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Research paper

Systemic optimization and structural evaluation of quinoline derivatives as transthyretin amyloidogenesis inhibitors

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ABSTRACT

Wild type transthyretin (TTR) and mutant TTR misfold and misassemble into a variety of extracellular insoluble amyloid fibril and/or amorphous aggregate, which are associated with a variety of human amyloid diseases. To develop potent TTR amyloidogenesis inhibitors, we have designed and synthesized a focused library of quinoline derivatives by Pd-catalyzed coupling reaction and by the Horner–Wadsworth–Emmons reaction. The resulting 2-alkynylquinoline derivatives, (*E*)-2-alkenylquinoline derivatives, and (*E*)-3-alkenylquinoline derivatives were evaluated to inhibit TTR amyloidogenesis by utilizing the acid-mediated TTR fibril formation. Among these quinoline derivatives, compound **14c** exhibited the most potent *anti*-TTR fibril formation activity in the screening studies, with IC_{50} values of 1.49 μM against WT-TTR and 1.63 μM against more amyloidogenic V30 M TTR mutant. That is comparable to that of approved therapeutic drug, tafamidis, to ameliorate transthyretin-related amyloidosis. Furthermore, rationalization of the increased efficacy of compound **14c** bearing a hydrophobic substituent, such as chloride, was carried out by utilizing *in silico* docking study that could focus on the region of the thyroid hormone thyroxine (T_4) binding sites. Additionally, the most potent compound **14c** exhibited good pharmacokinetics properties. Taken together, the novel quinoline derivatives could potentially be explored as potential drug candidates to treat the human TTR amyloidosis.

1. Introduction

Human amyloid diseases such as Alzeheimer's disease, Parkinson's disease, type II diabetes, light chain amyloidosis, and transthyretin amyloidosis are characterized by aberrant protein misfolding, misassembly, and deposition in the fibrillary cross-β-sheet amyloid fibril [1–3]. Transthyretin (TTR) is one of more than 30 nonhomologous human amyloidogenic proteins and peptides [4–6]. Amyloidogenesis of wild type (WT) TTR or one of over 100 thermodynamically less stable mutants of TTR appears to elicit the proteotoxicity and cell degeneration, which cause senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), or central nervous system selective amyloidosis (CNSA). SSA is associated with WT-TTR deposition in the heart and a late onset disease that affects up to 20% of the aged population [3], whereas tissue selective deposition of one of less stable mutants of TTR and cell degeneration results in FAP (e.g., V30M-TTR), FAC (e.g., V122I-TTR), and CNSA (e.g., D18G and A25T-TTR) [5–11].

TTR is a homotetrameric protein composed of 127-amino-acid, β-sheet-rich subunits [12]. The established physiological functions of TTR are to bind to and transport the thyroid hormone thyroxine (T_4) and *holo*-retinol binding protein in the blood and cerebrospinal fluid (CSF) [5,13]. TTR has two unique dimer-dimer interface, creating two funnel-shaped thyroxine binding sites as shown in Fig. 1A [14]. Because there are two major thyroxine carriers such as albumin and thyroid-binding globulin in the blood, more than 99% of thyroxine binding sites within TTR tetramer are unoccupied [5]. TTR fibril formation requires the rate-limiting tetramer dissociation

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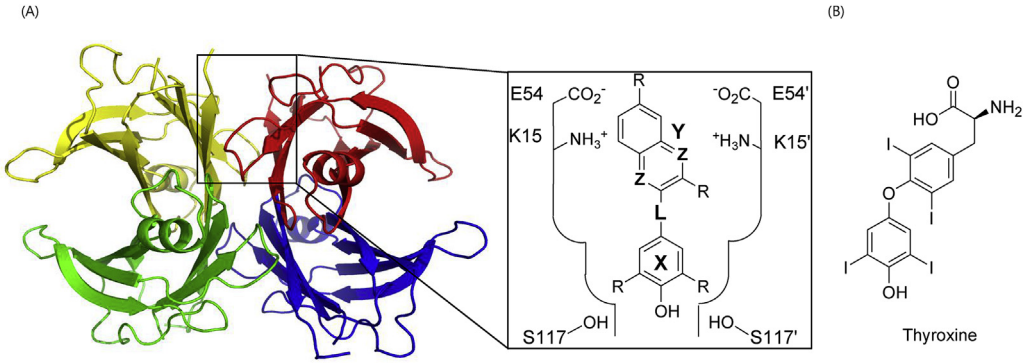

Fig. 1. (A) The X-ray crystal structure (PDB 2ROX) of WT-TTR with expanded view of one of thyroxine binding sites and proposed binding mode of small molecules. (B) Chemical structure of thyroxine.

and misassembly of partially denatured monomeric subunits into insoluble amyloid fibril and/or amorphous aggregate [5,15,16]. Reversible and irreversible small molecules that bind to one or two of thyroxine binding sites stabilize the ground state of tetrameric TTR and raise the kinetic barrier for dissociation, resulting in kinetic stabilization on TTR and preventing aggregation [5,17–37].

