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Thiophene substituted acylguanidines as BACE1 inhibitors

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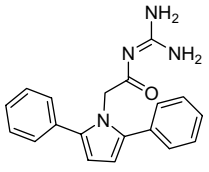
Abstract—A series of thiophene-substituted acylguanidines were designed from a pyrrole substituted acylguanidine HTS lead. This template allowed a greater flexibility, through differential Suzuki couplings, to explore the binding site of BACE1 and to enhance the inhibitory potencies. This exploration provided a 25-fold enhancement in potency to yield compound **10a**, which was 150 nM in a BACE1 FRET assay.

Alzheimer's disease (AD) is an age-related and irreversible brain disorder that occurs gradually and results in memory loss, behavior, and personality changes, a decline in cognition and eventually death.[1] These losses are related to the breakdown of the connections between nerve cells in the brain and the eventual death of many of these cells. The course of this disease varies from person to person, as does the rate of decline. On average, patients with AD live for 8–10 years after they are diagnosed, though the disease can last for up to 20 years.[2] One primary theory on the etiology of AD is that in AD patients, amyloid precursor protein (APP) is processed in the brain and converted to beta-amyloid protein, a precursor to amyloid plaques.[3] β-Secretase (β-site APP cleavage enzyme or BACE1) and γ-secretase are two important enzymes involved in the amyloid synthetic cascade. BACE1 is an aspartyl protease that initiates APP proteolytic cleavage generating the membrane bound C-terminal APP fragment (βCTF/C99). Further processing of this fragment by γ-secretase liberates the pathogenic $A\beta_{40-42}$ peptide.[4] Aggregation of $A\beta_{40-42}$ peptide eventually leads to oligomeric amyloid plaques, a hallmark of AD. It is believed that inhibition of BACE1 or γ-secretase will inhibit this amyloidogenic APP cleavage thus preventing the formation of β-amyloid.

Our goal was to produce a potent and selective non-peptidic small molecule inhibitor of BACE1. While there have been numerous reported substrate–based peptide inhibitors, there have been only a few examples of non-peptide based inhibitors.[5] In the course of running an HTS, the tri-substituted pyrrole **1**[6] was identified, which had an IC_{50} of 3.7 μM for BACE1 in a FRET assay and an ED_{50} of 8.9 μM in our cellular ELISA. Our initial efforts at examining the scope and breadth of the SAR of the pyrrole lead **1** were limited due to the difficulty in the substitution of a wide variety of aryl and heteroaryl moieties on the pyrrole ring. It became obvious to us very early that the sometimes unstable and



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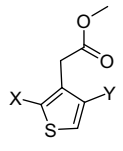

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difficult to prepare α-substituted halopyrrole precursors would prevent a rapid entry to divergent substitution patterns. Instead, we proposed that substitution of a thi-

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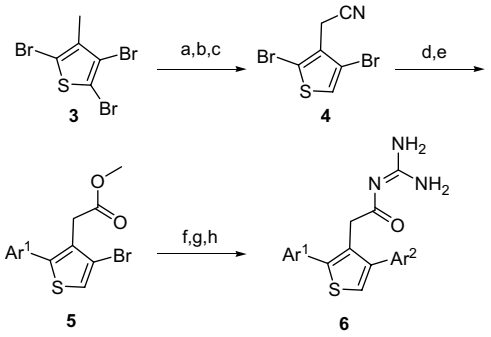
ophene ring for the core pyrrole would allow for a facile examination of the pendant aryl ring SAR while maintaining the disposition of these key moieties and complement the developing SAR around the pyrrole series. Thus, intermediate **2** with the aforementioned thiophene ring (X, Y = Br) would be a viable surrogate for the pyrrole. Previous experience with 2,4-dihalo-substituted thiophenes had shown that it was possible to perform Suzuki[7] couplings on the thiophene ring with either dibromo- or diiodothiophenes in a regiospecific manner. This proposed sequence would allow us to append the aryl substituents at a later stage in the synthesis, making the strategy more amenable to library generation.

