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First Selective Dual Inhibitors of Tau Phosphorylation and Beta-Amyloid Aggregation, Two Major Pathogenic Mechanisms in Alzheimer's Disease

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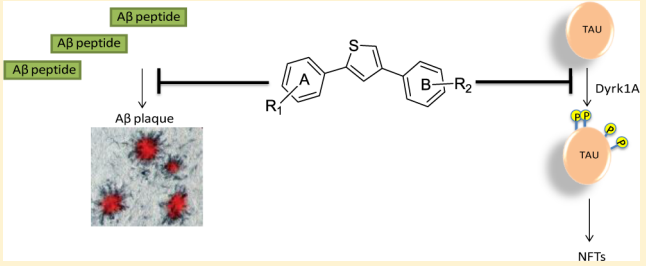
Supporting Information

ABSTRACT: In Alzheimer's disease (AD), multiple factors account for the accumulation of neurocellular changes, which may begin several years before symptoms appear. The most important pathogenic brain changes that are contributing to the development of AD are the formation of the cytotoxic β-amyloid aggregates and of the neurofibrillary tangles, which originate from amyloid-β peptides and hyperphosphorylated tau protein, respectively. New therapeutic agents that target both major pathogenic mechanisms may be particularly efficient. In this study, we introduce bis(hydroxyphenyl)-substituted thiophenes as a novel class of selective, dual inhibitors of the tau kinase Dyrk1A and of the amyloid-β aggregation.



KEYWORDS: *Alzheimer's disease, β-amyloid, tau protein, Dyrk1A, dual inhibitors*

Alzheimer's disease (AD) is the sixth leading cause of death in the United States[1] with an increasing incidence as the percentage of population aged over 65 is steadily growing. AD, as other chronic diseases, is a result of multiple factors rather than a single cause. Two of the most prominent pathological events in the appearance and development of Alzheimer's disease are the formation of insoluble amyloid plaques and neurofibrillary tangles.[2] The amyloid plaques are extracellular deposit of amyloid β (Aβ) fibrils, which are aggregates derived from oligomeric Aβ peptides, and thought to trigger the disease, probably in concert with neurofibrillary tangles (NFT).[2,3] NFT are insoluble aggregates of the microtubule-associated tau protein, which were also shown to promote neurodegeneration.[2] The formation of NFT is caused by hyperphosphorylation of the tau protein, for which several protein kinases are discussed to play major roles, including GSK3β, PKA, CDK5, and Dyrk1A.[4]

In people affected by Down syndrome (DS), AD occurs at a substantially earlier age of about 40 years.[5] The early amyloidosis-β in these patients was mainly attributed to an overexpression of the amyloid precursor protein (APP) due to the location of the APP gene in the so-called "Down syndrome critical region" (DSCR) on chromosome 21, of which three copies are present in all DS cases.[6] However, with respect to the early neurofibrillary degeneration, one of the major responsible factors was shown to be the dual specificity tyrosine phospho-regulated kinase 1A (Dyrk1A), which is encoded by another gene in the DSCR and therefore about 1.5-fold overexpressed in DS patients.[6−9] It has been demonstrated that Dyrk1A is involved in the premature development of AD in DS[8,9] because it phosphorylates several crucial substrates, such as the tau protein and α-synuclein (reviewed in ref 10). Phosphorylation of α-synuclein enhances the formation of neurotoxic intracellular inclusions, the so-called Lewy bodies, which are present in about 30−40% of the AD cases. It is assumed that in AD, Aβ, tau, and α-synuclein can promote each other's aggregation.[11] Dyrk1A is the major tau kinase in DS/AD patients, but it was also shown to be overexpressed in sporadic AD cases and may act as a priming kinase by creating the recognition sites for GSK3β, for example, via tau phosphorylation at Thr212.[12]

