Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1350–1352

Design, synthesis, and structure–activity relationship of novel thiophene derivatives for β-amyloid plaque imaging

Rajesh Chandra,[a] Mei-Ping Kung[a] and Hank F. Kung[a,b,*]

[a]*Department of Radiology, University of Pennsylvania, Room 305, 3700 Market Street, Philadelphia, PA 19104, USA*
[b]*Department of Pharmacology, University of Pennsylvania, Room 305, 3700 Market Street, Philadelphia, PA 19104, USA*

Received 11 October 2005; revised 14 November 2005; accepted 14 November 2005
Available online 1 December 2005

Abstract—Novel 2,5-diphenylthiophene derivatives were synthesized and structure activity relationship with regard to Aβ plaque binding was studied. Binding affinities of these compounds were found to range from 3.9 to >1000 nM, depending on the substitution patterns on the phenyl ring. The fluoroethyl-substituted thiophene derivatives showed excellent binding affinities. These compounds may be useful for the development of novel PET tracers for the imaging of β-amyloid plaques in the brain of patients with Alzheimer's disease.

Alzheimer's disease (AD) is a neurodegenerative disorder that impairs the normal functions of brain, ultimately leading to death. It is estimated that nearly 2% of the population in industrialized countries is affected and the risk is highest among older individuals. A central event in the pathology of Alzheimer's disease is the production of β-amyloid (Aβ) peptides and subsequent aggregation to form Aβ plaques.[1–3] Imaging study of β-amyloid plaque present in the brain offers one of the most potential diagnostic tools for the detection as well as evaluation of the advancement of AD. Thus, it transpires that the development of small molecular probes for labeling Aβ plaques in vivo is of major significance in Alzheimer's research.[4] It may also assist in the development of drugs targeting Aβ plaques for the treatment of AD and in ascertaining the effectiveness of the treatment.

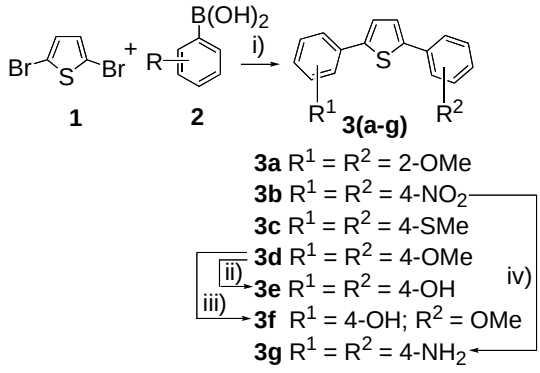
Several fluorescent probes, making use of the optical properties of highly conjugated dye molecules, have been reported to bind to amyloid plaques with high specificity. For instance, dye molecules such as Congo Red (CR) and Thioflavin-T and S have been used in the fluorescent staining of plaques and tangles in post-mortem AD brain sections.[5,6] Further studies showed that more abbreviated forms of chrysamine G, such as X-34,[7] ISB, BSB, and IMSB,[8,9] are equally potent in this regard. Positron emission tomography (PET) is a non-invasive imaging technique available for examining the brain functions, physiology, and metabolism. Previously, a stilbene derivative, [^{11}C]SB-13,[10] showing promise in detecting senile plaques in AD patients has been reported from our group. The short half-life (20 min) of C-11, however, may limit the usefulness of [^{11}C]SB-13 or even [^{11}C]PIB[11,12] for a widespread application. Comparable ^{18}F labeled agents may supplant the clinical need due to the longer half-life of the isotope (109.7 min). Recently, we have successfully reported two series of stilbene derivatives,[13] both of which can be labeled with F-18, as potential PET imaging agents for AD patients. In an attempt to test novel highly conjugated biphenyl derivatives, we have selected to investigate biphenyl thiophene derivatives. Our studies with the stilbene-based probes have established the minimum structural feature to be the presence of two phenyl rings, at least one of which should be electron rich, in order for these molecules to exhibit desirable Aβ plaque binding properties.[14] These two phenyl rings should be in conjugation with each other and the molecule as a whole should be neutral and planar. We replaced the double bond in stilbene-based probes with a cyclic moiety, at the same time without altering the above-mentioned structural features. Thiophene ring was an obvious choice, as it can be considered as a diene in *s-cis* conformation.[9] As for the attachment of phenyl rings to the thiophene core, 2 and 5 positions seemed to be the

Keywords: In vitro binding; PET imaging; Alzheimer's disease.
* Corresponding author. Tel.: +1 215 662 3096; e-mail: kunghf@sunmac.spect.upenn.edu

