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Novel Fluorinated 8-Hydroxyquinoline Based Metal Ionophores for Exploring the Metal Hypothesis of Alzheimer's Disease

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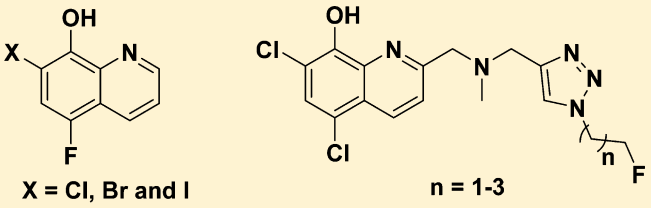
Supporting Information

ABSTRACT: Zinc, copper, and iron ions are involved in amyloid-beta (Aβ) deposition and stabilization in Alzheimer's disease (AD). Consequently, metal binding agents that prevent metal-Aβ interaction and lead to the dissolution of Aβ deposits have become well sought therapeutic and diagnostic targets. However, direct intervention between diseases and metal abnormalities has been challenging and is partially attributed to the lack of a suitable agent to determine and modify metal concentration and distribution *in vivo*. In the search of metal ionophores, we have identified several promising chemical entities by strategic fluorination of 8-hydroxyquinoline drugs, clioquinol, and PBT2. Compounds **15**−**17** and **28**−**30** showed exceptional metal ionophore ability (6−40-fold increase of copper uptake and >2-fold increase of zinc uptake) and inhibition of zinc induced Aβ oligomerization (EC_{50}s < ~5 μM). These compounds are suitable for further development as drug candidates and/or positron emission tomography (PET) biomarkers if radiolabeled with ^{18}F.



KEYWORDS: *8-Hydroxyquinoline, metal ionophore, positron emission tomography, Alzheimer's disease*

Alzheimer's disease (AD), a neurodegenerative disorder that affects approximately 44 million people worldwide, is the sixth leading cause of death with an estimated socio-economic burden of more than $200 billion. There is no cure for the debilitating disease with only few symptom-alleviating treatments.[1−4] Thus, understanding of disease etiology and development of therapeutics and biomarkers for AD is urgently needed.

AD is characterized by extracellular amyloid plaques containing Cu and Zn, which is accompanied by neuronal Cu deficiency and Zn dys-homeostasis.[5−9] It is known that Zn and Cu ions are involved in the Aβ deposition and stabilization and that metal chelating agents can lead to the dissolution of Aβ deposits by preventing metal−Aβ interaction.[10−13] Therefore, the metal hypothesis of AD has led to the search for diagnostic and therapeutic agents that are able to modulate or redistribute metal ions within the brain. A prototypical metal-chelating drug, 5-chloro-7-iodo-quinolin-8-ol (clioquinol; CQ (**11**); Chart 1), prevents Aβ toxicity. The metal ionophore activity of CQ (**11**) promotes cellular Zn and Cu uptake, initiating protective cell signaling events to degrade Aβ and prevent toxicity.[6,14] In a pilot phase II clinical trial, CQ (**11**) was well tolerated and attenuated the rate of cognitive decline in AD patients; however, further development was halted due to a contaminant during the manufacturing process.[15] A newer generation metal chelator, PBT2 (5,7-dichloro-2-((dimethylamino)methyl) 8-quinolinol; **18**), has also shown benefits in patients with Huntington's disease and patients with AD in phase II clinical trials.[16,17]

To identify a suitable metal chelator for AD drug development, a radiopharmaceutical based on a metal chelator would be useful to determine metal concentration and distribution in the living brain by positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Development of such agents would also advance our understanding of AD etiology that are affected by dysregulation of metal functions and may prove useful in