The production of Aβ peptides requires proteolysis of the APP by α-, β-, and γ-secretase. Of note, Dyrk1A was shown to phosphorylate APP and presenilin-1, a key component of the γ-secretase complex, thereby accelerating the formation of Aβ peptides.[10] In a pathological state, different Aβ peptides ranging from 39 to 42 amino acid residues are formed, with the prevalence of Aβ terminating at the amino acid 42 (Aβ_{42}), which is the form more prone to oligomerization and fibril formation.[13] In the brains of Down syndrome patients, Aβ_{42} can form numerous diffuse plaques already at the age of 12 years, whereas Aβ_{40} occurs first in plaques almost 20 years later.[14]

Not surprisingly, both these major neurotoxic processes, the formation of NFT and Aβ plaques, were proposed independently as pivotal targets for pharmacological inter-

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vention. However, attempts to inhibit either of these pathogenic mechanisms alone have so far been disappointing. Although the reasons for this are not fully clear, it can be hypothesized that a higher efficacy may arise from the simultaneous, specific inhibition of both major pathogenic processes, in line with the view that complex diseases may require polypharmacology approaches.[15] Only few examples of such dual inhibitors were hitherto described, and they were mainly restricted to approaches combining the inhibition of Aβ peptide aggregation with the inhibition of acetylcholinesterase (AChE).[16−19] These prototype inhibitors were believed to be superior to existing single-target agents; however, the AChE inhibitory activity aimed at treatment of the symptoms by amplification of the cholinergic neurotransmission, rather than at stopping the disease progression. Earlier reports on the inhibition of both tau protein phosphorylation and Aβ peptide aggregation involved the use of nonselective natural compounds such as curcumin or plant extracts (reviewed in ref 20). However, such plant-derived compounds are known to inhibit a plethora of enzymes; hence, the mechanism(s) of action remain poorly defined, and target-oriented optimization is not possible. In contrast, single small molecules able to specifically interfere with only the disease-relevant pathways, amyloid formation and tau phosphorylation, were—to the best of our knowledge—not reported yet.

Herein, we present the first selective dual inhibitors that inhibit a major tau kinase, Dyrk1A, and exhibit an anti-Aβ aggregating activity, thus targeting two of the major pathogenic mechanisms involved in AD progression.

Screening of an in-house library of enzyme inhibitors devoid of typical kinase inhibitor motifs identified compound **1** as a hit (Figure 1, IC_{50} = 1.8 μM).[22] Interestingly, we found that

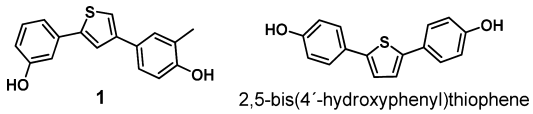
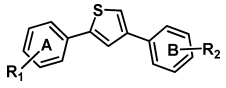


Figure 1. Hit and reference compound.[21]

structurally similar compounds were previously described to bind to Aβ peptide aggregates with high affinity, for example, 2,5-bis(4′-hydroxyphenyl)thiophene (K_i = 4.0 nM)[21] (Figure 1). Since it was possible that compounds with such a structural motif would also bind to the soluble monomeric or oligomeric Aβ peptides, thus potentially preventing aggregation, we tested the Dyrk1A inhibitor **1** and structurally related derivatives in Table 1 for this second biological activity. With a well consolidated experimental protocol in our hands,[18] we decided to use Aβ_{40} instead of the more toxic and aggregation prone Aβ_{42}, also because of its slower aggregation kinetics. We also explored the inverse approach, that is, testing of some 2,5-disubstituted thiophene isomers that were more similar to the described Aβ ligands,[21] and an analogous thiazole derivative against Dyrk1A; however, this group of analogues did not show any inhibitory activity (Table S1, Supporting Information). Hence, we focused on the 2,4-disubstituted thiophene isomers.

