrated dog in vivo cardiovascular model. Activity on the hERG channel has been observed for other BACE1 inhibitors containing a 2-amino heterocyclic warhead.[14,17−19] Concurrent efforts focused on modification of the xanthene core and optimization of the peripheral groups to identify BACE1 inhibitors with minimized hERG activity, improved BACE1 potency, and CNS exposure.[17−19] We report herein the design and optimization of a series of BACE1 inhibitors based on the 3-aza-4-fluoro-xanthene scaffold **2** that evolved from the lead xanthene compound **1** (Chart 1).

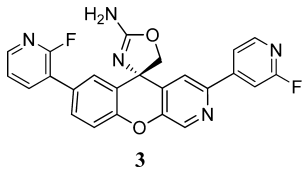
Chart 1. Early Aminooxazoline Xanthene Analogues



1

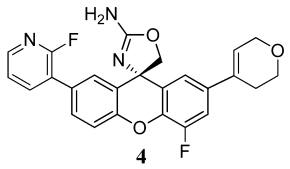
BACE1 IC_{50}: 2.2 nM
Cell IC_{50}: 25 nM
hERG K_i: 0.66 μM
Papp ($x10^{-6}$ cm/s): 13
Human/Rat Pgp ER: 1.5/0.9
Rat CSF/brain Aβ40 (POC):
-80%/-61% @30 mg/kg po

2



3

BACE1 IC_{50}: 0.9 nM
Cell IC_{50}: 21 nM
hERG K_i: >15 μM
Papp ($x10^{-6}$ cm/s): 14
Human/Rat Pgp ER: 1.2/1.8
Rat CSF/brain Aβ40 (POC):
-74%/-65% @10 mg/kg po
CSF effect $EC_{50,unbound}$: 15 nM
Brain effect $EC_{50,unbound}$: 21 nM



4

BACE1 IC_{50}: 0.3 nM
Cell IC_{50}: 4.1 nM
hERG K_i: 3.3 μM
Papp ($x10^{-6}$ cm/s): 6.9
Human/Rat Pgp ER: 1.0/1.0
Rat CSF/brain Aβ40 (POC):
-78%/-67% @10 mg/kg po
CSF effect $EC_{50,unbound}$: 5 nM
Brain effect $EC_{50,unbound}$: 10 nM

From the X-ray cocrystal structure of **1** in BACE1, it is observed that the isobutyl group extends into the P2′ pocket.[12] Structure−activity relationship (SAR) efforts involving P2′ substitution showed that introduction of polar P2′ groups could moderate the hERG activity, but typically with the consequence of increased Pgp efflux that resulted in reduced CNS penetration.[17] Further SAR studies on the xanthene core indicated that replacement of carbon with nitrogen at the 3-position decreased hERG activity and increased BACE1 potency. The gain in BACE1 potency was consistent with models based on the cocrystal structure of **1** suggesting that the 3-aza nitrogen could form a water-mediated hydrogen bond with the Trp76 side chain. Optimization of the 3-azaxanthene series produced **3** with robust reduction of CSF and brain Aβ levels in a rat pharmacodynamic (PD) model.[19] The Trp76 residue was also found to engage in a hydrogen bonding interaction with a fluorine substituent at the 4-position of the xanthene core, resulting in a significant improvement in BACE1 potency, as demonstrated by compound **4** that showed significant Aβ reduction in our PD assay.[17] Given the encouraging results obtained with the 3-aza and 4-fluoro xanthenes, we sought to combine the 3-aza and 4-fluoro moieties in one molecule (**2**). This resultant combination could potentially further improve the BACE1 potency and benefit from the 3-aza that was shown to mitigate hERG activity with the 4-fluoro group tempering the basicity of the 3-aza nitrogen with the expectation of moderating Pgp efflux.

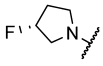
The 3-aza-4-fluoroxanthene analogues (**5**−**23**) were designed to keep the overall physicochemical properties (molecular weight, PSA, and clogP) within a range expected to be favorable for CNS penetration.[20] The synthesis of these compounds is discussed in the Supporting Information. The in vitro results are provided in Table 1. All the compounds (**5**−**23**) showed subnanomolar range BACE1 potency in the enzymatic assay and hERG potency >5 μM. Notably, compound **12** was more potent in the BACE1 enzymatic assay and the cell assay than its 3-aza-xanthene counterpart **3**, while **12** exhibited higher passive permeability than **3** and retained a low efflux ratio. Correspondingly, compound **16** showed enzymatic and cell potency comparable to its 4-fluoro-xanthene counterpart **4** and significantly improved passive permeability due to reduced lipophilicity. These results demonstrated that the 3-aza-4-fluoro-xanthene was a promising core structure with a profile superior to other xanthene cores.