Toward that end, the synthesis of key intermediate **2** (X, Y = Br) was initiated by lithium halogen exchange of the readily available 2,3,5-tribromo-4-methylthiophene **3** in ether at −78 °C and quenching with water to yield the 2,4-dibromo-3-methylthiophene (Scheme 1).[8] Radical induced bromination of the methyl group followed by displacement with cyanide yielded the (2,4-dibromothiophen-3-yl)-acetonitrile **4**. Acid catalyzed methanolysis of the nitrile and regioselective Suzuki coupling with a suitably substituted arylboronic acid at the 2-position of the thiophene yield a variety of intermediates **5** in 75–91% yield. This selective coupling is best achieved when only 1.1 equiv of boronic acid is used at a temperature of 45 °C. The second Suzuki coupling, at the 4-position of the thiophene ring, is significantly slower and occurs at elevated reaction temperatures (70 °C) and longer reaction times. While the yields are quite good (66–84%) the slower reaction and increased temperatures give rise to small amounts (3–6%) of dehalogenated adducts, which are easily separable by chromatography on silica gel. Basic hydrolysis of esters **5** with NaOH in ethanol, followed by carbonyl diimidazole (CDI) activation under basic conditions and displacement with guanidine, affords the desired final products **6**.

At the outset of our SAR exploration, our first goal was to differentiate the two pendant aryl rings from each other. That is, to define which aryl ring, Ar^1 or Ar^2, projects into the S1–S3 pocket and which occupies the S2′ pocket (see Fig. 1). An X-ray crystal structure analysis



Scheme 1. Reagents: (a) *n*-BuLi, Et_2O, −78 °C, H_2O (quench), 82%; (b) NBS, AIBN, hν, CCl_4, 91%; (c) KCN, EtOH, 86%; (d) H_2SO_4, MeOH, 88%; (e) $Ar^1B(OH)_2$, $PdCl_2(dppf)_2$, aq K_2CO_3, dioxane, 45 °C, 75–91% ; (f) $Ar^2B(OH)_2$, $PdCl_2(dppf)_2$, aq K_2CO_3, dioxane, 70 °C, 66–84%; (g) NaOH, EtOH, 95%; (h) CDI, DMF, guanidine hydrochloride, DIEA, 60–83%.

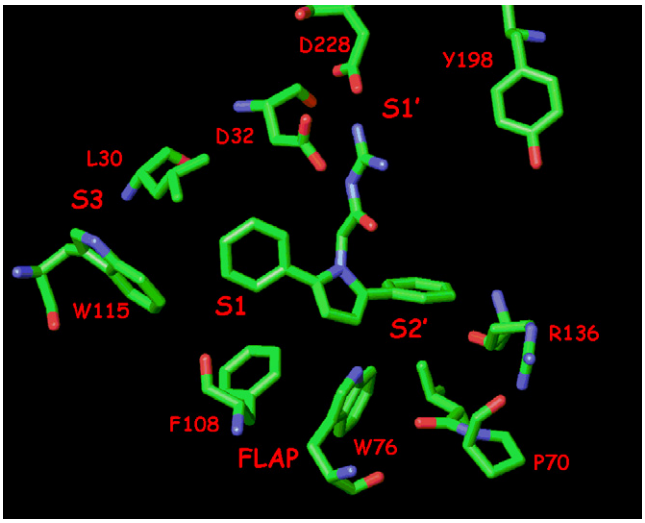
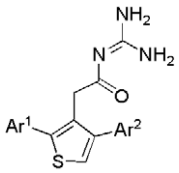


Figure 1. Depiction of an X-ray crystal structure of Compound **1** bound in the active site of BACE1. The relative locations of the different binding areas of the active site are labeled along with key amino acid residues. RCSB ID code: rcsb044055; PDB code: 2QU2.

of compound **1** co-crystallized with the BACE1 enzyme shows that the acylguanidine group binds to the key aspartates Asp32 and Asp228 as predicted. The surface area around the S1–S3 pocket is fairly large and has predominately hydrophobic residues while that of S2′ has more polar or charged residues. This led us to believe that it should be straightforward to discern between the two pockets. Our initial adduct, **6a** (Table 1), was meant to mimic the binding of the lead pyrrole, **1**, and to confirm the favorable substitution of the core heterocycle. Comparable binding affinity shows that this is indeed the case. A thorough examination of the molecular modeling of **6a** had suggested that the substitution pattern on the aryl group extending into the S1–S3 pocket would be better served by placing substituents on the *para*-position. Substitution of larger functionalities on the *para*-position that extend into the S3 pocket containing more polar functions had somewhat mixed results (e.g., **6b** and **6c**). While there is a 10-fold enhancement in binding for **6b**, attempts to further enhance affinity by extension of a pyrazole ring into S3, that is **6c**, were deleterious. Additional observations from the modeling predicted that a *para* propoxy group on the aryl targeted for the S1–S3 pocket would possibly enhance binding affinity. Furthermore, an examination of the S2′ pocket suggested that an *ortho* chlorine on the aryl group projecting into S2′ would increase binding through interactions with Trp76. Compounds **6d**–**6g** were prepared to challenge this hypothesis on the thiophene core nucleus. There are two alternate substitution patterns on the thiophene ring for these particular placements. The first has the proposed S1–S3 binding substituents on C-2 of the thiophene and the S2′ substituents on the C-4 (**6d** and **6f**), and the second has the exact opposite (**6e** and **6g**). The presence of the *ortho* chloro group on the S2′ aryl group of **6g** shows enhanced affinity for BACE1 in comparison to **6e**, demonstrating the positive effects of both substitutions. However, when the substituents are not favorably displaced, thus positioning the molecule with the proper aryl