Initially, we observed only a weak inhibition of the in vitro Aβ_{40} peptide aggregation by compound **1** (46% at 100 μM). We then explored whether it was possible to optimize this activity, starting with a variation of the hydroxyl position. Moving of the hydroxyl on the A ring from the *meta*- to the *para*-position clearly enhanced the potency to prevent the self-assembly of the Aβ peptide in all cases, and eventually gave rise to the most potent compounds (**3**, **5**−**8**, Table 1). Importantly, these modifications did not strongly affect the inhibition of Dyrk1A. The addition of an aliphatic moiety was identified as a key step for the enhancement of the Aβ aggregation inhibition. Especially the *meta*-methyl substitution of the A ring resulted in the most potent compounds in this regard (**7** and **8**). However, longer aliphatic chains were unfavorable (e.g., *m*-ethyl in **6**) and were even detrimental to the Dyrk1A inhibition (compare **6** vs **5**). Concerning the B ring, the hydroxyl in *meta*-position was favorable for the inhibition of Dyrk1A, whereas the *para*-hydroxyl conferred a higher potency against the Aβ aggregation (cf. **5** vs **7**). The comparison of **1** with **2** revealed that the inhibition of Dyrk1A by compounds that are *p*-hydroxy−substituted at the B ring could be increased by the introduction of *m*-methyl (**1**), while ethyl at the same position was already too large (**4**). Thus, compound **8** was synthesized aiming at creating the best balance between the two desired biological activities. The most favorable substitution pattern on the A ring was kept (from **5** and **7**), while an additional methyl group was introduced in the *meta*-position of the B ring. Indeed, **8** displayed a slightly enhanced inhibition of Dyrk1A compared with **7**, while the potency to prevent the Aβ aggregation was also improved. The comparison of **8** to its methoxy precursor **8i** showed that at least one H-bond donor function of the hydroxyl groups was essential to both biological activities (**8i**, Table 1). Altogether, our structure−activity relationship indicated that with the 2,4-diphenyl thiophene scaffold, the

Table 1. Biological Activities of 2,4-Diphenyl Thiophene Derivatives



name	R1	R2	Dyrk1A IC_{50} (μM)[ab]	Aβ_{40} % inhibition @ 100 μM
1	3-OH	3-CH_3; 4-OH	1.8	46 ± 2
2	3-OH	4-OH	4.0	38 ± 2
3	4-OH	3-OH	4.5	55 ± 2
4	3-OH	3-CH_2CH_3; 4-OH	5.0	49 ± 3
5	3-CH_3; 4-OH	3-OH	2.0	73 ± 2
6	3-CH_2CH_3; 4-OH	3-OH	28%[c]	62 ± 2
7	3-CH_3; 4-OH	4-OH	6.7	78 ± 1
8	3-CH_3; 4-OH	3-CH_3; 4-OH	5.0	91 ± 1
8i	3-CH_3; 4-OCH_3	3-CH_3; 4-OCH_3	n.i.[d]	37 ± 3

[a]SD < 10%. [b][ATP] = 100 μM. [c]Percent inhibition at 5 μM. [d]n.i., no inhibition.

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potency toward Aβ_{40} aggregation could be increased without loss of activity against Dyrk1A. In contrast, the previously described class of hydroxybenzothiophene analogues, derived from the bis(phenol)thiophenes following a ring condensation strategy,[23] proved to be markedly less potent in preventing the Aβ aggregation (Table S2, Supporting Information). Therefore, it failed to provide an appropriate balance between the inhibition of Dyrk1A and the Aβ aggregation, similarly to our previously published bis(pyrimidinyl)thiophene series[22] (Table S3, Supporting Information). Although it was suggested that phenolic compounds in general can interfere with Aβ peptide aggregation,[24] the lack of activity with the hydroxybenzothiophene analogs underlines that the spatial arrangement of the thiophene to the phenolic structures is important, also indicating a specific binding interaction.

Based on their highest potencies to prevent Aβ aggregation (cf. IC_{50} values in Table 2), compounds **5**, **7**, and **8** were selected for testing their cellular activity. To this end, we developed a cell-based assay that provided conditions for a strong tau phosphorylation specifically by Dyrk1A due to overexpression of both proteins (cf. Supporting Information, HEK293-tau-Dyrk1A cell assay). All three compounds were found to inhibit the intracellular, Dyrk1A-catalyzed tau phosphorylation with a remarkable efficacy, as there was only a slight decline of potency compared with that in the cell-free assay (Figure 2; cf. Table 1); the weakest Dyrk1A inhibitor **7** showed the lowest cellular potency. Altogether, compound **8** exhibited the best profile with a balanced inhibitory potency toward both targets (Table 2).