As previously observed, the P2′ region of the molecule was found to be fairly accommodating, accepting saturated and unsaturated ring systems including cyclic amines. The unsubstituted phenyl (**5**) and 3- and 4-pyridyl (**6** and **7**) all displayed single-digit nanomolar cellular activity. 2-Fluoro-4-pyridyl (**12**) retained the same level of potency, and the *o*-methyl substituent in both 4-pyridyl and 3-pyridyl groups (**8** and **9**) were also well tolerated, although **9** appeared to be more labile in human liver microsomes. Introducing an *o*-fluoro atom to the 3-pyridyl group, 2-fluoropyridin-3-yl (**10**) was 4- and 8-fold less potent than **6** in the BACE1 enzymatic and cell assays, respectively, and 2-fluoropyridin-5-yl (**11**) showed comparable potency to **6** in the enzymatic assay but was 3-fold less potent in the cell assay. All the P2′ dihydropyran analogues (**13**−**16**) exhibited single-digit nanomolar potency in both BACE1 enzymatic and cell assays. The permeability and efflux properties suggested exposure in the CNS should be easily achieved. The 3,6-dihydro-2*H*-pyrans (**15**−**16**) were more metabolically stable than the 3,4-dihydro-2*H*-pyrans (**13**−**14**) in both human and rat liver microsomal studies. The fully saturated tetrahydropyrans (**17**−**18**) showed improved metabolic stability but potency in the cell assay was decreased (5-fold **18** vs **16**, 25-fold **17** vs **13** and **15**). Replacing tetrahydro-2*H*-pyran with a morpholino group (**19**) improved both BACE1 enzymatic and cell potency by 2- and 4-fold, respectively. Compound **19** was metabolically stable in both human and rat liver microsomes and had good permeability and efflux properties. Further cyclic amine P2′ groups, 4,4-difluoropiperidyl (**20**), 3,3-difluoropyrrolidinyl (**21**), and 3-fluoropyrrolidinyl (**22**), were found to be well tolerated and exhibited excellent overall profiles. The 3-methylisoxazole (**23**) showed good BACE1 potency in the enzymatic assay but suffered from a high cell shift.

The cocrystal structure of compound **15** in BACE1 is shown in Figure 1.[21] As observed in previous structures,[12,19−21] the aminooxazoline moiety interacts with two catalytic aspartic

DOI: 10.1021/ml500458t
ACS Med. Chem. Lett. 2015, 6, 210−215

Table 1. 3-Aza-4-fluoroxanthenes

Cpd	R	IC_{50} (nM)[a] BACE1	IC_{50} (nM)[a] Cell[b]	CL_{int}(μL/min/mg) HLM/RLM[c]	Papp (nm/s)[d]	hER/rER[e]	hERG K_i (μM)[f]
5		0.7	8.0	28/21	11.0	1.2/1.9	>15
6		0.3	3.3	11/9	23.9	1.8/2.3	>15
7		0.4	6.0	28/22	20.9	2.6/3.3	>15
8		0.4	6.0	18/39	16.9	1.3/1.7	>15
9		0.8	12	115/14	16.0	1.6/2.8	>15
10		1.2	26	<14/<14	18.9	1.5/1.8	>15
11		0.3	19	<14/<14	13.2	1.0/1.0	>15
12		0.4	6.5	<14/<14	22.4	1.5/2.3	>15
13		0.2	3.4	91/99	17.6	1.7/1.6	>15
14		0.4	4.8	120/268	16.7	1.3/1.7	>15
15		0.3	4.0	44/65	21.3	1.9/2.2	>15
16		0.3	4.0	50/33	20.1	3.3/4.6	>15
17[g]		1.0	105	86/38	21.2	2.2/3.8	>15
18		0.7	19	30/19	18.6	6.4/6.7	>15
19		0.3	5.0	41/<14	14.9	1.0/1.2	>15
20		0.3	6.0	23/22	15.3	1.1/1.3	6.9
21		0.2	4.0	47/34	15.7	1.5/2.3	5.6
22		0.2	3.0	53/34	17.5	1.3/2.0	>15
23		0.3	23	24/15	18.5	1.7/2.4	>15