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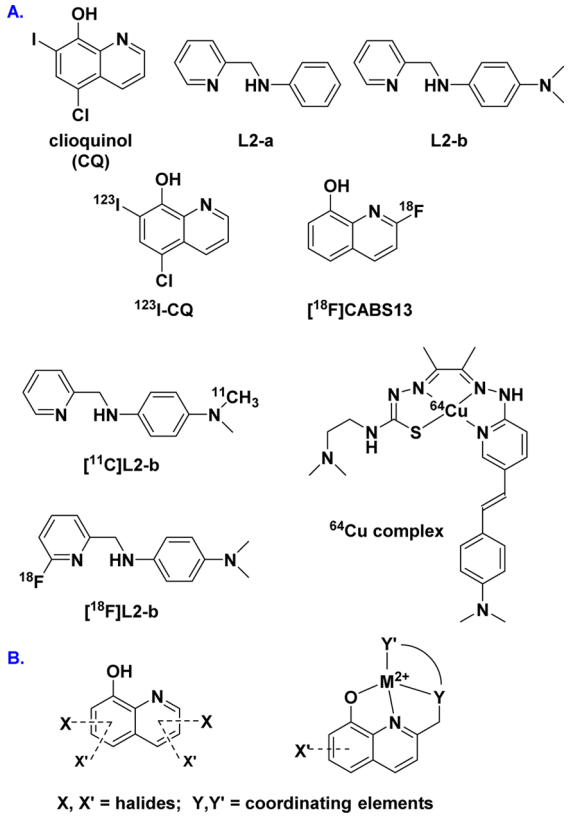
Chart 1. Representative Metal Chelators and Radiolabeled Derivatives for AD (A) and Design Strategy (B)



monitoring therapeutic response and disease progression for patients with AD. The first attempt to achieve this goal was carried out with iodine-123 labeled CQ and evaluated as an imaging marker in human subjects (Chart 1). Unfortunately, radio-deiodination *in vivo* and low brain uptake in human subjects precluded the use of this radiopharmaceutical for further development.[18,19] Our laboratory reported the first analogous PET radiotracer labeled with fluorine-18 (^{18}F, β^+, $t_{1/2}$ = 109.7 min) for this target, 2-[^{18}F]fluoro-8-hydroxyquinoline ([^{18}F]CABS13; Chart 1A), which demonstrated a significant temporal difference in brain uptake and retention among a transgenic mouse model of AD ($APP_{swe}/PSEN_{1dE9}$) compared with wild-type control.[20,21] However, PET imaging studies in normal nonhuman primates revealed low brain uptake of the radiotracer and fast metabolism, which may be attributed to species differences.[22] Notably, preliminary evaluations of other PET radiotracers to probe the metal hypothesis of AD were subsequently evaluated. Lim et al. recently reported two small molecules, namely, *N*-(pyridin-2-ylmethy)aniline (L2-a) and N^1,N^1-dimethyl-N^4-(pyridin-2-ylmethyl)benzene-1,4-diamine (L2-b), based on a hybrid design of CQ (**11**) and stilbene derivatives (Chart 1A). These compounds modulated metal induced Aβ aggregation and toxicity *in vitro*. In particular, L2-b demonstrated the ability to dissolve Aβ aggregates from brain tissue homogenates from AD patients.[23] Scott et al. radiolabeled compound L2-b with carbon-11 (^{11}C, β^+, $t_{1/2}$ = 20.3 min) or ^{18}F and performed a preliminary evaluation in healthy nonhuman primates,[24] and Donnelly et al. combined a functionalized styrylpyridine group and copper-64 (^{64}Cu, β^+, $t_{1/2}$ = 12.7 h) labeled thiosemicarbazone to detect Aβ *in vtiro* in post-mortem brain tissue of AD patients.[25]

The goal of present work is to synthesize a series of fluorinated 8-hydroxyquinolines as metal selective chelators for therapeutic applications and as potential PET imaging agents. Our design strategy is based on lead compounds 8-hydroxyquinoline (8HQ; **1**), CQ (**11**), and PBT2 (**18**). We expect to increase binding affinity, metal selectivity, and *in vivo* stability by strategically placing functional groups, including fluorine atom around hydroxyquinoline core, as well as design a better chelating interaction with metals, i.e., Zn and Cu, by increasing binding ability (Chart 1B). Two *in vitro* methods were used to evaluate these new compounds. Ionophore activity was assessed in cultured SH-SY5Y cells, a well-established neuronal model system. Cells were treated with each compound, and inductively coupled plasma mass spectrometry (ICP-MS) was used to identify compounds that promoted cellular uptake of Zn and Cu (Figures 1–3). While

