REVIEW

Potential synergy between tau aggregation inhibitors and tau chaperone modulators

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Abstract

Tau is a soluble, microtubule-associated protein known to aberrantly form amyloid-positive aggregates. This pathology is characteristic for more than 15 neuropathies, the most common of which is Alzheimer's disease. Finding therapeutics to reverse or remove this non-native tau state is of great interest; however, at this time only one drug is entering phase III clinical trials for treating tauopathies. Generally, tau manipulation by therapeutics can either directly or indirectly alter tau aggregation and stability. Drugs that bind and change the conformation of tau itself are largely classified as aggregation inhibitors, while drugs that alter the activity of a tau-effector protein fall into several categories, such as kinase inhibitors, microtubule stabilizers, or chaperone modulators. Chaperone inhibitors that have proven effective in tau models include heat shock protein 90 inhibitors, heat shock protein 70 inhibitors and activators, as well as inducers of heat shock proteins. While many of these compounds can alter tau levels and/or aggregation states, it is possible that combining these approaches may produce the most optimal outcome. However, because many of these compounds have multiple off-target effects or poor blood–brain barrier permeability, the development of this synergistic therapeutic strategy presents significant challenges. This review will summarize many of the drugs that have been identified to alter tau biology, with special focus on therapeutics that prevent tau aggregation and regulate chaperone-mediated clearance of tau.

Review

Therapeutic targeting of tau triage

Tauopathies, a class of neurodegenerative diseases including Alzheimer's disease, frontotemporal dementia, and progressive supranuclear palsy, are characterized by the pathological aggregation of hyperphosphorylated tau tangles in the human brain [1]. Because aberrant protein accumulation is a hallmark of many neurological diseases, and tau is one of many proteins that form disease-associated aggregates, this can present a new challenge for finding an aggregation inhibitor specific for tau.

Studies have shown that several molecular chaperone families, known as heat shock proteins (Hsps), are involved with preventing tau aggregation [2,3] or assisting in tau degradation [4]. These families, named for their general protein size in kiloDaltons, include Hsp70 and Hsp90, the smaller Hsp40, and small Hsps. Recently, a number of small molecule inhibitors have been developed and studied for their roles in regulating the ATPase activities of Hsp70 and Hsp90. In addition, much of the drug discovery efforts directed at tau are aimed at disrupting its aggregation; several aggregation inhibitors have been identified and their potential efficacy has been shown using model systems. This review will discuss drugs that have been developed to modulate the chaperone repertoire, as well as recent advances in therapeutics affecting tau aggregation. Table 1 summarizes all of the drugs discussed in this review. We speculate that these compounds could be synergistic, such that aggregation disruption followed by tau clearance could be more beneficial than either effect alone. By creating more soluble tau through inhibiting its aggregation, chaperones have a greater opportunity to bind to tau. This chaperone-bound tau can then be targeted for degradation.