[a]IC_{50} values were averaged values determined by at least two independent experiments, $n \geq 2$. [b]Human embryonic kidney cell, $n \geq 2$. [c]Human liver microsomal (HLM) and rat liver microsomal (RLM) clearance, $n = 1$. Compound concentration = 1 mM. Microsomal protein concentration = 250 mg/mL. [d]Apparent permeability measured in parental LLC-PK1 cells. Values are an average of apical to basolateral (A to B) and basolateral to apical (B to A) velocities and are reported as 10−6 cm/s, $n = 1$. [e]Efflux measured in LLC-PK1 cells transfected with either rat MDR1A/1B or human MDR1 and are reported as a ratio of (B to A)/(A to B), $n = 1$. [f]hERG filter binding assay using radiolabeled dofetilide as ligand, $n = 1$. [g]Racemic.

acids and Tyr71 engages in a π-stacking interaction with the xanthene core. The nitrogen atom of the 2-fluoropyridin-3-yl group interacts with Ser229 via a bridging water molecule in the P3 pocket. As anticipated, the 4-F substitution of xanthene-thene engages the indole NH of Trp76 in a hydrogen bonding interaction, with the nitrogen atom in the xanthene moiety involved in a water-mediated hydrogen bond to the Trp76 side chain. The oxygen atom in the P2′ dihydropyranyl group engages Tyr198 via a bridging water molecule.

On the basis of in vitro profiles, a number of compounds were profiled in a rat PD assay to study the effect on CNS Aβ reduction. Male Sprague−Dawley rats were administrated a single oral dose and the Aβ levels were measured in cerebrospinal fluid (CSF) and brain after 4 h. The unbound plasma EC_{50} was estimated for Aβ reduction in CSF and brain (Table 2). The aromatic P2′ analogues (**5**−**7**, **12**) showed 3- to 6-fold shifts of the unbound plasma EC_{50} for brain Aβ reduction compared to the cell IC_{50} (Table 1). The unbound

DOI: 10.1021/ml500458t
ACS Med. Chem. Lett. 2015, 6, 210−215

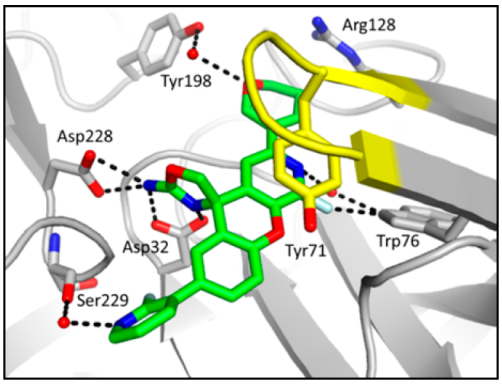


Figure 1. Co-crystal structure of BACE1 and compound **15**. Red spheres denote water molecules, and dashed lines indicate hydrogen bonds. The flap covering the active site is colored yellow.

Table 2. Reduction of CSF and Brain Aβ40 in Wild-Type Sprague–Dawley Rats

		CSF		brain	
Cpds	dose (mg/kg)	Aβ40 reduction (%)	unbound EC_{50} (mM)	Aβ40 reduction (%)	unbound EC_{50} (mM)
5	10	75	0.026	62	0.048
6	3	56	0.01	44	0.016
7	3	36	0.017	23	0.031
12	3	58	0.012	46	0.018
15	3	74	0.002	75	0.002
16	3	77	0.002	72	0.005
18	10	77	0.014	68	0.022
19	3	69	0.009	58	0.015
20	3	66	0.013	56	0.02
21	3	78	0.002	73	0.003
22	3	77	0.002	81	0.005

plasma EC_{50} for brain Aβ reduction of the dihydropyran analogous (**15**–**16**) and the tetrahydropyran **18** were consistent with their corresponding cell potencies. In particular, the dihydropyran analogues **15** and **16** demonstrated substantial Aβ40 reduction at the 3 mg/kg oral dose resulting in single-digit nanomolar EC_{50} values in both CSF and brain. We believe that these are the most orally efficacious BACE1 inhibitors in a rodent PD model published to date. Interestingly the plasma unbound EC_{50} of compound **15** was consistent in both brain and CSF while the other compounds exhibited about 1.5- to 2.5-fold shift between brain EC_{50} and CSF EC_{50}. The cyclic amine analogues (**19**–**22**) displayed robust Aβ40 reduction in CSF and brain and a 2- to 3-fold shift of the brain EC_{50} relative to the cell IC_{50}.