Table 1. SAR of substitution patterns for Ar^1 and Ar^2 on thiophene



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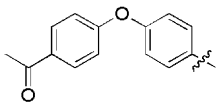
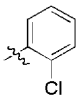
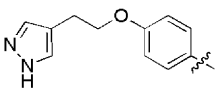
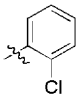
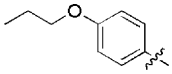
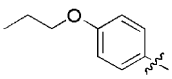
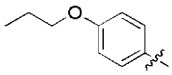
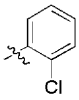
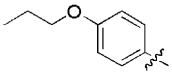
Compound	Ar^1	Ar^2	IC_{50} (μM)
6a			8.3
6b			0.75
6c			6.9
6d			0.68
6e			1.89
6f			0.63
6g			0.59

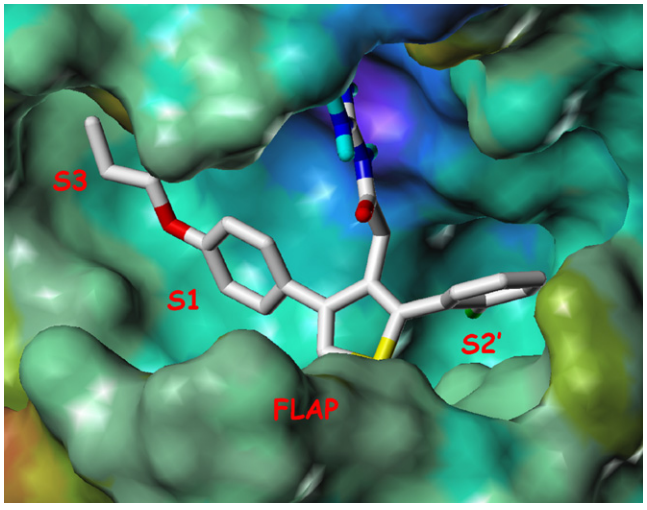
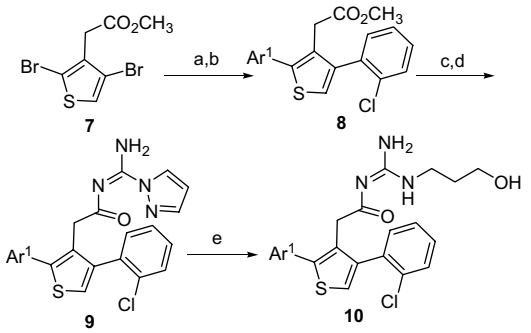


Figure 2. Molcad surface colored for the electrostatic potential of an X-ray of compound **6g** bound to BACE1. The green and light green surfaces indicate hydrophobic regions and the blue region represent negatively charged surfaces. RCSB ID code: rcsb044056; PDB ID code: 2QU3.



Scheme 2. Reagents: (a) $Ar_1B(OH)_2$ (1.1 equiv), aq K_2CO_3 (2.0 equiv), $PdCl_2(dppf)_2$ (3 mol%), dioxane 45 °C; (b) $Ar_1B(OH)_2$ (2.0 equiv), aq K_2CO_3 (4.0 equiv), $PdCl_2(dppf)_2$ (3 mol%), dioxane 70 °C; (c) NaOH, EtOH; (d) CDI, CH_2Cl_2, pyrazole carboxamidine hydrochloride, DIEA; (e) CH_2Cl_2, aminopropanol, DIEA.

moieties projecting into the proposed pockets, this added chloro group has no effect on the affinity (compare **6d** and **6f**). Molecular modeling suggests that the *ortho* chloro substituent on the C-4 aryl group on **6f** skews the aryl group because of interactions with the C-3 substituent and C-5 hydrogen to enhance π-stacking with Pro70. The absence of this substituent, as in **6e**, onto C2 of the thiophene nucleus is not optimized for this π-stacking and this may be the cause of decreased binding affinity.

