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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5518–5522

Thiazole-diamides as potent γ-secretase inhibitors

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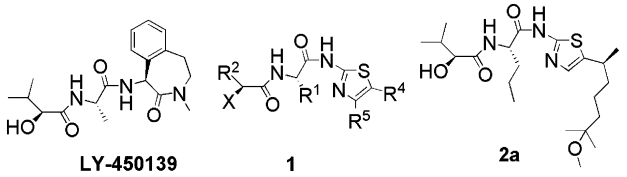
Received 19 July 2007; revised 13 August 2007; accepted 15 August 2007
Available online 19 August 2007

Abstract—The thiazole-diamide series (**1**) has been identified as highly potent γ-secretase inhibitors. Several representative compounds showed IC_{50} values of <0.3 nM. The synthesis and SAR, as well as a radiolabeled synthesis of [^{3}H]-**2a**, are described.

Alzheimer's disease (AD) is a neurodegenerative disease, which results in progressive memory loss and behavioral abnormalities. The standard current therapy is the use of acetylcholinesterase inhibitors as a palliative treatment. Recent research on AD is focused on disease modification therapy to slow down disease progression in AD patients. Amyloid-β peptides (Aβ) are thought to play a role in AD. Cleavage of amyloid precursor protein (APP) by β-secretase generates the membrane bound C-terminal fragment, C99, which is then cleaved by γ-secretase to release Aβ peptides, in which $Aβ_{1–42}$ is recognized to play a key role in the pathogenesis of AD.[1–3] The use of a β- or γ-secretase inhibitor or modulator to reduce the formation of the toxic Aβ peptides seems to be an attractive approach.[4] Several dipeptic γ-secretase inhibitors were reported.[4–8] Among these, LY450139 is currently in clinical trials to test this hypothesis.[9] During the course of our γ-secretase inhibitor program, we have identified highly potent γ-secretase inhibitors in the thiazole series (**1**). This paper describes the synthesis and biological data in series **1**, as well as the radiolabeled synthesis of [^{3}H]-**2a**.



The starting 2-amino-thiazoles were either obtained from commercial sources or prepared by literature methods[10,11] as shown in Scheme 1. In general the 2-amino-5-alkyl-thiazoles were prepared by α-bromination of an aldehyde, followed by thiazole ring formation as exemplified by **3**–**6**. Alternatively 2-amino-thiazoles were prepared by reaction of the anion generated from 2-amino-thiazole **7**[11] with an electrophile to give **8**–**11** as illustrated in Scheme 1. Bromination of 7-methoxy-3,7-dimethyloctanal, followed by reaction with thiourea, provided a 1:1 mixture of **3** and **4** in 19% and 23% isolated yield, respectively, after purification by column chromatography. Compound **4** was converted to the hydroxyl analog **5** by stirring with an excess of sulfuric acid and water. The 5-acetyl-2-amino-thiazole **6** was prepared by a method analogous to that described in the literature.[10]

These 2-amino-thiazoles were used for the next amide coupling reaction using standard peptide coupling agents as illustrated in Scheme 2. Unfortunately not all the

Keywords: γ-Secretase inhibitors; Alzheimer's disease; Thiazole.
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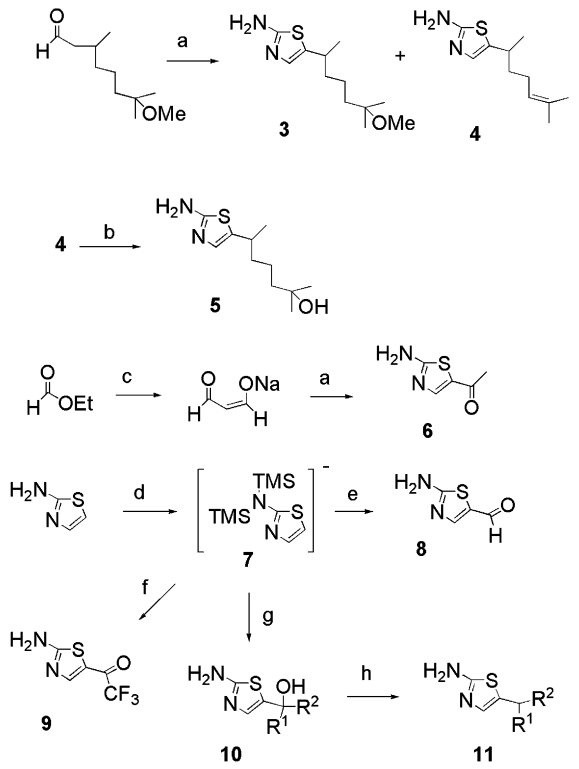
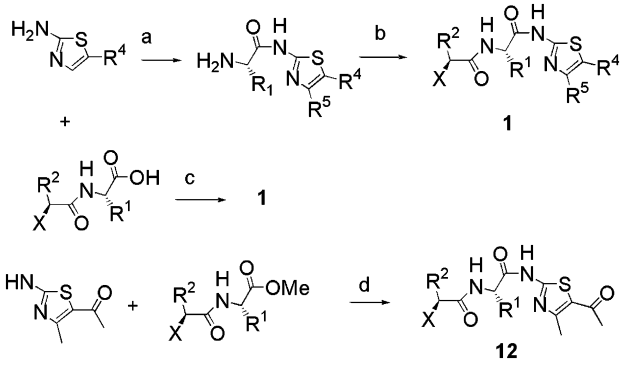
doi:10.1016/j.bmcl.2007.08.035