Table 2. IC_{50}'s of the Best Compounds for the Inhibition of Aβ_{40} Aggregation and Intracellular Tau Phosphorylation

name	Aβ_{40} inhibition (cell free assay, IC_{50} ± SEM, μM)	Inhibition of tau phosphorylation in cells (IC_{50}, μM)[a]
5	33 ± 4	8.5
7	16 ± 2	11.6
8	11 ± 3	8.3

[a]SD < 15%.

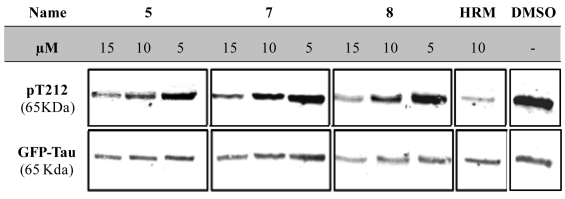

Figure 2. HEK293-tau-Dyrk1A cell assay. Compounds **5**, **7**, and **8** inhibit Dyrk1A-catalyzed tau phosphorylation in stably transfected HEK293 cells. The test compounds, DMSO, or the reference inhibitor harmine (HRM)[25,26] were added to the cell medium at the indicated concentrations. After immunoblotting, the level of phospho-tau-Thr212 was detected using a phosphospecific antibody. To normalize the signals, total recombinant tau protein was quantified using an anti-GFP antibody. One representative experiment out of two is shown.

A major obstacle for the application of kinase inhibitors is often their poor selectivity. Therefore, we tested the selectivity of **8** against a panel of carefully selected kinases, which not only included a member of each branch of the human kinome but also the complete list of frequently reported off-targets for Dyrk1A inhibitors.[27] This challenging screening revealed that **8** was rather selective for the Dyrk family of kinases, with the only exception of Clk4 (Table S4, Supporting Information). Coinhibition of the latter kinase might be advantageous with respect to its involvement in the alternative splicing of tau, which results in a pathogenic imbalance between 3R-tau and 4R-tau isoforms through skipping of exon 10.[28] As Dyrk1A shares this pathogenic role,[29] coinhibition of Dyrk and Clk kinases by our compounds might efficiently correct the tau splicing isoform imbalance. It should be noted that the clean selectivity profile versus the chosen kinase panel ruled out any nonselective inhibitory properties such as those found with plant−derived compounds like curcumin.[30]

In conclusion, using a focused multitarget approach, we identified a new bis(hydroxphenyl)thiophene-based family of dual Dyrk1A and β-amyloid inhibitors. Of note, the chemical class of bis(hydroxyphenyl)-substituted thiophenes was previously reported to display a good metabolic stability in rat liver microsomes and a high permeability in the CaCo-2 assay,[31] and was orally available in rats.[32] Although the substitution pattern of those derivatives was different, these studies demonstrated that bis(hydroxyphenyl)-substituted thiophenes can show favorable pharmacokinetic properties in spite of the phenolic substructure. Moreover, the penetration of the blood-brain barrier is another important requirement for AD therapeutics; interestingly, bisphenolic compounds with comparable physicochemical properties (bis(hydroxyphenyl) bis-styrylbenzenes) were shown to readily cross the blood-brain barrier.[33]

For proof-of-concept studies, it is preferable to optimize the potencies of our lead compounds; however, **8** might be considered to be just at the border of being potentially applicable to in vivo AD models—provided that no dose-limiting toxicities occur. For comparison, Hirohata and coworkers reported that ibuprofen inhibits α-synuclein fibril formation with an IC_{50} of 12.1 μM, similar to the potency range of our compounds, and pointed out that such levels can be reached in cerebrospinal fluid after an oral dose of 800 mg in humans.[34] While the kinase inhibitory activity can certainly be optimized, it is unclear to what extent the antifibrillogenic potency can be increased, since the underlying mode of action may involve stoichiometric binding rather than a catalytic mechanism. Thus, the in vivo efficacy might also depend on the concentration of the Aβ peptides in the affected neurons, which may, at least partially, be lower than the 30 μM in our cell-free assay.