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Table 1 List of drugs

Tau aggregation inhibitors	Family	Mechanism	Toxicity	BBB permeability	Other notes
AQ2S	Anthraquinone	β-sheet inhibitor	Nontoxic	Likely	Laxative
Emodin	Anthraquinone	β-sheet inhibitor	Nontoxic	Likely	Laxative
Daunorubicin	Anthraquinone	β-sheet inhibitor	Nontoxic	Likely	Laxative
Mitoxantrone	Anthraquinone	β-sheet inhibitor	Nontoxic	Likely	Laxative
Pixantrone	Anthraquinone	β-sheet inhibitor	Nontoxic	Likely	Laxative
C11	Carbocyanine	β-sheet inhibitor	Low toxicity	Likely	
N744	Carbocyanine	β-sheet inhibitor	Low toxicity	Unknown	
PR-619	Diaminopyridine	Hsp70 inhibitor	Some toxicity	Unknown	
17-AAG	Natural product derivative	Hsp90 inhibitor	Some toxicity	Permeable	Low bioavailability
Geldanamycin	Natural product	Hsp90 inhibitor	Highly toxic	Not permeable	Toxic
Hydroxytyrosol	Natural product	β-sheet inhibitor	Nontoxic	Permeable	
Novobiocin	Natural product	Hsp90 inhibitor	Low toxicity	Poorly permeable	
Oleuropein	Natural product	β-sheet inhibitor	Nontoxic	Not permeable	
Oleuropein aglyxone	Natural product	β-sheet inhibitor	Nontoxic	Unknown	
Radicicol	Natural product	Hsp90 inhibitor	Some toxicity	Poorly permeable	Low bioavailability
Withaferin A	Natural product	Cdc37 inhibitor	Some toxicity	Permeable	
Paclitaxel	Natural product	Microtubule stabilizer	Highly toxic	Poorly permeable	
Curcumin	Natural product; flavonoid	Hsp70 inhibitor	Nontoxic	Permeable	Low bioavailability
Myricetin	Natural product; flavonoid	Hsp70 inhibitor	Low toxicity	Permeable	
Quercetin	Natural product; flavonoid	Hsp70 inhibitor	Low toxicity	Permeable	
B1C11	N-Phenylamine	β-sheet inhibitor	Low toxicity	Unknown	
B4A1	N-Phenylamine	β-sheet inhibitor	Low toxicity	Unknown	
B4D3	N-Phenylamine	β-sheet inhibitor	Low toxicity	Unknown	
B4D5	N-Phenylamine	β-sheet inhibitor	Low toxicity	Unknown	
Methylene blue	Phenothiazine	Hsp70 inhibitor	Low toxicity	Permeable	Blue color
Quinoxalines	Phenothiazine	Hsp70 inhibitor	Selective toxicity	Permeable	Low bioavailability
BSc3094	Phenylthiazolyl-hydrazides	β-sheet inhibitor	Some toxicity	Unknown	
Epalrestat	Rhodanine	β-sheet inhibitor	Some toxicity	Permeable	Aldose reductase inhibitor
Troglitazone	Rhodanine	β-sheet inhibitor	Highly toxic	Likely	
MKT-077	Rhodocyanine	Hsp70 inhibitor	Selective toxicity	Not permeable	
YM-01	Rhodocyanine	Hsp70 inhibitor	Selective toxicity	Not permeable	
YM-08	Rhodocyanine	Hsp70 inhibitor	Selective toxicity	Permeable	Less potent than YM-01
Macrocycles	Synthetic	β-sheet inhibitor	Unknown	Unknown	

17-AAG, 17-(allylamino)-17-demethoxygeldamycin; AQ2S, anthraquinone-2-sulfonic acid; BBB, blood–brain barrier; C11, 3,3′-diethyl-9-methyl-thiacarbocyanine iodide; Cdc37, cell division control 37 kDa; Hsp, heat shock protein; MKT-077, 1-ethyl-2-((3-ethyl-5-(3-methylbenzothiazolin-2-yliden))-4-oxothiazolidin-2-ylidenemethyl) pyridinium chloride; N744, 3,3′-bis(β-hydroxyethyl)-9-ethyl-5,5′-dimethoxythiacarbocyanine iodide; PR619, 2,6-diamino-3,5-dithiocyanopyridine, thiocyanic acid C,C′-(2,6-diamino-3,5-pyridinediyl) ester; 2,6-diaminopyridine-3,5-bis(thiocyanate).

Assays and rationale for tau aggregation inhibitors

Tau aggregation has been defined using multiple techniques, but three primary assays are traditionally used. Two of these techniques, the thioflavin fluorescence stain and the Gallyas silver stain, are typically used to examine tau aggregates in tissue. These stains bind beta sheets, allowing for measurements of tau amyloidogenicity [5]. Tau filaments are also often measured using electron microscopy, both in tissue and *in vitro* [6]. Biochemically, tau aggregation is measured using sequential extractions with detergents, such as sarkosyl and sodium dodecyl sulfate [7]. Currently, there is no direct measurement that can be performed to assess tau aggregation in living organisms; however, brain imaging using single photon emission computed tomography scans is in development.