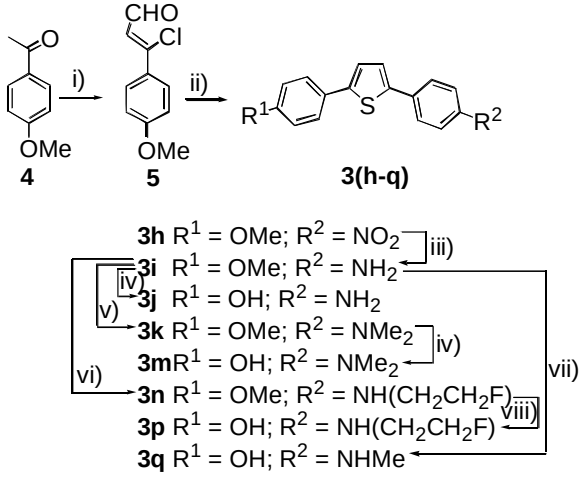
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.055

obvious choices as it will avoid any steric interactions between the phenyl rings. Reported herein are the synthesis and the structure–activity relationship of a novel series of potential Aβ plaque imaging agents based on the biphenyl thiophene core.

The 2,5-disubstituted thiophene derivatives (**3a**–**q**) employed in the study were prepared according to Schemes 1 and 2. The key step in the synthesis of N,N-, O,O-, and S,S-disubstituted derivatives is the Suzuki coupling reaction of 2,5-dibromothiophene with the corresponding phenylboronic acids. It is worthmentioning that the compound **3d** had been prepared by the multi-step sequence involving the Pd(0) catalyzed coupling of theinylzinc chloride with iodoanisole,[15] while compound **3b** had been synthesized starting from the acyclic precursor 4-nitroacetophenone.[16] However, we opted the Suzuki coupling procedure, as the target compounds are readily obtained from commercially available starting materials in one step, often in good yields.[17] The subsequent functional group transformations of the resulting primary products (**3b**–**d**) afforded compounds (**3e**–**g**) (Scheme 1). Compound **3c** has been prepared according to de Boer et al.[18]



Scheme 1. Reagents and conditions: (i) 2 M Na_2CO_3/$Pd(PPh_3)_4$, DMF, 100 °C, 24 h; (ii) BBr_3 (2 equiv)/CH_2Cl_2, 50 °C, 6 h; (iii) BBr_3 (1 equiv)/CH_2Cl_2, 50 °C, 6 h; (iv) $SnCl_2$/ethanol, 85 °C, 12 h.



Scheme 2. Reagents and conditions: (i) $POCl_3$/DMF, 60 °C, 3 h; (ii) a—Na_2S/DMF, rt, 2 h; b—4-nitrobenzylbromide, 50 °C, 3 h; c—NaOMe; 10 min; (iii) $SnCl_2$/ethanol, 85 °C, 12 h; (iv) BBr_3 (1 equiv)/CH_2Cl_2, 50 °C, 6 h; (v) $(CH_2O)_n$, AcOH, $NaCNBH_3$, 18 h; (vi) K_2CO_3/DMF, 90 °C, 1 h then $BrCH_2CH_2F$, 90 °C, 12 h; (vii) a—$(Boc)_2O$, THF, reflux, 18 h; b—NaH, DMF, 50 °C, 30 min then MeI, 50 °C, 3 h; c—BBr_3/CH_2Cl_2, microwave, 140 °C, 5 min; (viii) BBr_3 (1 equiv)/CH_2Cl_2, −78 °C to rt, 18 h.

The synthesis of N,O-disubstituted derivatives started from the acyclic precursor 4-methoxy acetophenone **4**, which upon treatment with $POCl_3$ and DMF afforded β-chloroacrolein **5**. Treatment of **5** sequentially with $Na_2S{\cdot}9H_2O$ and 4-nitrobenzyl bromide followed by cyclization with NaOMe yielded 2-(4-nitrophenyl)-5-(4-methoxyphenyl)-thiophene **3h**.[19] Further manipulations with the nitro and methoxy groups in **3h** as depicted in Scheme 2 resulted in compounds (**3i**–**q**). For the preparation of *N*-methyl derivative **3q**, the routinely used method of reductive alkylation[20] was found to be inefficient. In order to achieve it, the aniline nitrogen in **3i** was initially protected with Boc, subsequently deprotonated using NaH and methylated using MeI. The deprotection of the *N*-Boc group and O-demethylation were achieved in one step using microwave heating to afford **3q**.[21]

The in vitro binding studies of the thiophene derivatives (**3a**–**q**) have clearly established the functional group tolerance of these molecules (Table 1).