Scheme 1. Reagents and conditions: (a) i—1 equiv Br_2 in dioxane, 0 °C over 1 h, then room temperature, 2 h; ii—1.4 equiv thiourea, ethanol, overnight; (b) excess H_2SO_4, H_2O, over weekend; (c) NaOMe, Et_2O, acetone; (d) i—1 equiv *n*-BuLi in THF at −78 °C, 10 min; ii—1.02 equiv trimethylsilyl chloride at −78 °C for 10 min, −5 °C, 10 min; iii—1 equiv *n*-BuLi −78 °C, 10 min; iv—1.02 equiv trimethylsilyl chloride at −78 °C, 10 min, −5 °C, 10 min; v—1.3 equiv *n*-BuLi in THF, −78 °C, 30 min; (e) i—DMF, −78 °C, then −5 °C, 30 min; ii—1 N HCl, 44%; (f) i—CF_3COOEt, −78 °C, then 0 °C; ii—satd NH_4Cl, 62%; (g) i—$R^1R^2C(=O)$; 50% for 1-benzyl-piperidin-4-one; 27% for 3-pentanone; 27% for 4-heptanone; (h) Et_3SiH, BF_3Et_2O, CH_2Cl_2 to give a mixture of **11** (32%) and the corresponding elimination product, an olefin (50%) that was hydrogenerated with 10% Pd/C, H_2 to give **11**, 90%.



Scheme 2. Reagents and conditions: (a) i—*t*-BOC-NH-CH(R^1)COOH, HOBt, EDC, triethylamine, CH_2Cl_2; ii—4 N HCl in dioxane; (b) R^2CH(X)COOH, HOBt, EDC, triethylamine, CH_2Cl_2; (c) HOBt, EDC, triethylamine, CH_2Cl_2; (d) i—2 equiv ketone and 2 equiv $AlMe_3$ in toluene, THF for 1 h, room temperature; ii—1 equiv ester, reflux overnight, 20%.

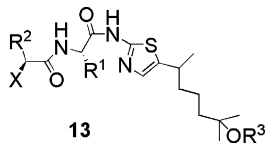
aminothiazoles coupled well with a carboxylic acid derivative. For example several attempts at coupling 2-amino-4-methyl-5-acetyl thiazole with an acid using various coupling agents, solvents or temperatures failed to provide the desired product, perhaps due to poor solubility. This synthetic hurdle was overcome by an alternative method, in which a 2-aminothiazole was reacted with trimethylaluminum at room temperature, followed by heating with an ester to provide the desired product as illustrated in the preparation of **12** (Scheme 1). The structural type **13** shown in Table 1 has a chiral center at C_5 attached to the thiazole ring. Two diastereomers in **2**, **20**, and **23** were separated by chiral HPLC to give the corresponding diastereomers, **2a**–**b**, **20a**–**b**, and **23a**–**b**, respectively.[12] Because these isomers were isolated as a glass foam, the absolute stereochemistry cannot be determined without further work. Based on the X-ray structural analysis of the related analogs in a different heteroaryl series with a similar SAR pattern to the thiazole series (data not shown), the absolute stereochemistry for more active diastereomers **2a**, **20a**, and **23a** was tentatively assigned the (*S*)-configuration.

Scheme 3 shows the reductive amination of an aldehyde or a ketone with an amine in the presence of sodium triacetyloxyborohydride to give compounds in series **14** shown in Table 2.