Compound **15** was further evaluated in a time-course PD assay in rat using a 3 mg/kg oral dose (Figure 2). Maximum Aβ reduction in both CSF and brain was observed at 4 h postdose. An indirect response model was used to model the PK–PD relationship (Figure 3). The generated in vivo unbound plasma IC_{50} values for CSF and brain Aβ reduction (3.7 and 4.3 nM, respectively) were in agreement with the estimated plasma EC_{50} obtained from the single dose PD study. Compound **15** was subsequently studied in a Rhesus monkey time-course PD assay. A robust Aβ40 reduction in CSF was observed at a 5 mg/kg dose. The generated in vivo unbound plasma IC_{50} values for CSF was at 0.5 nM, 7-fold more potent than in rats.

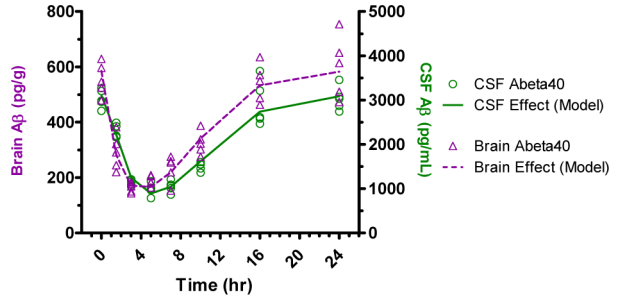


Figure 2. Time course of brain and CSF Aβ lowering in rat following a 3 mg/kg dose of **15**.

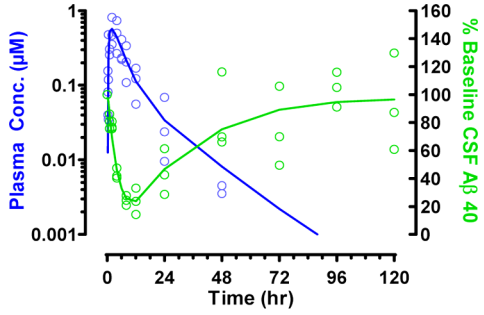


Figure 3. PK–PD relationship of **15** in Rhesus monkey following a 5 mg/kg po dose.

The pharmacokinetic profile of compound **15** was evaluated in rat, dog, and Cynomolgus monkey (Table 3). Compound **15** demonstrated a good pharmacokinetic profile in all species. Consistent with the in vitro microsomal stability data, **15** showed the lowest in vivo clearance and the longest half-life in dog. When dosed orally, the bioavailability was 50%, 121%, and 43% in rat, dog, and Cynomolgus monkey, respectively.

Table 3. Pharmacokinetic Properties of 15

		iv			po	
species	dose iv/po (mg/kg)	CL (L/h/kg)	Vdss (L/kg)	$T_{1/2}$ (h)	AUC (μM·h)	*F* (%)
rat	2.0/2.0	0.68	1.8	4	3.49	50
dog	1.0/2.0	0.26	2.50	8.9	23.9	121
cyno	1.0/2.0	0.9	2.1	4.3	2.18	43

To evaluate the cardiovascular safety profile, **15** was administered intravenously to anesthetized Beagle dogs in a series of three consecutive 30 min infusions at doses of 1, 3, and 12 mg/kg. No significant effect on the QTc interval was observed at these doses. The safety margin over the PD effect (IC_{50}) in rats was determined to be 223-fold for electrocardiographic effects and 51-fold for hemodynamic effects. The margins observed in this study represent a significant improvement over compound **1**.

In conclusion, application of structure-based design with simultaneous balance of physicochemical parameters enabled the discovery of a series of orally highly potent 3-aza-4-fluoroxanthene BACE1 inhibitors. Compound **15** emerged as an optimized lead that demonstrated robust reduction of CNS Aβ levels in both rats and monkeys and provided significant safety margins in a canine cardiovascular safety model.

DOI: 10.1021/ml500458t
ACS Med. Chem. Lett. 2015, 6, 210–215

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization data for new compounds, and methods for in vitro, in vivo, and pharmacokinetic assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Kyung Gahm, Samuel Thomas, and Wesley Barnhart for chiral separation and Alexander Long for protein purification. We gratefully acknowledge Shamrock Structures LLC for data collection at the Advanced Photon Source (APS). Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

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DOI: 10.1021/ml500458t
ACS Med. Chem. Lett. 2015, 6, 210−215