In general, efforts aimed at either preventing or reversing tau aggregation are much further advanced than those targeting chaperones. Tau contains a well-characterized,

aggregation-prone peptide sequence in the third micro-tubule binding repeat domain of exon 10+ tau. This hexapeptide motif, VQIVYK, located at amino acids 306 to 311, has been shown to exhibit the highest propensity to aggregate [8]. Drugs that directly bind near this hexapeptide region have been the most effective at preventing tau aggregation [9]. Interestingly, however this hexapeptide domain is also the region that chaperones most readily recognize [10]. This further suggests that there is some interplay between tau aggregation and its regulation by chaperones.

Heat shock proteins as therapeutic targets

Hsps are a group of molecular chaperones that assist in protein folding, transport, and degradation. These chaperones have been extensively studied for their ability to regulate aberrant intracellular proteins. Typically, misfolded tau collects in the soma and accumulates in the somatodendritic compartment [11,12]. Intracellular factors that can readily access this compartment are therapeutic options for preventing tau aggregation or degrading aberrant tau species. Molecular chaperones are naturally able to maintain a gateway that could determine the fate of a non-native protein, such as aggregated tau; however, with aging, this system begins to slow and proteins unnaturally accumulate. This age-induced blockade can be caused by many factors, including dysfunctional or decreased degradation mechanisms [13] or declining chaperone levels coupled with rising levels of misfolded proteins [14].

The Hsp70, small Hsp, and Hsp90 families are notably known to play roles in tau pathology [15,16]. Currently, therapeutics targeting Hsp90 have shown promise as tau reducing agents [17,18]; however, a common problem with Hsp90 inhibitors is that they stimulate the transcription factor heat shock factor protein 1 (HSF1). Upon stress, HSF1 activation leads to induced expression of a whole panel of other Hsps that can antagonize the effects of Hsp90 inhibition. Hsp70 inhibitors have also shown promise as anti-tau therapeutics [19], and they do not cause a heat shock induction. However, there are more Hsp70 variants in the cell, each with different functions, and these inhibitors are not selective for one over the other. This non-specific inhibition could lead to unwanted side effects. There is also a need to increase blood–brain barrier (BBB) permeability of these compounds in order to create drugs that can be delivered peripherally yet treat centrally, a process that is in development [20].

Hsp90 inhibition decreases tau

The Hsp90 chaperone is a highly conserved, ATP-requiring protein that functions as part of a large protein complex. Among others, this complex includes the co-chaperones cell division control 37 kDa (Cdc37), FK506 binding protein 51 kDa (FKBP51), and carboxyl terminus of Hsc70-interacting protein (CHIP), all of which contain tetratricopeptide repeat domains that allow Hsp90 binding and aid in directing Hsp90 function. Since the original discovery that Hsp90 inhibitors could facilitate tau clearance [17,18], many Hsp90 inhibitors have been identified as potential tau therapeutics. These inhibitors include radicicol and its multiple derivatives, geldanamycin, the geldanamycin analog 17-(allylamino)-17-demethoxygeldamycin (17-AAG), and N-(7-((2R,3R,4S,5R)-3,4-dihydroxy-5-methoxy-6,6-dimethyl-tetrahydro-2H-pyran-2-yloxy)-8-methyl-2-oxo-2H-chromen-3-yl)acetamide (KU-32). (For a comprehensive review of Hsp90 inhibitors, see [21]).

Of these inhibitors, geldanamycin has been the most studied as a tau therapeutic. An Hsp90-inhibiting antibiotic used to cause alterations in tau, geldanamycin has been shown to decrease levels of insoluble tau aggregates by 80% while leaving total tau levels unchanged [22]. Recently, a group showed that geldanamycin was able to activate the proteasome to cause tau degradation [23], and another study showed in a primary neuron model that geldanamycin treatment led to decreased tau phosphorylation by downregulating aberrant kinase activity [24]. Geldanamycin inhibits the Hsp90 homodimer by binding to its two N-terminal nucleotide binding domains [25]. However, geldanamycin proved to be quite toxic [26], prompting the development and testing of the geldanamycin analog 17-AAG.