Scheme 4 describes the synthesis of [^{3}H]-**2a**. The precursor **17** was prepared by coupling of racemate **15** and racemate **16** to give a mixture of four diastereomers that were separated by chiral HPLC. Because all four diastereomers are oils, it will be challenging to determine the absolute stereochemistry without additional work. Fortunately one of the diastereomers **17** was found to be much more potent than the other three isomers (0.55 vs 6.57, 8.10, and 9.20 nM) in the γ-secretase whole cell inhibition assay. Compound **17** was carried on to hydrogenation and the product was co-injected with standard **2a** to confirm that **17** is the desired isomer. The radiolabeled material, [^{3}H]-**2a**, was prepared by tritiation of the precursor **17** with tritium in the presence of 10% Pd/C in ethanol at atmospheric pressure to incorporate a mixture of 1–7 tritium atoms in the *i*-Pr group, which provided 164.34 Ci/mmol specific activity with 99.27% radiochemistry purity. [^{3}H]-**2a** was used as a radiolabeled ligand for a γ-secretase binding assay to evaluate the inhibition from several different classes of γ-secretase inhibitors and modulators (data not shown).

γ-Secretase inhibition data in cell free and whole cell assays in series **1** are shown in Tables 1–3. Table 1 lists the SAR in series **13**. Several compounds in series **13** represent the most potent compounds discovered in the thiazole series, in which the long side chain at C_5 attached to the thiazole ring increases the whole cell potency dramatically with IC50 <0.3 nM. Most compounds were tested as a mixture of two diastereomers, except **2**, **20**, and **23**. The (*S*,*S*,*S*)-isomer of those compounds shows significantly greater potency than the corresponding (*S*,*S*,*R*)-isomer, indicating the desired chirality may direct the side chain conformation into the binding site of the enzyme complex. The SAR indicates that an R^1 group with *n*-Pr is an optimal group compared to a Me or Et. In general the best group for CHX in series

Table 1. SAR in aminothiazole series **13**

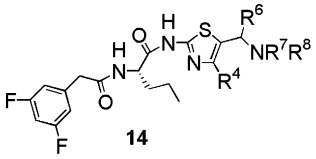


13

R^2	X	R^1	OR^3	Compound[a]	CFA IC_{50}[c] (nM)	WCA IC_{50}[c] (nM)
i-Pr	OH	Pr	OMe	**2**	5.9	0.30
				2a (*S*,*S*,*S*)[b]	9	0.30
				2b (*S*,*S*,*R*)[b]	117	7.49
3,5-Di-F-$PhCH_2$	H	Pr	OH	**18**	6.0	0.05
			OMe	**19**	10.0	0.14
		Et	OMe	**20**	13.6	0.08
				20a (*S*,*S*,*S*)[b]	30.0	0.10
				20b (*S*,*S*,*R*)[b]	727.0	81.50
		Me	OMe	**21**	30	0.22
t-Bu	OH	Pr	OH	**22**	7.4	0.22
	(*S*)	Pr	OMe	**23**	13.0	0.43
				23a (*S*,*S*,*S*)[b]	10.0	0.11
				23b (*S*,*S*,*R*)[b]	9.9	1.14

[a] All compounds were tested as a mixture of two diastereomers, unless otherwise indicated.
[b] The absolute stereochemistry is tentatively assigned.
[c] IC_{50}s were determined using a cell free assay (CFA)[13] or whole cell assay (WCA).[14] Values are geometric means of at least two experiments with six data points in each experiment.

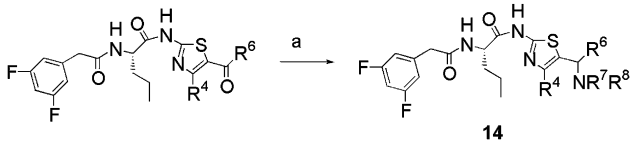
Table 2. SAR in 5-aminoalkylene-thiazole series **14**



