Potential pharmacological chaperones targeting cancer-associated MCL-1 and Parkinson disease-associated α-synuclein

Miseok Oh\textsuperscript{a,b,1}, Ji Hoon Lee\textsuperscript{b,1,2}, Wei Wang\textsuperscript{a}, Hui Sun Lee\textsuperscript{c}, Woo Sirl Lee\textsuperscript{b}, Christopher Burlak\textsuperscript{d}, Wonpil Im\textsuperscript{c}, Quyen Q. Hoang\textsuperscript{a,b,3}, and Hyun-Suk Lim\textsuperscript{a,b,3}

\textsuperscript{a}Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202; \textsuperscript{b}Department of Chemistry, Pohang University of Science and Technology, Pohang 790-784, South Korea; \textsuperscript{c}Department of Molecular Biosciences and Centre for Bioinformatics, The University of Kansas, Lawrence, KS 66604; and \textsuperscript{d}Department of Surgery, Indiana University School of Medicine, Indianapolis, IN 46202

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Pharmacological chaperones are small molecules that bind to proteins and stabilize them against thermal denaturation or proteolytic degradation, as well as assist or prevent certain protein–protein assemblies. These activities are being exploited for the development of treatments for diseases caused by protein instability and/or aberrant protein–protein interactions, such as those found in certain forms of cancers and neurodegenerative diseases. However, designing or discovering pharmacological chaperones for specific targets is challenging because of the relatively featureless protein target surfaces, the lack of suitable chemical libraries, and the shortage of efficient high-throughput screening methods. In this study, we attempted to address all these challenges by synthesizing a diverse library of small molecules that mimic protein α-helical secondary structures commonly found in protein–protein interaction surfaces. This was accompanied by establishing a facile “on-bead” high-throughput screening method that allows for rapid and efficient discovery of potential pharmacological chaperones and for identifying novel chaperones inhibitors against a cancer-associated protein, myeloid cell leukemia 1 (MCL-1), and a Parkinson disease-associated protein, α-synuclein. Our data suggest that the compounds and methods described here will be useful tools for the development of pharmaceuticals for complex-disease targets that are traditionally deemed “undruggable.”

Results and Discussion

Design of a Triazine-Piperazine-Triazine Scaffold as α-Helix Mimetics. α-Helices are involved in mediating many PPIs in which short α-helical peptide segments spanning two to three helical turns play a key role as a recognition motif, and side chains at $i$, $i+3$ or $i+4$, and $i+7$ are often crucial for PPIs (Fig. 1A). Because α-helix-mediated PPIs are involved in various cellular signaling pathways, there have been tremendous efforts to develop molecules that can mimic helical peptide structures to modulate such interactions (18, 22). These include stabilized helical peptides (15–17, 23) and peptidomimetic foldamers (24–29). Alternatively, nonpeptidic, small-molecule α-helix mimetics have attracted significant interest. In general, small molecules are more

Significance

Many complex diseases are caused by aberrant protein–protein interactions or protein misfolding, which present immense challenges for drug discovery. Here, we present the design, synthesis, and usage of a diverse library of potential pharmacological chaperones that could interfere with undesirable protein–protein interactions and prevent aggregation of misfolded proteins.


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1M.O. and J.H.L. contributed equally to this work.
2Present address: New Drug Development Center, Daegu Gyeongbuk Medical Innovation Foundation, Daegu 701-310, South Korea.
3To whom correspondence may be addressed. Email: hslim@postech.ac.kr or qhoang@iu.edu.

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preferable over peptides for their cell permeability, proteolytic resistance, and bioavailability. Hamilton and colleagues pioneered the development of nonpeptidic α-helix mimetics. They demonstrated the proof of concept that appropriately functionalyzed terphenyl 1 can physically mimic α-helical structures and functionally active as inhibitors of α-helix-mediated PPIs (19). This pioneering work inspired considerable efforts devoted to developing small-molecule α-helix mimetics (18, 19). However, the majority of these efforts were stymied by the relatively poor water solubility and synthetic difficulty of α-helix mimetic molecules. To overcome this critical barrier, we set out to develop α-helix mimetic scaffolds with greater synthetic accessibility that yield products with favorable physicochemical properties.