Recent developments of Hsp90 inhibitors

Compared with its parent molecule, 17-AAG showed lower toxicity and had more potent effects at decreasing tau phosphorylation in primary neurons [24]. 17-AAG was also able to decrease tau in an *in vitro* model of tauopathy [27]. Although the analog compound was unable to alter tau phosphorylation at serines 396 and 404 or rescue a motor deficit, Sinadinos and colleagues recently showed that treating Drosophila larvae expressing human 3R tau with 17-AAG dramatically decreased total tau levels [28].

In addition to 17-AAG, radicicol is another Hsp90 inhibitor that was discovered after geldanamycin. Radicicol is a natural product that inhibits Hsp90 while inducing Hsp40 and Hsp70. Again in a Drosophila model, radicicol has been shown to dose-dependently decrease the levels of tau [28]. Analogs of radicicol, originally made for use in oncogenic research, have yet to be evaluated for their effects on tau [29].

Owing to the potentially toxic effects of N-terminal Hsp90 ATPase inhibitors, C-terminal ATPase inhibitors are now thought to be preferred. These C-terminal inhibitors are currently in development through new research on novobiocin inhibitors. Novobiocin is an

antibiotic that binds to the two C-terminal ATPase sites of the Hsp90 homodimer. Analogues of novobiocin were developed by the Blagg group to test whether C-terminal ATPase inhibition of Hsp90 would yield fewer toxic side effects. From these studies, the new lead compound KU-32 showed the greatest potential for efficacy against diseases of the central nervous system because it could cross the BBB, and caused an attenuated heat shock response compared with N-terminal inhibitors [30,31]. The effects of KU-32 on tau biology *in vivo* have not yet been evaluated, but it appears to be a promising drug candidate for tauopathies.

Because inhibition of Hsp90 in many cases activates HSF1, it is very difficult to elucidate the mechanism by which Hsp90 inhibition decreases tau levels or aggregates. Additionally, as Hsp90 is involved in many diseases and is ubiquitously expressed, striving for one specific result through global Hsp90 inhibition may lead to many off-target effects. More effort has been placed recently on developing drugs that target co-chaperones of Hsp90 for increased specificity. This development, however, is in its infancy. Withaferin A, one known inhibitor of the Hsp90 co-chaperone Cdc37, was shown to decrease aggregated tau in a mouse model, but it also led to the induction of Hsp27 and Hsp70 [28]. Other Hsp90 co-chaperones, such as FKBP51 [32] and CHIP [22], have been identified as being good targets for altering tau phosphorylation states and levels. However, compounds directed at these targets have yet to be developed.

Targeting Hsp70 with small molecules

Along with the Hsp90 family, Hsp70 has been extensively studied as a therapeutic target for modulating tau [15,22,33]. The Hsp70 family is a ubiquitously expressed group that can prevent protein aggregation through ATP hydrolyses [34]. This family includes more than 10 members. The most commonly studied are the constitutively expressed Hsp73 (heat shock cognate (Hsc)70), the stress induced Hsp72 (Hsp70), glucose-regulated protein (GRP)78 (BiP), which is expressed in the endoplasmic reticulum, and mitochondrial GRP75 (mortalin) [35]. These ~70 kDa proteins have three functional domains: an N-terminal ATPase domain, a substrate binding domain, and a C-terminal domain that functions as a lid.

Although many of the Hsp90 inhibitors discussed above can be also included in the Hsp70 modulating category because they induce Hsp70 expression, recent work from our group and others have identified Hsp70-specific modulating drugs that can potently facilitate tau clearance [20,33,36]. Both activators and inhibitors of Hsp70 ATPase function have been identified, and these compounds appear to regulate the association of Hsp70 proteins with either DnaJ/Hsp40 proteins or nucleotide

exchange factors, which in turn alters the ATPase activity of Hsp70. Currently, these compounds are derived from three drug families; the flavonoids, the phenothiazine dyes, and the rhodocyanine dyes [37,38]. Importantly, these compounds have few known side effects. Altering ATPase function has been shown to affect tau biology, so these Hsp70 ATPase modulators may be relevant targets for tauopathies.