METHODS

HEK293-Tau-Dyrk1A Cell Assay. HEK293-tau-Dyrk1A cells, generated as described in the Supporting Information, were seeded in 12-well plates and grown in full medium without G418 until 70% confluence was reached. The medium was then exchanged by DMEM containing 0.1% of FCS and tetracycline (0.3 μg/mL) to induce the expression of Dyrk1A, resulting in a strong increase of Dyrk1A activity in the cells (see experimental validation of the assay, Figure S1 in the Supporting Information). The test compounds or DMSO (control) were then added from stock solutions in DMSO to the desired final concentration (DMSO concentration ≤ 0.2%). Initially, potential cytotoxicity of the compounds was checked; no signs of toxicity were observed after incubation for 2 days at the highest concentrations (20 μM) of the compounds. Under the assay conditions, the incubation with the compounds was carried out overnight in an incubator at 37 °C and 5% CO_2. The next day, the medium was removed from each well and the plate frozen at −80 °C for 10−15 min to facilitate the cell disruption. Then, 200 μL of SDS-PAGE sample buffer per well was added to the frozen cells and cell lysis was completed by passing several times through a micro syringe (Hamilton). Aliquots of 24 μL of the samples were then separated by SDS-PAGE (10% gels), and the

proteins blotted on a PVDF membrane (Immobilon-FL, Millipore) using a semidry blotting apparatus (Biometra). Free binding sites on the membrane were then blocked for 1 h using blocking buffer (3% bovine serum albumin in Tris buffered saline (TBS), pH 7.2), followed by an incubation with the first primary antibody (pT212) diluted 1:1000 in blocking buffer overnight at 4 °C. On the following day, the membrane was washed with TBS containing 0.1% Tween-20 for three times and one time with TBS. Then it was incubated with the second primary antibody (anti-GFP, dilution 1:1000) in blocking buffer for 1 h at room temperature. After a second wash, the membrane was incubated with secondary antibodies (IR-Dye-labeled anti-mouse and anti-rabbit, both diluted 1:7500 in blocking buffer) for 1 h at room temperature. The signals of the washed membrane were finally detected by using an Odyssey infrared imager (LI-COR).

■ ASSOCIATED CONTENT

Supporting Information

Biological activities of 2,5-bis(hydroxyphenyl)thiophenes, hydroxybenzothiophenes, and bis(pyridinyl)thiophenes (Tables S1−S3); selectivity screening (Table S4); experimental details for all assays and syntheses; HEK293-tau-Dyrk1A assay validation (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

M.M. developed and performed the cellular tau phosphorylation assay and repeated kinase inhibition assays; C.S. and P.M. contributed to inhibitor design and performed synthetic chemistry; C.S. performed most kinase inhibition assays; M.C. conducted Aβ aggregation assays and interpreted data; R.W.H., A.C., and M.E. conceived the project, interpreted data, and oversaw the research. The manuscript was written by M.M., M.C., and M.E..

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AD, Alzheimer's disease; DS, Down syndrome; Dyrk, dual specificity tyrosine (Y) phosphorylation regulated kinase; Aβ, amyloid β; NFT, neurofibrillary tangles; DSCR, Down syndrome critical region; APP, amyloid precursor protein; GSK3β, glycogen synthase kinase 3β; PKA, protein kinase A; CDK5, cyclin dependent kinase 5; HRM, harmine

■ REFERENCES

(1) Murphy, S. L., Xu, J., and Kochanek, K. D. (2013) Deaths: Final Data for 2010. *National Vital Statistics Reports* , Vol. *61*, National Center for Health Statistics, Hyattsville, MD.