Table 1. Inhibition constants (K_i, nM) of compounds (**3a**–**q**) on ligand binding to AD brain homogenates[a]

2,5-Diphenylthiophene derivative	Inhibition constants (K_i, nM)
3a $R^1 = R^2$ = 2-OMe	625 (±20)
3b $R^1 = R^2$ = 4-NO_2	185 (±30)
3c $R^1 = R^2$ = 4-SMe	>1000
3d $R^1 = R^2$ = 4-OMe	108 (±22)
3e $R^1 = R^2$ = 4-OH	4.0 (±0.6)
3f R^1 = 4-OH; R^2 = 4-OMe	6.1 (±0.5)
3g $R^1 = R^2$ = 4-NH_2	6.1 (±0.8)
3h R^1 = 4-NO_2; R^2 = 4-OMe	18.5 (±5.0)
3i R^1 = 4-NH_2; R^2 = 4-OMe	5.6 (±0.4)
3j R^1 = 4-NH_2; R^2 = 4-OH	9.6 (±0.8)
3k R^1 = 4-NMe_2; R^2 = 4-OMe	500 (±20)
3m R^1 = 4-NMe_2; R^2 = 4-OH	7.5 (±0.4)
3n R^1 = 4-NH(CH_2CH_2F); R^2 = 4-OMe	21.5 (±0.5)
3p R^1 = 4-NH(CH_2CH_2F); R^2 = 4-OH	3.9 (±.05)
3q R^1 = 4-NHMe; R^2 = 4-OH	31.2 (±3.0)

[a] Values are means ± SEM of three independent experiments, each in duplicate. Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. For the competition binding, the reaction mixture contained 50 μL tissue homogenates (20–50 μg), 50 μL of [^{125}I]IMPY (diluted in PBS, 0.02–0.04 nM) and 50 μL of inhibitors (10^{-5} to 10^{-10} M diluted serially in PBS containing 0.1% bovine serum albumin) in a final volume of 1 mL. Non-specific binding was defined in the presence of 600 nM IMPY in the same assay tubes. The mixture was incubated at 37 °C for 2 h, and the bound and the free radioactivity were separated by vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester followed by 2 × 3 mL washes of PBS at room temperature. Filters containing the bound I-125 ligand were counted in a gamma counter (Packard 5000) with 70% counting efficiency. Protein determinations were performed with Lowy's method using bovine serum albumin as a standard. The results of inhibition experiments were subjected to non-linear regression analysis using EBDA by which K_i values were calculated.

In the case of **3b**, where both the phenyl rings carry strongly electron withdrawing NO_2 groups ($R^1 = R^2 = NO_2$), the K_i was found to be 185 nM. However, the replacement of these NO_2 groups with NH_2 (compound **3g**) resulted in a steep decrease in the K_i value (6 nM). It proves that the phenyl rings should be electron rich in order for these compounds to exhibit high binding affinities. One other important factor emerges by comparing the K_i values of compounds (**3d–f**) all of which carry electron rich phenyl rings. In the case of **3d** where both R^1 and R^2 are OMe, the compound showed a low binding affinity ($K_i = 108$ nM). When either one or both of these OMe groups is replaced by an OH group, the resulting compounds showed very high binding affinities (**3e**: $R^1 = R^2 = OH$, $K_i = 4$ nM and **3f**: $R^1 = OH$, $R^2 = OMe$, $K_i = 6$ nM). It can be surmised that at least one ring should carry either NH or OH group for these molecules to exhibit effective binding to Aβ plaques. This is further proven by the K_i value (500 nM) of compound **3k**, in which the rings are substituted with OMe and NMe_2 moieties. It is important to note that *N*-fluoroethyl-substituted derivative **3p** exhibits a very high binding affinity (3.9 nM); the corresponding [^{18}F] labeled compound can be utilized to image Aβ plaques in brain using PET.

In conclusion, we have synthesized several novel 2,5-diphenylthiophene derivatives with various substitution patterns on the phenyl rings. The SAR studies of these compounds clearly vindicated our assumption that the phenyl rings should be electron rich in order for these compounds to exhibit desirable binding affinities. Further, all compounds, which exhibited high binding affinities, carried at least one OH or NH moiety in the phenyl ring. The *N*-fluoroethyl-substituted compound showed very good binding to Aβ plaques in vitro, which is very important for the development of PET tracers. Further studies to develop 2,5-diphenylthiophene-based PET markers for the imaging of Aβ plaques is currently under way.

Acknowledgments

This work was supported by grants awarded from the National Institutes of Health (R21-AG021868, M.P.K. and R01-AG022559, H.F.K.).