To obtain such molecules, we designed a triazine-piperazine-triazine scaffold 2 (Fig. 1C). This scaffold is composed of a piperazine ring between two triazine rings. Piperazines are found in many biologically active molecules and can be a good replacement of a phenyl ring in terphenyl structures (30–32). Triazine is a widely used privileged scaffold because of its broad range of biological activities and ease of synthetic manipulation (33, 34). We used this scaffold as the backbone and functionalized its R1, R2, and R3 groups to form α-helix mimetics. To determine the physical resemblance of peptide α-helices, we used in silico molecular dynamics simulation to generate and energy-minimize potential rotamers of the scaffold with arbitrary functional groups. This computational study predicted that spatial arrangements of the three functional groups (R1, R2, and R3) precisely matched the locations of side chains (i, i + 3 or i + 4, and i + 7) on peptide α-helices (Fig. 1D), suggesting our scaffold could mimic α-helical structures with appropriate placement of side chains.

To gain insight into the properties of our scaffold in solution, we determined its aqueous partition coefficient. The calculated log Poctanol/water (Clog P) value for triazine-piperazine-triazine scaffold 2a is 1.36, which is within the desirable range of hydrophilicity. This is a significant improvement over the current terphenyl scaffold 1a, the Clog P of which is 6.21 (Fig. 1E), suggesting the improved aqueous solubility of the triazine-piperazine-triazine scaffold has more “drug-like” properties.

Moreover, in addition to three variable locations for side chain mimetic functional groups (R1, R2, and R3), scaffold 2 also contains two diversification sites (R4 and R5) for modulating its overall hydrophilicity (Fig. 1C). Most biologically active helical peptides are amphipathic, with one side containing hydrophobic residues (usually at protein-contacting areas) and with hydrophilic residues on the other side, which presumably improves solubility. Such helical peptides bearing solvent-exposed hydrophilic surfaces are energetically favorable when binding to a target protein. In addition, charged residues on amphipathic helical peptides often participate in hydrophilic interactions with charged residues on the protein surface, resulting in an additional contribution to binding affinity and specificity (35). Surprisingly, among many nonpeptidic small-molecule α-helix mimetics, there are only a few amphipathic α-helix mimetic scaffolds (31, 36–38). Given the two additional sites (R4 and R5) on the opposite side, our scaffold 2 can effectively mimic amphipathic α-helix structures by introducing various hydrophilic functional groups at R4 and R5, thereby potentially leading to more potent and selective ligands.

**Solid-Phase Synthesis.** We have developed a concise, divergent solid-phase synthetic route to prepare the triazine-piperazine-triazine scaffold (SI Appendix, Scheme S1). First, monosubstituted dichlorotriazines 3 were loaded onto Rink-Amide resin. 2-Nitro-benzensulfonyl-protected piperazine derivatives 5 were coupled to the resin-bound triazine derivatives 4. After removing the N-nitrobenzenesulfonyl protecting group on 6, 2-ethylamino-4,6-dichloro-[1,3,5]triazine 7 was introduced to afford 8. The chloride was displaced with various amines (R2,NH). Finally, cleavage reaction with trifluoroacetic acid furnished trifunctionalized compounds 9.

To assess the feasibility of the solid-phase synthesis, a series of compounds was prepared, using various building blocks. The purity and identity of the released crude products were analyzed by liquid chromatography/MS (SI Appendix, Figs. S1 and S2). The average purity of the final products was >92%, demonstrating the efficiency of our solid-phase synthesis. Compared with convergent, lengthy solution-phase synthesis of most terphenyl-based structures, our solid-phase synthetic route is simple and divergent, allowing for the rapid creation of large numbers of diversely functionalized compounds by using many readily available building blocks.