(2) Ittner, L. M., and Götz, J. (2011) Amyloid-B and Tau—A Toxic Pas de Deux in Alzheimer's Disease. *Nat. Rev. Neurosci. 12*, 65−72.

(3) Hawkes, C. A., Carare, R. O., and Weller, R. O. (2014) Amyloid and Tau in the Brain in Sporadic Alzheimer's Disease: Defining the Chicken and the Egg. *Acta Neuropathol. 127*, 617−618.

(4) Martin, L., Latypova, X., Wilson, C. M., Magnaudeix, A., Perrin, M.-L., Yardin, C., and Terro, F. (2013) Tau Protein Kinases: Involvement in Alzheimer's Disease. *Ageing Res. Rev. 12*, 289−309.

(5) Wisniewski, K. E., Wisniewski, H. M., and Wen, G. Y. (1985) Occurrence of Neuropathological Changes and Dementia of Alzheimer's Disease in Down's Syndrome. *Ann. Neurol. 17*, 278−282.

(6) Wegiel, J., Dowjat, K., Kaczmarski, W., Kuchna, I., Nowicki, K., Frackowiak, J., Mazur Kolecka, B., Wegiel, J., Silverman, W. P., Reisberg, B., Deleon, M., Wisniewski, T., Gong, C.-X., Liu, F., Adayev, T., Chen-Hwang, M.-C., and Hwang, Y.-W. (2008) The Role of Overexpressed DYRK1A Protein in the Early Onset of Neurofibrillary Degeneration in Down Syndrome. *Acta Neuropathol. 116*, 391−407.

(7) Liu, F., Liang, Z., Wegiel, J., Hwang, Y.-W., Iqbal, K., Grundke-Iqbal, I., Ramakrishna, N., and Gong, C.-X. (2008) Overexpression of Dyrk1A Contributes to Neurofibrillary Degeneration in Down Syndrome. *FASEB J. 22*, 3224−3233.

(8) Kimura, R., Kamino, K., Yamamoto, M., Nuripa, A., Kida, T., Kazui, H., Hashimoto, R., Tanaka, T., Kudo, T., Yamagata, H., Tabara, Y., Miki, T., Akatsu, H., Kosaka, K., Funakoshi, E., Nishitomi, K., Sakaguchi, G., Kato, A., Hattori, H., Uema, T., and Takeda, M. (2007) The DYRK1A Gene, Encoded in Chromosome 21 Down Syndrome Critical Region, Bridges between Beta-Amyloid Production and Tau Phosphorylation in Alzheimer Disease. *Hum. Mol. Genet. 16*, 15−23.

(9) Park, J., Yang, E. J., Yoon, J. H., and Chung, K. C. (2007) Dyrk1A Overexpression in Immortalized Hippocampal Cells Produces the Neuropathological Features of Down Syndrome. *Mol. Cell. Neurosci. 36*, 270−279.

(10) Tejedor, F. J., and Hämmerle, B. (2011) MNB/DYRK1A as a Multiple Regulator of Neuronal Development. *FEBS J. 278*, 223−235.

(11) Larson, M. E., Sherman, M. A., Greimel, S., Kuskowski, M., Schneider, J. A., Bennett, D. A., and Lesné, S. E. (2012) Soluble A-Synuclein Is a Novel Modulator of Alzheimer's Disease Pathophysiology. *J. Neurosci. 32*, 10253−10266.

(12) Ferrer, I., Barrachina, M., Puig, B., Martínez de Lagrán, M., Martí, E., Avila, J., and Dierssen, M. (2005) Constitutive Dyrk1A Is Abnormally Expressed in Alzheimer Disease, Down Syndrome, Pick Disease, and Related Transgenic Models. *Neurobiol. Dis. 20*, 392−400.

(13) Roher, A. E., Lowenson, J. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E., and Ball, M. J. (1993) Beta-Amyloid-(1−42) Is a Major Component of Cerebrovascular Amyloid Deposits: Implications for the Pathology of Alzheimer Disease. *Proc. Natl. Acad. Sci. U.S.A. 90*, 10836−10840.