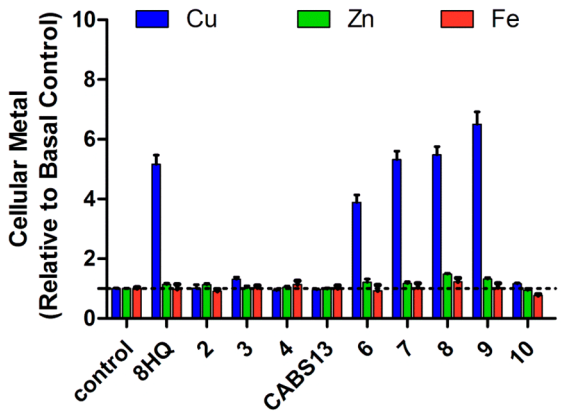


Figure 1. Ionophore assay of 8HQ derivatives. Dashed line indicates control level. SH-SY5Y cells were treated with each compound (20 μM) for 6 h, and cellular metal levels were measured with ICP-MS. Dashed line indicates control level.

the compounds were not expected to be Fe chelators, cellular Fe uptake was also measured. Aggregation of Aβ oligomers was assessed fluorometrically with 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonate (bis-ANS). The effective concentration that reversed Aβ aggregation by 50% (EC_{50}) was determined with nonlinear regression analysis (Table 1 and Supporting Information).

Herein we describe an array of candidate compounds that showed superior binding affinity, metal selectivity and Cu and Zn ionophore activity over the therapeutic drugs CQ (**11**) and PBT2 (**18**). These promising compounds provide a candidate pool for the development of drug candidates and/or PET ligands. As shown in Chart 2, efforts were first focused on fluorinated 8-hydroxyquinoline derivatives to improve the ionophore ability. We discovered that the binding pocket was sensitive to different heteroaromatic rings (compounds **2** and **3**). We then systematically evaluated fluorine substituents around the hydroxyquinoline because fluorine contribution to the electron density of the heterocyclic ring is a function of its position on 8HQ (**1**). CABS13 (compound **5**) and compound **6** were inferior to 8HQ (**1**) in terms of Cu uptake, while compounds **7**–**9** showed equal or superior Cu uptake (Figure 1). Neuronal Zn and Fe uptake were largely unaffected. Modification of CQ was carried out in attempt to discover lead candidate radiotracers, which can reveal higher brain uptake than [^{123}I]CQ.[19] Therefore, we replaced labile iodine on CQ

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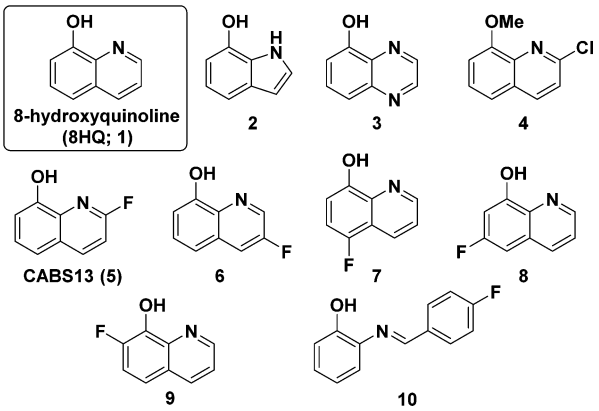
Table 1. Reversal of Aggregation of Soluble Aβ Oligomers by Fluorinated Hydroxyquinoline Derivatives[a]

Cpd. No.	EC_{50} ± SD (μM)	Cpd. No.	EC_{50} ± SD (μM)
CABS13 (**5**)	>120	15	5.2 ± 0.5
6	43.5 ± 4.4	16	3.7 ± 0.4
7	21.5 ± 15.9	17	2.5 ± 2.8
8	37.5 ± 10.1	PBT2 (**18**)	2.0 ± 0.3
9	14.0 ± 3.2	23	2.4 ± 0.4
10	15.4 ± 3.4	24	2.0 ± 0.4
CQ (**11**)	1.8 ± 0.4	28	2.3 ± 0.4
12	4.9 ± 1.8	29	4.5 ± 1.3
13	3.5 ± 0.6	30	2.8 ± 0.1
14	2.3 ± 0.4		

[a]Aggregation of Zn-induced Aβ oligomers was assessed fluorometrically with bis-ANS. CQ and PBT2 were used as positive controls. EC_{50} ± SD were determined with nonlinear regression analysis (Prism 6, Graphpad). All compounds had an EC_{50} significantly lower than CABS13, $P < 0.01$.