References and notes

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
2. Selkoe, D. J. *JAMA* **2000**, *283*, 1615.
3. Selkoe, D. J. *Ann. Intern. Med.* **2004**, *140*, 627.
4. Selkoe, D. J. *Nat. Biotechnol.* **2000**, *18*, 823.
5. Klunk, W. E.; Pettegrew, J. W.; Abraham, D. J. *J. Histochem. Cytochem.* **1989**, *37*, 1273.
6. Elhaddaoui, A.; Pigorsch, E.; Delacourte, A.; Turrell, S. *Biospectroscopy* **1995**, *1*, 351.
7. Styren, S. D.; Hamilton, R. L.; Styren, G. C.; Klunk, W. E. *J. Histochem. Cytochem.* **2000**, *48*, 1223.
8. Skovronsky, D. M.; Zhang, B.; Kung, M.-P.; Kung, H. F.; Trojanowski, J. Q.; Lee, V. M.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7609.
9. Lee, C.-W.; Zhuang, Z.-P.; Kung, M.-P.; Plössl, K.; Skovronsky, D.; Gur, T. L.; Hou, C.; Trojanowski, J. Q.; Lee, V. M.-Y.; Kung, H. F. *J. Med. Chem.* **2001**, *44*, 2270.
10. Verhoeff, N. P.; Wilson, A. A.; Takeshita, S.; Trop, L.; Hussey, D.; Singh, K.; Kung, H. F.; Kung, M.-P.; Houle, S. *Am. J. Geriatr. Psychiat.* **2004**, *12*, 584.
11. Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergstrom, M.; Savitcheva, I.; Huang, G.-f.; Estrada, S.; Ausen, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langstrom, B. *Ann. Neurol.* **2004**, *55*, 306.
12. Mathis, C. A.; Wang, Y.; Klunk, W. E. *Curr. Pharm. Des.* **2004**, *10*, 1469.
13. (a) Zhang, W.; Oya, S.; Kung, M.; Hou, C.; Zhuang, Z.; Maier, D.; Kung, H. *J. Med. Chem.* **2005**, *48*, 5980; (b) Zhang, W.; Oya, S.; Kung, M. P.; Hou, C.; Maier, D. L.; Kung, H. F. *Nucl. Med. Biol.* **2005**, *32*, 799.
14. Kung, H. F.; Lee, C.-W.; Zhuang, Z. P.; Kung, M. P.; Hou, C.; Plossl, K. *J. Am. Chem. Soc.* **2001**, *123*, 12740.
15. Takahashi, K.; Suzuki, T.; Akiyaka, K.; Ikegami, Y.; Fukuzawa, Y. *J. Am. Chem. Soc.* **1991**, *113*, 4576.
16. Ling, C.; Lahti, P. M. *J. Am. Chem. Soc.* **1994**, *116*, 8784.
17. General procedure for the Suzuki reaction: to a mixture of 2,5-dibromothiophene (1 mmol) and phenylboronic acid (1.66 mmol) in 10 mL of anhydrous DMF was added 2 M Na_2CO_3 (5 mL). After degassing the mixture for 15 min, $Pd(PPh_3)_4$ (5 mol %) was added and the mixture was heated at 100 °C for 24 h and cooled to room temperature. The solvent was removed under reduced pressure and the residue was taken in ethyl acetate. The ethyl acetate layer was washed successively with water and brine, and dried over anhyd $MgSO_4$. The crude, after the evaporation of the solvent, was purified by column chromatography on silica gel (50% DCM in hexane) to afford products (**3a–d**).
18. de Boer, B.; Meng, H.; Perepichk, D. F.; Zheng, J.; Frank, M. M.; Chabal, Y. J.; Bao, Z. *Langmuir* **2003**, *19*, 4272.
19. Kirsch, G.; Prim, D.; Leising, F.; Mignani, G. *J. Heterocycl. Chem.* **1994**, *31*, 1005.
20. Barluenga, J.; Bayon, A. M.; Asensio, G. *J. Chem. Soc., Chem. Commun.* **1984**, 1334.
21. Preparation of **3q**: compound **3i** (325 mg, 1.16 mmol) and di-*tert*-butyldicarbonate (266 mg, 1.22 mmol) were dissolved in anhyd THF (12 mL) and the solution was refluxed overnight. Subsequent workup and column chromatography afforded *N*-Boc-protected **3i**. The *N*-Boc **3i** (198 mg, 0.6 mmol) was taken in 4 mL anhyd DMF, NaH (20 mg, 0.8 mmol, 95% powder) was added, and the mixture was warmed at 50 °C for 30 min. It was then cooled to room temperature, MeI (426 mg, 3 mmol) was added and stirred at room temperature for 3 h to afford *N*-methyl-*N*-Boc **3i** after workup and purification. Microwave heating (140 °C, 5 min, Biotage initiator microwave oven) of *N*-methyl-*N*-Boc **3i** (39 mg, 0.1 mmol) and BBr_3 (IM solution in DCM, 0.1 mL, 0.1 mmol) in DCM (4 mL) afforded compound **3q**.