(14) Lemere, C. A., Blusztajn, J. K., Yamaguchi, H., Wisniewski, T., Saido, T. C., and Selkoe, D. J. (1996) Sequence of Deposition of Heterogeneous Amyloid Beta-Peptides and APO E in Down Syndrome: Implications for Initial Events in Amyloid Plaque Formation. *Neurobiol. Dis. 3*, 16−32.

(15) Anighoro, A., Bajorath, J., and Rastelli, G. (2014) Polypharmacology: Challenges and Opportunities in Drug Discovery. *J. Med. Chem.*, DOI: 10.1021/jm5006463.

(16) Rizzo, S., Rivière, C., Piazzi, L., Bisi, A., Gobbi, S., Bartolini, M., Andrisano, V., Morroni, F., Tarozzi, A., Monti, J.-P., and Rampa, A. (2008) Benzofuran-Based Hybrid Compounds for the Inhibition of Cholinesterase Activity, Beta Amyloid Aggregation, and Abeta Neurotoxicity. *J. Med. Chem. 51*, 2883−2886.

(17) Brogi, S., Butini, S., Maramai, S., Colombo, R., Verga, L., Lanni, C., De Lorenzi, E., Lamponi, S., Andreassi, M., Bartolini, M., Andrisano, V., Novellino, E., Campiani, G., Brindisi, M., and Gemma, S. (2014) Disease-Modifying Anti-Alzheimer's Drugs: Inhibitors of Human Cholinesterases Interfering with β-Amyloid Aggregation. *CNS Neurosci. Ther. 20*, 624−632.

(18) Catto, M., Berezin, A. A., Lo Re, D., Loizou, G., Demetriades, M., De Stradis, A., Campagna, F., Koutentis, P. A., and Carotti, A. (2012) Design, Synthesis and Biological Evaluation of Benzo[*e*]-