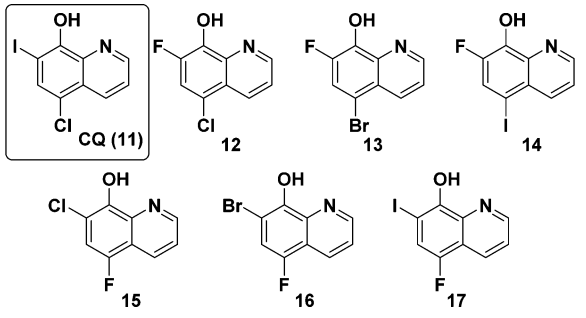
Chart 2. Structures of 8-Hydroxyquinolines and Related Compounds



with other halides, such as fluoride, chloride, or bromide, at various positions to improve the stability of the molecule due to higher C−X (X = F, Cl or Br) bond energies (Chart 3). Among six analogues screened, positional isomers **15**−**17** are of particular interest because of improved Cu and Zn ionophore activity (Figure 2).

Since PBT2 (**18**) is characterized as a monoprotic tridentate ligand, we proposed several new molecular entities by changing the nature of the coordination site or denticity to achieve better metal binding affinity and selectivity toward Cu and Zn ions. Specifically, substitution with chelating properties on the 2-position of the pyridine ring would expect to increase the

Chart 3. Structures of 8HQ Derivatives



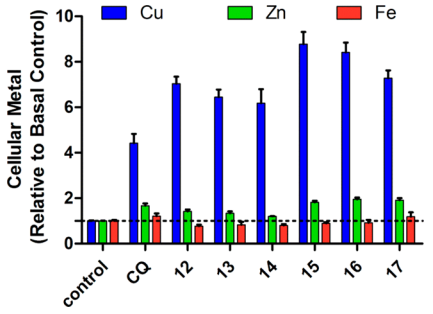
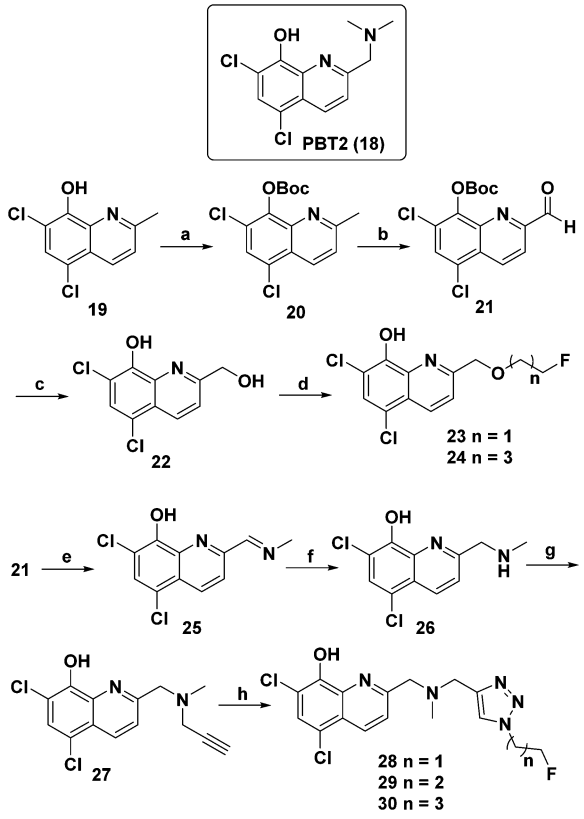


Figure 2. Ionophore activity of CQ derivatives. SH-SY5Y cells treated were treated with each compound (20 μM) for 6 h, and cellular metal levels were measured with ICP-MS. Dashed line indicates control level.

interaction with metals, i.e., Zn or Cu, in brain. The change from bidentate to tridentate, even tetradentate, could ensure enhanced ionophore ability greater than that of CQ. Two parallel approaches, namely, fluoroalkyl (compounds **23** and **24**) and fluorotriazole (compounds **28**−**30**) derivatives, were prepared. As indicated in Scheme 1, compound **20** was first protected with a Boc group, followed by SeO_2 oxidation, to yield aldehyde **21**. After $NaBH_4$ reduction and subsequent alkylation with the corresponding fluoroalkyl tosylate, compounds **23** and **24** with different aliphatic fluorine atom were achieved in 16−18% yield. Three triazole derivatives were

Scheme 1. Synthesis of PBT2 derivatives[a]



[a]Conditions: (a)Boc_2O, DMAP, THF, 90%; (b) SeO_2, 1,4-dioxane; (c) $NaBH_4$, then TFA, 80% from step b; (d) NaH, DMF, fluoroalkyl tosylate, 16% ($n = 1$), 18% ($n = 3$); (e) CH_3NH_2, THF, 93% from step b; (f) $NaBH_4$, MeOH, 58%; (g) 3-bromopropyne, DIPEA, DMF, 33%; (h) fluoroalkyl azide, $CuSO_4$, Na ascorbate, THF/H_2O, 45% ($n = 1$), 49% ($n = 2$), 39% ($n = 3$).