**Peptoid-Encoded One-Bead-One-Compound Combinatorial Library.** Although many different HTS methods are available for traditional drug targets such as enzymes, there are limited numbers of screening methods for detecting PPI regulators (20, 21). Indeed, there are only a few reports on the use of HTS to identify α-helix mimetic small molecules (39–41). The most frequently used method to identify α-helix mimetics is a competitive binding assay, using a fluorescence polarization (FP). This assay is designed to find compounds that disrupt a pairwise interaction between a target protein and a fluorescently labeled helical peptide. However, such binding peptides do not always exist, and identifying them is another challenge (especially when high-resolution structural information for protein complexes is not available). More importantly, this type of competitive assay may not be suitable for detecting PPI stabilizers. Hence, development of convenient HTS methods is of considerable interest for facilitating the identification of pharmacological chaperones.

Using our divergent solid-phase synthesis protocol, a large, combinatorial library is readily accessible. For the rapid and efficient selection of best compounds among a library of α-helix mimetics, we developed a convenient HTS method by taking advantage of a bead-based screening platform, which was originally developed by Lam and colleagues for screening peptides (42) and has been successfully applied to cyclic peptides and peptidomimetics such as β-peptides and peptoids (29, 43–47). Relative to conventional HTS assays, on-bead screening has several advantages (48). This method enables a large number of library molecules (literally more than millions) to be screened simultaneously by incubating with a target protein in a single test tube. In addition, bead-based screening does not require any prior knowledge on structures of target proteins and binding partners (either proteins or peptides). Importantly, many protein surfaces often alter their conformation on binding with partner proteins or ligands, creating new binding pockets (49). Such dynamic pockets, induced by the adaptive nature of protein
surfaces, are difficult to predict. In this context, unbiased approaches through on-bead screening of a large one-bead one-compound (OBOC) library could be a powerful tool for finding compounds that induce and bind to such unpredictable binding pockets on protein interfaces.

Inspired by Lam’s peptide-based encoding method (50), we constructed a peptoid-encoded OBOC combinatorial library of α-helix mimetics on topologically segregated bilayer beads (Fig. 2). Peptoids would offer several attractive features as coding materials (44, 51): Peptoids can be efficiently synthesized under very mild conditions (52–54), which would be compatible with various reaction conditions used for synthesis of library molecules, and in addition, a vast number of structurally diverse primary amines, as the diversity-generating element in peptoid synthesis, are readily available from commercial sources.

A peptoid-encoded combinatorial library was synthesized on bifunctional beads, in which triazine-piperazine-triazines are displayed on the outer layer of the beads, and peptoids as coding tags are separately incorporated in the interior of the beads (SI Appendix, Figs. S3 and S4). Therefore, only α-helix mimetics on the bead surface can be exposed to a target protein during on-bead screening, and the structure of hit compounds will be revealed by sequencing the corresponding peptoids (Fig. 2). The diversity of this OBOC combinatorial library was $18 \times 3 \times 27 = 1,458$ (SI Appendix, Figs. S3 and S4).

**High-Throughput Screen Against MCL-1.** To demonstrate the efficacy of our peptoid-encoded OBOC library of α-helix mimetics, MCL-1 was chosen as a target protein. MCL-1 is a member of the antiapoptotic B-cell lymphoma 2 (BCL-2) family of proteins that includes BCL-2, BCL-X$_L$, A1, and BCL-w. BCL-2 proteins interact with BCL-2 homology domain 3 (BH3) protein family members such as BAK and block their apoptosis-inducing activity, thereby promoting cell survival. Given the critical roles of BCL-2 in cancer progression, inhibition of the PPIs between BCL-2 prosurvival proteins and BH3 proapoptotic proteins is emerging as an attractive strategy for cancer treatment (55).