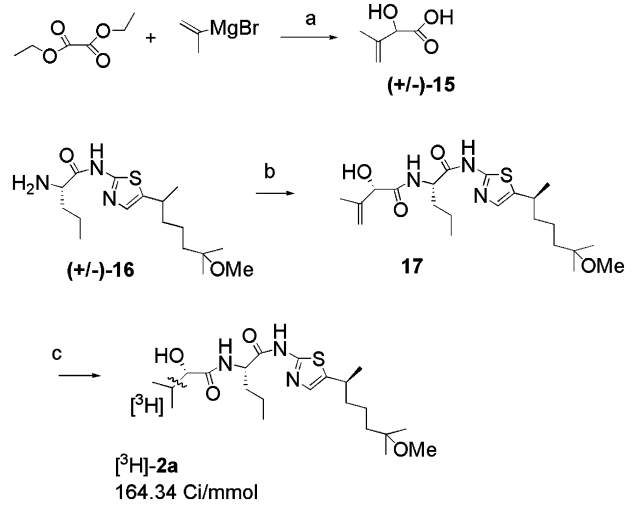
14

NR^7R^8	R^6	R^4	Compound	CFA IC_{50}[a] (nM)	WCA IC_{50}[a] (nM)
NH_2	H	H	**24**	200.0	93.0
NHMe	Me	Me	**25**	51.8	50.5
NHEt	H	H	**26**	63.2	104
NHEt	Me	Me	**27**	155.0	42.0
$NHCH_2CF_3$	Me	Me	**28**	10.0	4.5
NH*n*-Pr	Me	H	**29**	15.9	10.1
NH*i*-Pr	Me	H	**30**	42.4	29.8
NH*n*-Bu	Me	H	**31**	6.0	2.0
NH*n*-Bu	Me	Me	**32**	7.1	3.7
$NHCH_2$*i*-Pr	Me	H	**33**	4.2	4.2
$NHCH_2$*i*-Pr					
Mixture	Me	Me	**34**	10	4.8
Isomer a			**34a**	3.1	2.5
Isomer b			**34b**	33.0	31.6
$NHCH_2$*t*-Bu	Me	H	**35**	134	231
$NHCH_2CH_2$*i*-Pr	Me	H	**36**	2.6	1.2
$NHCH_2CH_2$*i*-Pr	Me	Me	**37**	5.0	2.0
$NHCH_2CH_2$*t*-Bu	Me	H	**38**	2.7	1.2
$NHCH_2CH_2$*t*-Bu	Me	Me	**39**	5.9	2.0
NMe_2	Me	Me	**40**	28.3	39.7
$NHCH_2Ph$	Me	H	**41**	3.0	1.4
NH-(*S*)-CH(*i*-Pr)COOMe	Me	H	**42**	4.0	0.7
NBuEt	H	H	**43**	30.0	12.7
Pyrrolidinyl	Me	H	**44**	10.0	10.6
Morpholinyl	H	H	**45**	30.0	12.7
Morpholinyl	Me	H	**46**	3.8	2.4
4-Me-1-piperidinyl	Me	Me	**47**	28.3	26.1

[a] IC_{50}s were determined using a cell free assay (CFA)[13] or whole cell assay (WCA).[14] Values are geometric means of at least two experiments with six data points in each experiment.



Scheme 3. Reagents and conditions: (a) 2–12 equiv NHR^7R^8, 1–2 equiv AcOH, 2–7 equiv $NaBH(OAc)_3$ in dichloroethane, room temperature, overnight.



Scheme 4. Reagents and conditions: (a) (i) −78 °C in Et_2O:THF (3:1); (ii) neutralization with 1 N HCl, 87% crude yield; (iii) 1 equiv $NaBH_4$ in methanol, 0 °C, 10 min, 59% crude yield; (iv) LiOH in dioxane/water (1:1), 86% crude, used as it is; (b) (i) (+/−)-**15**, 1.2 equiv HOBt, 1.5 equiv EDC, 4 equiv triethylamine, CH_2Cl_2, 15%; (ii) chiral HPLC separation; (c) tritium, 10%Pd/C, EtOH, 1 atm, then purified by YMC ODS AQ S-3 column and the structure was confirmed by co-injection with the reference standard **2a**. MS: M+1 showed 444, 446, 448, 450, 452, 454, and 456, indicating a mixture of 1–7 tritium atoms was incorporated.

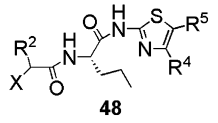
1 is CH_2 or (*S*)-CHOH, or replacement of CHX with a C(=O) as we found that compounds with X of an alkyl, alkoxy, or amino group were weak or inactive in our assay (data not shown). The long side chain at C_5 of the thiazole in series **13** can be replaced with a basic amino group to improve the physicochemical properties, such as increased solubility and basicity, while retaining good potency as shown in examples **36**–**39** and **42** in Table 2.