Flavonoids as anti-tau therapeutics

Natural products research has yielded many unique, active compounds. A class of these known as flavonoids have had broad implications in many diseases. While the flavonoid myricetin possesses anti-Hsp70 activity and reduces tau levels [33], other flavonoids that lack anti-Hsp70 activity can still reduce tau levels, including curcumin [39] and quercetin [40]. Curcumin has been shown to alter tau phosphorylation in primary neurons [41]. Moreover, this yellow compound has recently been implicated to have therapeutic value in mouse models of tauopathies [42]. Just last year, curcumin was shown to elevate Hsp90 and Hsc70 without affecting HSF1, while suppressing soluble tau but not insoluble tau [43]. Moreover, curcumin is BBB permeable and showed a significant behavioral deficit rescue. Curcumin has been involved with many clinical trials and is well tolerated, but its efficacy in the treatment or prevention of tauopathies has yet to be clearly demonstrated. Quercetin has shown protective effects against amyloid-beta pathology and may have a therapeutic role for tau aggregation.

Phenothiazines: efficacy through pleiotropy

Phenothiazines have been studied for their therapeutic potential for over a century. One phenothiazine, methylene blue (MB; phenothiazine methylthionium chloride), has been involved in multiple clinical trials testing its efficacy for many diseases ranging from psychiatric disorders to cancer [44]. In addition, MB was originally identified as a potent beta-pleated sheet inhibitor [45] and more recently as an Hsp70 inhibitor [33].

Regardless of the mechanism, MB has been shown to be protective against tau accumulation in several models. Specifically, our group showed that MB given *ad libitum* in drinking water or through osmotic pump into the brain decreased tau phosphorylation and rescued learning impairment in rTg4510 mice [46]. This has since been validated by several other groups [37,47,48]. Another group showed that MB treatment on a tau *C. elegans* model alleviated tau-induced neuronal toxicity [49]. A proprietary formulation of MB has successfully passed through phase 1 and phase 2 clinical trials according to reports from TauRx Therapeutics (Singapore, Republic of Singapore) [50]. This year, the drug will go

into phase 3 trials, for which patients are currently being recruited. The study is scheduled to conclude in 2015 [51].

Although MB is progressing in the clinic and has low toxicity, there are some drawbacks of this drug. MB is blue in color and causes discoloration of the eyes and urine; occasionally, it can also induce nausea.

Another member of the phenothiazine family, quinoxalines have a structure similar to MB. These compounds have been shown to have potent activity in preventing and even reversing the aggregation of tau. However, quinoxalines, while able to permeate the BBB, were found to have very low absorption *in vivo*, making them inadequate as possible therapeutics for tau aggregation [39].

Rhodocyanines: a promising Hsp70 inhibitor scaffold

Rhodocyanine dyes have been studied for over a century for their therapeutic potential. Recent work from our group shows that one example of these dyes, 1-ethyl-2-((3-ethyl-5-(3-methylbenzothiazolin-2-yliden))-4-oxothiazolidin-2-ylidenemethyl) pyridinium chloride (MKT-077), can inhibit Hsp70 ATPase activity and facilitate tau clearance [36,52,53]. Researchers have been working to alter the chemical structure of MKT-077 to increase its potency, BBB permeability and target specificity, and to reduce its off-target effects [20,54].

A new family of MKT-077 compounds has been created, including the recently published YM compounds [20]. Two of these analogs, YM-01 and YM-08, showed anti-tau activity. Compared with the mostly mitochondrial MKT-077, YM-01 is more concentrated in the cytosol – potentially allowing it to be more accessible to cytosolic Hsp70 [54], conferring improved anti-tau efficacy. YM-08 is a further refinement of YM-01 that has reduced potency but dramatically improved BBB permeability [20].

Small heat shock proteins: no enzymatic activity, yet major functionality

Small Hsps are ATP-independent molecular chaperones with a molecular mass under 43 kDa. The small Hsps have two primary domains, one of which is an alpha crystalline C-terminal domain. These small Hsps are known to self-dimerize and oligomerize [55]. Upon a stress event, they become phosphorylated and dissociate to act upon non-native proteins [56].