[1,2,4]triazin-7(1*H*)-one and [1,2,4]-Triazino[5,6,1-Jk]carbazol-6-one Derivatives as Dual Inhibitors of Beta-Amyloid Aggregation and Acetyl/butyryl Cholinesterase. *Eur. J. Med. Chem. 58*, 84−97.
(19) Rook, Y., Schmidtke, K.-U., Gaube, F., Schepmann, D., Wünsch, B., Heilmann, J., Lehmann, J., and Winckler, T. (2010) Bivalent Beta-Carbolines as Potential Multitarget Anti-Alzheimer Agents. *J. Med. Chem. 53*, 3611−3617.
(20) Calcul, L., Zhang, B., Jinwal, U. K., Dickey, C. A., and Baker, B. J. (2012) Natural Products as a Rich Source of Tau-Targeting Drugs for Alzheimer's Disease. *Future Med. Chem. 4*, 1751−1761.
(21) Chandra, R., Kung, M.-P., and Kung, H. F. (2006) Design, Synthesis, and Structure-Activity Relationship of Novel Thiophene Derivatives for Beta-Amyloid Plaque Imaging. *Bioorg. Med. Chem. Lett. 16*, 1350−1352.
(22) Schmitt, C., Kail, D., Mariano, M., Empting, M., Weber, N., Paul, T., Hartmann, R. W., and Engel, M. (2014) Design and Synthesis of a Library of Lead-like 2,4-Bisheterocyclic Substituted Thiophenes as Selective Dyrk/Clk Inhibitors. *PLoS One 9*, e87851.
(23) Miralinaghi, P., Schmitt, C., Hartmann, R. W., Frotscher, M., and Engel, M. (2014) 6-Hydroxybenzothiophene Ketones: Potent Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) Owing to Favorable Molecule Geometry and Conformational Preorganization. *ChemMedChem*, DOI: 10.1002/cmdc.201402050.
(24) Porat, Y., Abramowitz, A., and Gazit, E. (2006) Inhibition of Amyloid Fibril Formation by Polyphenols: Structural Similarity and Aromatic Interactions as a Common Inhibition Mechanism. *Chem. Biol. Drug Des. 67*, 27−37.
(25) Frost, D., Meechoovet, B., Wang, T., Gately, S., Giorgetti, M., Shcherbakova, I., and Dunckley, T. (2011) B-Carboline Compounds, Including Harmine, Inhibit DYRK1A and Tau Phosphorylation at Multiple Alzheimer's Disease-Related Sites. *PLoS One 6*, e19264.
(26) Bain, J., Plater, L., Elliott, M., Shpiro, N., Hastie, C. J., McLauchlan, H., Klevernic, I., Arthur, J. S. C., Alessi, D. R., and Cohen, P. (2007) The Selectivity of Protein Kinase Inhibitors: A Further Update. *Biochem. J. 408*, 297−315.
(27) Schmitt, C., Miralinaghi, P., Mariano, M., Hartmann, R. W., and Engel, M. (2014) Hydroxybenzothiophene Ketones Are Efficient Pre-mRNA Splicing Modulators Due to Dual Inhibition of Dyrk1A and Clk1/4. *ACS Med. Chem. Lett. 5*, 963−967.
(28) Hartmann, A. M., Rujescu, D., Giannakouros, T., Nikolakaki, E., Goedert, M., Mandelkow, E. M., Gao, Q. S., Andreadis, A., and Stamm, S. (2001) Regulation of Alternative Splicing of Human Tau Exon 10 by Phosphorylation of Splicing Factors. *Mol. Cell. Neurosci. 18*, 80−90.
(29) Yin, X., Jin, N., Gu, J., Shi, J., Zhou, J., Gong, C.-X., Iqbal, K., Grundke-Iqbal, I., and Liu, F. (2012) Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase 1A (Dyrk1A) Modulates Serine/arginine-Rich Protein 55 (SRp55)-Promoted Tau Exon 10 Inclusion. *J. Biol. Chem. 287*, 30497−30506.
(30) Aggarwal, B. B., and Sung, B. (2009) Pharmacological Basis for the Role of Curcumin in Chronic Diseases: An Age-Old Spice with Modern Targets. *Trends Pharmacol. Sci. 30*, 85−94.
(31) Bey, E., Marchais-Oberwinkler, S., Werth, R., Negri, M., Al-Soud, Y. A., Kruchten, P., Oster, A., Frotscher, M., Birk, B., and Hartmann, R. W. (2008) Design, Synthesis, Biological Evaluation and Pharmacokinetics of Bis(hydroxyphenyl) Substituted Azoles, Thiophenes, Benzenes, and Aza-Benzenes as Potent and Selective Nonsteroidal Inhibitors of 17beta-Hydroxysteroid Dehydrogenase Type 1 (17beta-HSD1). *J. Med. Chem. 51*, 6725−6739.
(32) Bey, E., Marchais-Oberwinkler, S., Negri, M., Kruchten, P., Oster, A., Klein, T., Spadaro, A., Werth, R., Frotscher, M., Birk, B., and Hartmann, R. W. (2009) New Insights into the SAR and Binding Modes of Bis(hydroxyphenyl)thiophenes and -Benzenes: Influence of Additional Substituents on 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) Inhibitory Activity and Selectivity. *J. Med. Chem. 52*, 6724−6743.
(33) Flaherty, D. P., Kiyota, T., Dong, Y., Ikezu, T., and Vennerstrom, J. L. (2010) Phenolic Bis-Styrylbenzenes as β-Amyloid Binding Ligands and Free Radical Scavengers. *J. Med. Chem. 53*, 7992−7999.
(34) Hirohata, M., Ono, K., Morinaga, A., and Yamada, M. (2008) Non-Steroidal Anti-Inflammatory Drugs Have Potent Anti-Fibrillogenic and Fibril-Destabilizing Effects for Alpha-Synuclein Fibrils in Vitro. *Neuropharmacology 54*, 620−627.