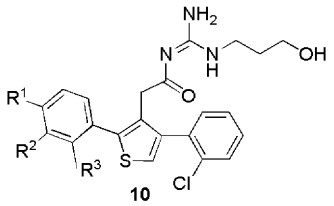
The original hypothesis that the propyloxyphenyl substituent would enhance affinity and project into the S1–S3 pocket of the BACE1 site was confirmed when an X-ray co-crystal structure of analog **6g** with the BACE1 enzyme was attained (Fig. 2).

In an attempt to optimize the potency and to identify opportunities to exploit additional space within the enzyme active site, the S1′ area was explored. Structure–activity relationships (SAR) being developed concurrently with the pyrrole series (Ref. 6) of acylguanidines, focused on exactly that goal, revealed this opportunity. On those derivatives it had been shown that substitution of a functionalized alkyl group, in particular 3-hydroxypropyl, on the terminus of the guanidine portion of the molecule projecting into the S1′ pocket of BACE1 enhanced binding. Synthesis of the analogous derivatives from this series was undertaken and is described in Scheme 2.

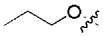
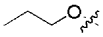
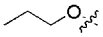
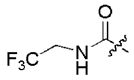
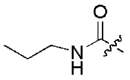
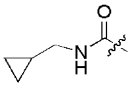
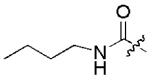
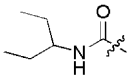
Routine Suzuki coupling of an aryl boronic acid with (2,4-dibromo-thiophen-3-yl)-acetic acid methyl ester **7** (prepared by methanolysis of compound **4**) at the 2-position followed by a second Suzuki coupling at the 4-position with 2-chlorophenyl boronic acid gave excellent yields of the diaryl substituted thiophenyl esters **8**. Hydrolysis of the ester followed by activation with carbonyl diimidazole, as before, then coupling with pyrazole carboxamidine yielded the pyrazole substituted acylguanidines **9**.[9] Displacement of the pyrazole with 3-aminopropanol readily occurs and affords the desired final products.

It can be seen from the data in Table 2 that substitution of the hydroxyl containing 3-carbon fragment that reaches into the S1′ pocket enhances the BACE1 FRET

Table 2. SAR of substitution patterns for Ar^1 on thiophene–propanol substituted acylguanidines



10

Compound	R^1	R^2	R^2	IC_{50} (μM)
10a		H	H	0.15
10b		CH_3	H	1.54
10c		H	CH_3	1.55
10d		H	H	0.20
10e		H	H	0.26
10f		H	H	0.14
10g		H	H	0.28
10h		H	H	0.93

activity by a factor of 4-fold (**10a**, compare to **6f**) and afforded one of the most potent compounds in the series. Utilizing this optimized binding of S1′ and S2′, further examination of small substituents on the S1 aryl group showed that this pocket is fairly narrow and intolerant of even small changes. Substitution of a simple methyl group at either the *ortho*- or *meta*-position of the phenyl ring decreases activity by 10-fold (**10b** and **10c**). Reaching out further to the S3 pocket, with the synthesis of several substituted amides (**10d**–**10h**), afforded results that were supported by molecular modeling. Not surprisingly, each pocket has tolerances and addition of a branched alkyl chain reaching into the S3 pocket reduced binding by 6-fold (**10h**). Furthermore, it was shown that substitution on the 4-position of the phenyl group could tolerate five to six atoms before binding is adversely affected. Of the amides synthesized the cyclopropylmethyl substituted compound **10f** was the most closely related to **10a** in activity in the in vitro assay.

Compound **6a**, our initial thiophene compound, had an IC_{50} of 8.3 μM for BACE1 in a FRET assay, and was similar in activity to the pyrrole substituted compound **1** found through HTS, which had an IC_{50} of 3.7 μM. In terms of selectivity, compound **10a**, which had an IC_{50} of 0.15 μM, was 7-fold selective versus BACE2, 23-fold selective versus cathepsin D, and displayed only 16% inhibition at 100 μM for pepsin.

In conclusion, we have investigated the substitution of a thiophene ring for the key pyrrole nucleus in a series of substituted acylguanidines. This replacement allowed us to more easily probe the size constraints of the S1 and S3 pockets of the BACE1 enzyme. Furthermore, utilizing results from the thiophene SAR allowed for optimized derivatives possessing S1′ moieties to be prepared which showed potencies below 200 nM. These potent inhibitors of the enzyme supplied us with molecules to co-crystallize with the protein and with X-ray analysis further expand our understanding of the requirements for small molecule inhibition of BACE1.

Acknowledgments

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