Our group and others have shown that Hsp27 has relevant therapeutic potential. *In vitro*, Hsp27 was able to prevent the aggregation of tau filaments, as measured by dynamic light scattering and atomic force microscopy. Hsp27 is also able to prevent tau accumulation and rescue long-term potentiation deficits [16,36].

Hsp22 and Hsp25 have not been extensively explored for their effects on tau aggregation. However, they have been shown to prevent other amyloid accumulation such as amyloid-beta [57] and alpha-synuclein [58] aggregation. While compounds that regulate the activity of these small Hsps are not yet available, it is possible that they may be useful as therapeutics in recombinant form or via gene delivery. This is a developing area that could hold great promise for tauopathies.

Small molecule inhibitors of tau aggregation

While chaperone modulation of tau is an emerging field, preventing tau aggregation directly with compounds that bind tau is much further developed. Rhodanine drugs, including epalrestat and troglitazone, have been extensively developed and characterized by the Mandelkow group for their ability to alter tau aggregation. These low-toxicity compounds are actually able to disaggregate insoluble tau fibrils, known as paired helical filament tau [39]. This group performed an extensive characterization of over 50 derivatives, several of which proved to be potent compounds that prevented tau aggregation in neuronal cell lines [38]. Further developments of highly membrane permeable analogs are necessary to test these drugs *in vivo*.

Anthraquinones are synthetic, organic compounds that can also potently prevent and reverse tau aggregation [59]. A few derivatives that have exhibited anti-amyloidogenic properties include emodin, daunorubicin, mitoxantrone, and pixantrone [60]. Recently, the nontoxic analog anthraquinone-2-sulfonic acid (AQ2S) was identified to have not only anti-aggregation properties but was also found to be neuroprotective [61,62]. The structurally similar N-phenylamine also possesses anti-amyloidogenic properties. Derivatives of this compound, B1C11, B4D3, B4A1, and B4D5, have been shown to not only significantly prevent tau polymerization, but also to disassemble tau fibrils with relatively low toxicity [60]. However, there are no data that display the efficacy of these drugs *in vivo*.

Another aromatic scaffold, phenylthiazolyl-hydrazide was identified from a drug screen as a tau aggregation inhibitor. In one screen, the phenylthiazolyl-hydrazide derivative BSc3094 was identified to most effectively prevent and reverse tau aggregation out of dozens of phenylthiazolyl-hydrazide analogs produced, many of which showed some anti-amyloid activity. This family has low toxicity and their activity was also shown to be cytoprotective in a neuronal cell model of tauopathy [63].

Natural products have not only been shown to effect tau through chaperone modulation, as described above, but also through direct interaction with tau. Oleuropein, hydroxytyrosol, and oleuropein aglycone were isolated from olive extraction and showed anti-tau aggregation efficacy, with oleuropein having the greatest potency [5]. Future *in vivo* studies are needed to look at efficacy in the brain. Data suggest that oleuropein cannot pass the

BBB, but the aglycone analog has remained untested. Treatment of hydroxytyrosol, which is BBB permeable, in an *in vivo* tau mouse model would be necessary to determine aggregation inhibitory activity in the brain. Important to note is that administration of some phenols extracted from foods have been shown to exhibit different activity than that predicted *in vivo* [64].

Members in the carbocyanine scaffold, a group of blue–green dyes, have been shown to block tau aggregation; however, the potency of these drugs is variable. The structural composition of the linker chains in bisthiacarbocyanine derivatives was recently shown to lead to changes in the efficacy of aggregation inhibition [65]. One small molecule inhibitor in this family, 3,3′-bis(β-hydroxyethyl)-9-ethyl-5,5′-dimethoxythiacarbocyanine iodide (N744), has been shown to have biphasic effects on tau aggregation [66]. This is not a surprising result, since other dye-based inhibitors are effective at preventing tau polymerization at specific concentrations, and not dose dependently [67]. N744 has additionally been shown to disaggregate tau filaments in a recombinant system [66]. Other analogs in this family, such as 3,3′-diethyl-9-methyl-thiacarbocyanine iodide (C11), exhibited inhibitory aggregate activity in *ex vivo* tissue slices from a mouse line expressing human tau [68].