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accessed by a similar manner using Huisgen cycloaddition (click chemistry) to give compounds **28**−**30** in 39−49% yields. To our delight, fluorinated compound **24** exhibited similar ionophore behavior compared to PBT2 (**18**). Compounds **28**−**30** showed exceptional Cu uptake (10-fold increase for compound **28**, 16-fold increase for compound **29**, and 8-fold increase for compound **30**) greater than that of PBT2 (**18**). For Zn neuronal uptake, compounds **28**−**30** showed comparable results to those of PBT2 (**18**) (Figure 3). While none of the

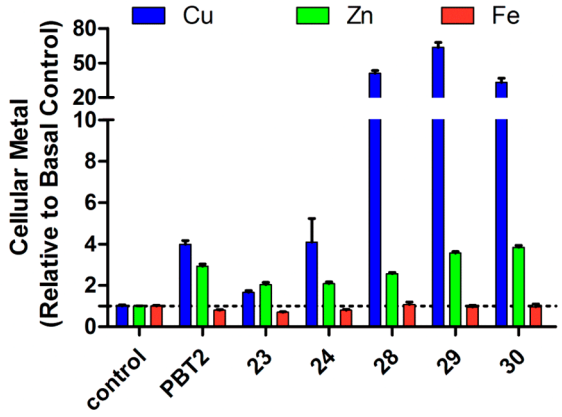

Figure 3. Ionophore activity of PBT2 derivatives. SH-SY5Y cells were treated with each compound (20 μM) for 6 h, and cellular metal levels were measured by ICP-MS. Dashed line indicates control level.

fluorinated compounds promoted Fe uptake in these assays, Fe ionophore activity cannot be excluded. Importantly, the Cu and Zn ionophore activity of several compounds was comparable to the clinically relevant 8-hydroxyquinolines CQ (**11**) and PBT2 (**18**).

Fluorinated compounds were also evaluated for their ability to reverse the aggregation of soluble Aβ oligomers *in vitro* (Table 1 and Supporting Information). From our previous PET imaging studies, [^{18}F]CABS13 detects Aβ *in vivo*,[20,25] but the present study showed that this compound does not affect Aβ aggregation *in vitro* and metal uptake. However, fluorinated 8-hydroxyquinolines (Chart 2, compounds **6**−**10**) improved anti Aβ aggregation activities compared to CABS13 (compound **5**). Three fluorinated CQ analogues (Chart 3, compounds **12**−**14**) and another set of fluoro analogues (compounds **15**−**17**) have EC_{50} values similar to CQ in the range of 2.3−5.2 μM. PBT2 derivatives **23**, **24**, and **28**−**30** (EC_{50} = 2.0−4.5 μM) are also excellent candidates. Compounds **28**−**30** may be regarded as an improvement on PBT2, as they display improved Cu ionophore activity while retaining similar Aβ disaggregation activities.

In summary, we have synthesized an array of fluorinated hydroxyquinolines based on the clinical CQ (**11**) and PBT2 (**18**) scaffolds. Several equipotent lead compounds, **15**−**17** and **28**−**30**, identified from the ionophore and Aβ reversal assays are worthy of further evaluation as potential therapeutics and/or PET ligand development to probe the metal hypothesis of AD.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00281.

Synthetic details and characterization of all new compounds and assay methods (PDF)

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Author Contributions

#These authors contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest: Dr. Bush is a shareholder in Prana Biotechnology Ltd., Cogstate Ltd., Mesoblast Ltd., Eucalyptus Ltd. and Cogstate Ltd., and a consultant for Collaborative Medicinal Development Pty Ltd.

■ ABBREVIATIONS

PET, positron emission tomography; AD, Alzheimer's disease; Aβ, amyloid-β peptide; CQ, clioquinol; PBT2, 5,7-dichloro-2-((dimethylamino)methyl) 8-quinolinol; Boc_2O, di-*tert*-butyl dicarbonate; DMAP, 4-dimethylaminopyridine; THF, tetrahydrofuran; TFA, trifluoroacetic acid; DMF, dimethylformamide; DIPEA, *N*,*N*-diisopropylethylamine

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