Many efforts have been made to develop BH3 mimetics to disrupt the BCL-2/BH3 interactions, which are mediated by binding an α-helical peptide segment in BH3 into the hydrophobic cleft of BCL-2 (55). One of the most potent BCL-2 inhibitors is ABT-737 (56). Interestingly, ABT-737 binds to BCL-2 and BCL-X$_L$ with high affinity, but not to MCL-1. Not surprisingly, ABT-737 is not effective in cancer cells overexpressing MCL-1 (57). Given that overexpression of MCL-1 is observed in many cancers and is associated with cancer progression and drug resistance, it is generally accepted that inhibition of MCL-1, as well as BCL-2, is a promising strategy for effective cancer treatment (58).

MCL-1 Binding Assays. To validate the binding ability of the hit compounds to MCL-1ΔNAC, FP experiments were executed. 9a-FL, 9b-FL, and 9c-FL bound MCL-1ΔNAC with $K_D$ values of 2.8, 3.0, and 1.1 μM, respectively. The binding affinity of a known BH3 peptide (58) used as a positive control was 0.3 μM, which is similar to the reported binding affinity ($K_D = 245$ nM) (Fig. 3B). To verify whether the fluorescein and linker conjugated to 9a–9c have any effects on the binding between the compounds and MCL-1ΔNAC, we synthesized fluorescein-linker moiety, Fluor-linker (SI Appendix, Figs. S7 and S9), and used it as a negative control. As shown in Fig. 3B, Fluor-linker did not exhibit any binding to MCL-1ΔNAC, showing that the fluorophore and the linker have no contribution to the binding affinity of 9a–9c to MCL-1. We then performed a competitive FP assay using nonlabeled compounds 9a–9c to determine whether they are able to antagonize the interaction between MCL-1 and BH3 (Fig. 3C). As expected, 9b and 9c dissociated the MCL-1ΔNAC/BH3 interaction with $K_i$ values of 9.3 and 17.5 μM, respectively, whereas 9a had weak activity. 9a-nc, a negative control compound, did not bind to MCL-1ΔNAC (Fig. 3C). We chose 9c for more detailed investigation because it has the highest binding affinity among the three hits. To determine whether 9c has selectivity for MCL-1 over other structurally similar proteins, we used competitive FP assays to measure the binding affinity of 9c against BCL-X$_L$ΔC (amino acids 1–212), which is a deletion construct of human BCL-X$_L$, another BCL-2 protein member, with the BH3 binding pocket similar to MCL-1. We found that 9c did not significantly inhibit the interaction between BCL-X$_L$ΔC and a BH3 peptide (SI Appendix, Fig. S10), indicating that 9c is selective for MCL-1. To understand how 9c might bind to MCL-1ΔNAC, we computationally docked 9c into the binding site for BH3. The three functional groups at R$_1$, R$_2$, and R$_3$ on the...
To examine whether the hit compounds were found to bind to MCL-1. (Fig. 3. A) Structures of hit compounds 9a–9c and a negative control 9-nc. (B) Binding curves for 9a–9c binding to MCL-1. (C) Inhibition curves of 9a–9c for fluorescein-labeled BH-3 peptide binding to MCL-1. Error bars represent SD from three independent experiments.

**MCL-1 Cellular Assays.** To examine whether 9c is cell-permeable and can disrupt native interactions between MCL-1 and proapoptotic proteins inside cells, we performed coimmunoprecipitation experiments. Jurkat T cells were treated with DMSO as a carrier control or increasing concentrations of 9c, and cell lysates were immunoprecipitated with anti-MCL-1 antibody and subjected to Western blot, using anti-BAK and anti-MCL-1 antibodies (Fig. 4A). The BAK Western blot analysis showed that immunoprecipitated BAK was dose-dependently decreased in 9c-treated cells. In contrast, 9-nc, a negative control, did not inhibit the MCL-1/BAK interaction (SI Appendix, Fig. S12). This result ensures that 9c is cell permeable and competitively binds to MCL-1, liberating BAK protein. Cellular uptake of 9c was confirmed using confocal microscopy (Fig. 4B).