Table 2 shows the SAR of NR^7R^8 in series **14**. Increasing the length from a small NH_2 in **24** to a longer side chain shown in **36**–**39** improved the potency. The best side chain 5-C(Me)$NH_2CH_2CH_2CMe_2$(R) shown in **14** has similar length to 5-C(Me)$CH_2CH_2CH_2CMe_2(OR^3)$ shown in **13**, indicating that the methylene group in the C_5 side chain can be replaced with a basic polar amino group without losing much potency. The two diastereomers in **34** were separated by chiral HPLC to give isomers **34a**–**b**. Consistent with previous findings, isomer **34a** is 10 times more potent than **34b**. Similar potency was found for analogs with R^4 of Me or H. In general compounds with R^6 of Me are equal or slightly more potent than those with R^6 of H.

Table 3 shows the SAR of various positions in which X of OH seems to improve potency slightly as seen in example **50** (2.2 nM) versus **56** (0.3 nM), where the (*S*)-isomer is much more potent than the corresponding (*R*)-isomer (see examples **54** and **55** with 2.2 vs 183 nM). The hydroxyl analog **61** shows similar activity to the corresponding ketone **62**.

In summary, potent γ-secretase inhibitors in the thiazole series were discovered. Several analogs in **13** are highly potent γ-secretase inhibitors with $IC_{50} < 0.3$ nM. The SAR in various positions was explored and optimized. The 5-alkyl-thiazole side chain shown in series **13** and the corresponding series in **14** provided enhanced potency. The SAR described in the thiazole series provided

Table 3. The SAR of representative compounds in series **1**



48

$CHR^2(X)$	R^4	R^5	Compound	CFA IC_{50}[a] (nM)	WCA IC_{50}[a] (nM)
3,5-Di-F-$PhCH_2$	H	$CHMe_2$	**49**	30.0	5.1
		$CHEt_2$	**50**	26.3	2.2
		$C(OH)Et_2$	**51**	1.0	1.2
		CH*n*-Pr_2	**52**	<34.6	0.8
		$CH(Me)CH_2$*t*-Bu	**53**	64.8	1.6
(*S*)-PhCH(OH)	H	$C(OH)Et_2$	**54**	2.8	2.2
(*R*)-PhCH(OH)	H	$C(OH)Et_2$	**55**	141.0	183.0
(*S*)-3,5-Di-F-PhCH(OH)	H	$CHEt_2$	**56**	6.2	0.3
3,5-Di-F-$PhCH_2$	Me	Me	**57**	141.0	94.1
	Me	Et	**58**	30.0	12.9
	Me	C(=O)Me	**59**	20.0	27.7
(*S*)-*t*-BuCH(OH)	H	$CHMe_2$	**60**	45.1	20.1
(*S*)-*t*-BuCH(OH)	H	$C(OH)Me_2$	**61**	94.9	37.3
t-BuC(=O)	H	$CHMe_2$	**62**	116.0	16.8

[a] IC_{50}s were determined using a cell free assay (CFA)[13] or whole cell assay (WCA).[14] Values are geometric means of at least two experiments with six data points in each experiment.

the ground work for that in other heteroaryl series as γ-secretase inhibitors. [^{3}H]-**2a** was synthesized as a radiolabeled ligand for γ-secretase binding assays.

Acknowledgments

The authors thank D.E. Virtue and S.F. Matis for chiral HPLC separation and Drs. F.W. White, E. Callegari, and B. Bronk for helpful discussion.

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12. Diastereomers of **20** were separated by chiral HPLC using Chirobiotic T column to give **20a** (100.0% ee) and **20b** (98.5% ee) and **23** and **2** were each separated by Chiralpak AD column to give **2a** (92.1% ee) and **2b** (95.3% ee), and **23a** (96.2% ee) and **23b** (95.4% ee), respectively.
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14. H4 cells (human brain neuroglioma) were stably transfected with human APP695 containing the Swedish Familial Alzheimers Disease mutation. Cells were distributed into 96-well plates at a density of about 30,000 cells/well and allowed to attach to the plate surface for approximately 6 h at 37 °C. After thitime, cells were washed to remove secreted Aβ and fresh media containing test compound were added. Following incubation with compound overnight at 37 °C, media were harvested and subjected to immunoassay to determine the amount of Aβ secreted from the cells. A two-site sandwich ELISA utilizing commercially available monoclonal antibodies, 6E10 and 4G8-biotinylated, is employed to provide an estimate of the amount of total Aβ, predominantly $A\beta_{1-40}$ and $A\beta_{1-42}$. Signals from these samples are compared to standard curves generated with synthetic $A\beta_{1-40}$ peptide and used to calculate IC_{50} values for each test compound.