Oral tafamidis (Vyndaqel®) is only one approved therapeutic drug to ameliorate transthyretin-related amyloidosis. Tafamidis was initially used in Europe in 2011 for adult patients with early stage symptomatic polyneuropathy and has since been approved in Argentina, Japan, and Mexico for the treatment of TTR familial amyloid polyneuropathy (TTR-FAP). The drug selectively binds to thyroxine binding sites within TTR tetramer with negative cooperativity and kinetically stabilize the WT-TTR and the variant TTR tetramer through prevention of TTR dissociation, the rate-limiting step of TTR amyloid fibril formation [38–40]. In this study, numerous structure-activity relationship studies were carried out to identify TTR amyloidogenesis inhibitors with desirable pharmacological properties: aromatic X ring bearing polar substituents for hydrogen bond interaction with the Ser-117 or Thr-119 hydroxyl groups and van der Waals interaction in the inner binding cavity, hydrophobic linker L, and aromatic Y ring bearing polar substituents for electrostatic and/or hydrogen bond interactions with the Lys 15 and/or the Glu-54 in the outer binding cavity (Fig. 1B). On the basis of previous structural data [17,18,22–30,32,34,36], we chose focused substituents on the two aromatic rings (X and Y) and two kinds of linker to design potent WT- and V30M-TTR amyloidogenesis inhibitors and compared their potency to that of tafamidis. We investigated the molecular mechanism for the *anti*-TTR fibril formation activity of quinoline derivatives by performing *in silico* docking studies and reported the PK evaluations of two potent inhibitors *in vivo*.

2. Results and discussion

2.1. Chemistry

The general procedure for the synthesis of 2-alkynylquinoline derivatives **7a-i** was outlined in Scheme 1. Intermediates **2a**-**e**, obtained by the protection of hydroxyl group with MEM group or TIPS group, were treated with TMS-acetylene under Pd/Cu catalyzed Sonogashira coupling conditions to afford TMS-protected alkynes **3a**-**e** in 40%–94% yield. TMS-deprotected alkynes **4a**-**e**, which were prepared from deprotection of TMS group with K_2CO_3 in MeOH, were coupled with 2-chloroquinoline derivatives **5a-d** via a second Pd/Cu catalyzed Sonogashira coupling reactions. The final 2-alkynylquinoline derivatives **7a-h** were conveniently obtained by the deprotection of MEM or TIPS groups and 2-alkynylquinoline derivatives **7h** was subjected to reduction with Sn powder in AcOH and HCl to give 2-alkynylquinoline derivatives **7i**.

The Heck coupling reaction of 2-chloroquinoline derivatives **5a** and **5d** and terminal alkene **8** [34] in the presence of $Pd_2(dba)_3$, a variety of ligands (P(*o*-Tol)$_3$, PPh_3, *t*ButylXPhos), and a variety of bases (Et_3N, K_2CO_3, Cy_2NMe) was utilized as shown in Scheme 2. Among them, *N,N*-Dicyclohexylmethylamine (Cy_2NMe) and *t*ButylXPhos were found to be very effective additives to give (*E*)-2-alkenylquinoline derivatives **9a** and **9b**. Deprotection of MEM groups of **9a** and **9b** using concentrated HCl afforded the corresponding (*E*)-2-alkenylquinoline derivatives **10a** and **10b** and subsequently, the nitro compound **10b** was treated with $SnCl_2 \cdot 2H_2O$ in EtOH to produce the amine compound **10c**.

Finally, the (*E*)-3-alkenylquinoline derivatives **14a** and **14b** were prepared by the Horner–Wadsworth–Emmons reactions between substituted 2-chloroquinoline-carbaldehydes **11a** and **11b** and diethyl phosphonate **12** [37] followed by the deprotection of MEM groups using concentrated HCl as shown in Scheme 3. Subsequently, the demethylation of **14b** with BBr_3 provided (*E*)-3-alkenylquinoline derivatives **14c**.

2.2. Biological evaluation

2.2.1. Inhibition of WT- and V30M-TTR amyloidogenesis by quinoline derivatives

The newly synthesized quinoline derivatives are evaluated their ability to inhibit TTR amyloidogenesis using the previously established acid-mediated TTR fibril formation assay [41]. Briefly, a candidate inhibitor (3.6 or 7.2 μM) was preincubated with a physiological concentration of WT-TTR or V30M-TTR (each 3.6 μM) and amyloidogenesis was triggered by adjusting the pH to 4.4. The turbidity of the sample solution was measured after 72 h incubation at 37 °C to evaluate their ability to prevent WT-TTR or V30M-TTR amyloidogenesis. The potency of candidate inhibitors was expressed as the percentage of TTR fibril formation, compared to TTR fibril formation in the absence of inhibitor, as shown in Tables 1–3.

The first study was focused on the effect of a variety of hydrophobic substituents flanked by 4-hydroxyl group on an aryl-X ring. It is clear that the hydrophobic group such as methyl group play a critical role in enhancing binding potency (**7a** vs **7b**). However, the decrease in potency of compounds **7c**-**e** with bigger hydrophobic groups over compound **7b** suggests that the small hydrophobic group is essential for WT-TTR binding, due to spatial limitation in the inner binding cavity. We then shift our attention to the effect of substituents on a quinoline-Y ring, while holding the 3,5-dimethyl-