Blocking the MCL-1/BH3 interaction by 9c would liberate proapoptotic proteins such as BAK from MCL-1, resulting in apoptosis induction and cell-growth inhibition. To test this, Jurkat human leukemia T cells and human multiple myeloma U266 cells were treated with DMSO or varying concentrations of 9c and 9-nc, and cell viability was evaluated by MTS assays. Indeed, 9c killed Jurkat T cells and U266 cells in a dose-dependent manner, but not normal cells such as WS-1 human fibroblast cells and human embryonic kidney (HEK) 293 cells (Fig. 4C and SI Appendix, Fig. S13A), indicating that selective toxicity to malignant cells with MCL-1 overexpression over normal cells. 9-nc, a negative control, had no significant effect on both cancerous cells and normal cells. To validate whether the selective cytotoxicity of 9c is a result of caspase-dependent apoptosis, we conducted apoptosis assays. U266 cells and WS-1 cells were exposed to DMSO or various concentrations of 9c, and caspase activity was monitored by Caspase-Glo 3/7 assay kit. As expected, 9c induced caspase activation in U266 cells, but not in WS-1 cells (SI Appendix, Fig. S13C).

As a consequence, we have successfully demonstrated that a cell-permeable PPI inhibitor can be identified through on-bead screening of a combinatorial library of α-helix mimetics. 9c can be a starting point for development of potent inhibitors targeting MCL-1. Further biological evaluation and structure–activity relationship studies are currently in progress to obtain more potent and selective MCL-1 inhibitors.

**α-Synuclein Screening.** To determine whether our compounds are effective pharmacological chaperones in stabilizing protein folding and/or preventing protein aggregation, we used α-synuclein as a representative of protein-folding-related diseases. α-Synuclein aggregation leading to the formation of Lewy bodies is the pathological hallmark of Parkinson disease (60). The mechanism of Lewy body formation is still unclear. However, various lines of evidence indicate that misfolding of α-synuclein and subsequent inappropriate self-association are key steps in Lewy body formation (61). α-Synuclein was traditionally thought to be a natively unfolded protein (62); however, recent evidence by our group and others suggest it also exists as folded tetramers (63, 64), which implies that destabilization of the tetramer might lead to more aggregation-prone species. These findings opened venues for testing new therapeutic approaches for this devastating disease for which there is no cure and for which there are no effective treatments. Here, we use our α-helix-mimetic compounds to test the approach of preventing α-synuclein aggregation by stabilizing its folded structure and/or preventing aberrant self-association with pharmacological chaperones. This is an ideal test case for our chemical library because α-synuclein consists mostly of α-helices (~65%), and its aggregation could be monitored in real-time (63, 64).

For the screening assay, we engineered a construct of α-synuclein consisting of an N-terminal extension MKCK quad-peptide to introduce a reactive cysteine residue for chemical linking with biotin (wild-type α-synuclein contains no cysteine). The cysteine-containing α-synuclein (cys-Syn) was biotinylated (SI Appendix, Fig. S14) and used for screening, as described earlier for MCL-1. On-bead screening identified two hit compounds, 9Q1 and 9Q2, as potential α-synuclein binders (Fig. 5A). We then synthesized and purified these compounds for further experimentation (SI Appendix, Fig. S9).

**α-Synuclein Binding Assay.** To confirm the binding of the hit compounds, we used FP assay as described earlier for MCL-1. Q1 and Q2 were found to bind to α-synuclein with K9 values of 68 and 148 nM, respectively (Fig. 5B). Q1 and Q2 share two identical side chains at R1 and R2 and similar features at R3, R4, R5, and R6.