A new small molecule, 2,6-diamino-3,5-dithiocyanopyridine, thiocyanic acid C,C′-(2,6-diamino-3,5-pyridinediyl) ester, 2,6-diaminopyridine-3,5-bis(thiocyanate) (PR-619), is an inhibitor of ubiquitin isopeptidases that upregulate Hsp70. An *in vitro* system showed that PR-619 was able to stabilize the microtubule network [69]. This same study showed that treatment led to small tau aggregates surrounding the microtubule organizing center. Importantly, tau phosphorylation at both serines 396 and 404 and serines 262 and 356 (12E8) was decreased, which increased the ability of tau to bind the microtubules [69]. Translation of this drug to an *in vivo* rodent model would be important to understand its therapeutic potential.

Conclusion
Tau aggregation contributes to the pathogenesis of many neurodegenerative diseases. Finding therapeutics that can prevent or reverse this non-native accumulation is thus highly desirable. Although many compounds have been recently identified to have anti-aggregative effects on tau, the large majority of these small molecule inhibitors is not specific for tau aggregates, but rather targets all proteins that can form beta-sheet amyloids. This lack of specificity may alter pharmacodynamics between individuals, making the appropriate dose difficult to assess based on the total amyloid burden in the body. The macrocyclic drugs are the first to be synthetically designed to specifically bind tau [9]. Further development of these compounds may help identify a tau specific inhibitor that is active *in vivo*.

Currently many aggregation inhibitors would need to be used at high concentrations to be effective against the high levels of tau present in neurons [70]. Tau is typically found in axons, but is thought to be further concentrated into somatodendritic aggregates in disease [71]. Delivering aggregation inhibitors with assistance from nanoparticle encapsulation [72] could therefore boost their efficacy by increasing their concentration in the brain. Other strategies that preferentially and specifically target tau aggregates within neurons or areas within the neuron where tau aggregates are present could also increase the potential for success. Perhaps combining therapies that specifically target tau aggregates and then facilitate tau clearance would further overcome this potential problem associated with high focal concentrations of tau.

The combination of tau aggregation inhibitors with compounds that can facilitate tau clearance could be advantageous in a clinical setting, possibly producing true synergy. Molecular chaperones are a prime target for regulating tau turnover; many drugs have been identified that alter the expression or activities of chaperone proteins, and advances in the last decade have increased drug efficacy and BBB permeability. These developments have allowed us to advance our understanding of the role of tau accumulation in disease, but concerns about specificity and off-target effects have slowed the progress of these compounds to the clinic. In addition, drugs that prevent aggregation, disaggregation, degradation, or increased expression may not be effective in preventing or reversing tauopathy phenotypes. Constructing the next generation of small molecule drugs to selectively eliminate only abnormal tau may be essential, a strategy that may now be possible given our advanced understanding of tau triage biology.

Abbreviations
17-AAG: 17-(allylamino)-17-demethoxygeldamycin; AQ2S: Anthraquinone-2-sulfonic acid; BBB: Blood–brain barrier; C11: 3,3′-diethyl-9-methyl-thiacarbocyanine iodide; Cdc37: Cell division control 37 kDa; CHIP: Carboxyl terminus of Hsc70-interacting protein; FKBP51: FK506 binding protein 51 kDa; GRP: Glucose-regulated protein; HSF1: Heat shock factor protein 1; Hsc: Heat shock cognate; Hsp: Heat shock protein; KU-32: *N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-methoxy-6,6-dimethyl-tetrahydro-2*H*-pyran-2-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-yl)acetamide; MB: Methylene blue (phenothiazine methylthionium chloride); MKT-077: 1-ethyl-2-((3-ethyl-5-(3-methylbenzothiazolin-2-yliden))-4-oxothiazolidin-2-ylidenemethyl) pyridinium chloride; N744: 3,3′-bis(β-hydroxyethyl)-9-ethyl-5,5′-dimethoxythiacarbocyanine iodide; PR619: 2,6-diamino-3,5-dithiocyanopyridine, thiocyanic acid C,C′-(2,6-diamino-3,5-pyridinediyl) ester, 2,6-diaminopyridine-3,5-bis(thiocyanate).

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