**Cellular activity of 9c.** (A) Coimmunoprecipitation assay. Jurkat T cells were treated with DMSO or varying concentrations of 9c for 2.5 h. The effect of 9c on the MCL-1/BH3 interaction was analyzed by MCL-1 immunoprecipitation and BAK western analysis. The results are representative of three independent experiments. (B) Confocal microscopic images of A549 cells treated with 1 μM 9c-FL or 5 (6)-carboxyfluorescein (Flu), for 4 h. (C) Effect of 9c and 9-nc (a negative control) on cell viability of Jurkat T leukemia cells (Left) and WS-1 normal fibroblast cells (Right).
and their α values Q2 On the basis of the confirmed shift the melting temperature of 9a as a negative control for structural (Q2 Q2 to probably also bind to A53T, we affects the aggregation also showed similar binding patterns as those observed with compounds, to showed very weak binding, and Q2 11011 α Q2 HTS. The proof-of-concept screens against a cancer-associated protein MCL-1 and a Parkinson disease-associated protein α-synuclein demonstrated that that triazine-piperazine-triazine-based α-helix mimetics are effective at binding and modulating the activity and behavior of distinctly different helical proteins. MCL-1 is an established canonical helical protein that interacts with other helical structures, suggesting that the hit compounds that we identified likely mimicked certain features of MCL-1’s natural binding partners. α-Synuclein, in contrast, has no known structures; its interacting partners are unknown (aside from self-associating), and aggregation mechanism is still a mystery. What we do know is that when partially folded, it consists of dynamic helical structures, thus demonstrating that our method is capable of obtaining hit compounds even without prior knowledge of the structure of the target protein or its binding partners. Given the convenience of the screening method and structural diversity of the library, we believe our strategy could be a useful approach to developing pharmacological chaperones against a wide range of different helical proteins.

Conclusions

Despite their great potential as therapeutic candidates for the treatment of many diseases caused by protein instability and/or aberrant protein–protein interactions, discovering pharmacological chaperones for specific target proteins is among the most difficult challenges in drug discovery and chemical biology. Here, we have presented an approach that allows for large-scale synthesis of a peptoid-encoded OBOC combinatorial library of α-helix mimetic small molecules and rapid identification of potential pharmacological chaperones through a facile “on-bead” HTS. The proof-of-concept screens against a cancer-associated protein MCL-1 and a Parkinson disease-associated protein α-synuclein demonstrated that that triazine-piperazine-triazine-based α-helix mimetics are effective at binding and modulating the activity and behavior of distinctly different helical proteins. MCL-1 is an established canonical helical protein that interacts with other helical structures, suggesting that the hit compounds that we identified likely mimicked certain features of MCL-1’s natural binding partners. α-Synuclein, in contrast, has no known structures; its interacting partners are unknown (aside from self-associating), and aggregation mechanism is still a mystery. What we do know is that when partially folded, it consists of dynamic helical structures, thus demonstrating that our method is capable of obtaining hit compounds even without prior knowledge of the structure of the target protein or its binding partners. Given the convenience of the screening method and structural diversity of the library, we believe our strategy could be a useful approach to developing pharmacological chaperones against a wide range of different helical proteins.

Fig. 5. (A) Structure of Q1 and Q2, hit compounds to α-synuclein. (B) FP assays. Binding affinity of hit compounds Q1, Q2, negative controls 9a–9c, and Fluor-linker against wild-type α-synuclein were determined using FP assay. (C) Circular dichroism thermal denaturation assay. Unfolding of α-synuclein in the presence or the absence of hit compounds Q1 and Q2 were monitored using ellipticity at 222 nm, with respect to temperature.

with both consisting of 1,2-dialkoxylanobenzene (Fig. S4). To investigate the contributions of compound backbone and roof linker to the observed binding of Q1 and Q2 to α-synuclein, we measured the binding of MCL-1 hit compounds 9a–9c, which have the same backbone structure as Q1–Q2, and Fluor-linker. As shown in Fig. 5B, 9a and 9b showed very weak binding, and Fluor-linker did not show detectable binding. Compound 9c, which contains two identical side chains as the Q compounds, showed moderate affinity to α-synuclein, as anticipated. Conversely, we also examined whether α-synuclein-interacting compounds bind to MCL-1. Consistent with the structural features, Q1, which has similar structure to 9c, moderately inhibited MCL-1/BiH3 interactions, whereas Q2 showed little effect (SI Appendix, Fig. S15). In the subsequent cell viability assay, both Q1 and Q2 had no significant effect on the viability of both cancerous leukemia cells and normal cells (SI Appendix, Fig. S15). Taken together, these results indicate that the binding and selectivity of the compounds for their target proteins are conferred by the compounds’ side chains. To further confirm the binding of Q compounds and their effects on α-synuclein thermal stability, we used circular dichroism thermal stability assay. As shown in Fig. 5C and SI Appendix, Fig. S16, Q1 and Q2 shifted the melting temperature of α-synuclein from 37 °C to 40 °C and 41 °C, respectively. The α-helices on α-synuclein are amphiphilic, with long stretches of hydrophobic surfaces (64, 65); the relatively hydrophobic Q1 and Q2 probably interact with the hydrophobic surfaces on α-synuclein. How the binding of these compounds affects the structure of α-synuclein warrants further investigation; however, our objectives here are to test whether our library consists of molecules that could bind and inhibit α-synuclein aggregation, which is detailed here.

α-Synuclein Aggregation Assay. On the basis of the confirmed binding affinity of Q1 and Q2 to α-synuclein, we set out to determine whether the binding of Q1 and Q2 affects the aggregation propensity of α-synuclein. As mentioned earlier, we initially screened for Q compounds using wild-type α-synuclein; however, the wild-type does not aggregate readily, so we used an aggregation-prone, disease-associated mutant (A53T) to perform aggregation assays. To ascertain that Q1 and Q2 also bind to A53T, we repeated the FP binding assay using A53T protein and we found that Q1 and Q2 also bind strongly to the A53T mutant (Kd values = 268 and 372 nM, respectively), albeit at slightly lower affinity compared with wild-type α-synuclein (Fig. 6A). Compounds 9a–9c also showed similar binding patterns as those observed with wild-type α-synuclein. We selected 9a as a negative control for aggregation assays because it showed no binding to α-synuclein.

We preincubated Q1, Q2, 9a, and DMSO with A53T mutant α-synuclein and monitored its aggregation using Thioflavin-T fluorescence aggregation assay, as previously described (64). We found that both Q1 and Q2 significantly delayed the onset of aggregation and dramatically decreased the total amounts of aggregated fibrils within the 30-h duration of our experiment (Fig. 6B), whereas DMSO and 9a did not inhibit aggregation of A53T mutant α-synuclein.

Although the mechanisms by which the Q-compounds prevent α-synuclein aggregation are still unclear, it is consistent with the idea that the binding of Q1 and Q2 to α-synuclein prevented it from aggregating, either by masking the surfaces that directly involve in aggregation or by preventing its conversion/misfolding into aggregation prone species. In any case, our data demonstrate that our chemical library consists of molecules that effectively inhibited α-synuclein fibrillation and suggests these compounds might be useful also for other proteins associated with protein-misfolding and aggregation-prone related diseases.

Fig. 6. (A) FP assays. Binding affinity of Q1, Q2, 9a–9c, and Fluor-linker against the disease-associated mutant of α-synuclein A53T were measured using FP assay. (B) Thioflavin-T aggregation assays. Effects of Q1, Q2, and negative-control 9a on aggregation of α-synuclein A53T were determined by using Thioflavin-T fluorescence aggregation assay.
Methods

Synthesis and characterization of 9a–9c and Q1–Q2 can be found in SI Appendix. Protocols for OBOC library synthesis and on-bead screening are detailed in SI Appendix. Methods of molecular docking, protein purification, binding assays, cell viability assays, caspase assays, confocal microscopy experiment, coimmunoprecipitation assays, circular dichroism thermal stability assay, and thiolflavin-T aggregation assay are described in SI Appendix.


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