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Microtubule-Stabilizing Agents as Potential Therapeutics for Neurodegenerative Disease

Kurt R. Brunden[1], **John Q. Trojanowski**[1], **Amos B. Smith III**[2], **Virginia M.-Y. Lee**[1], and **Carlo Ballatore**[1,2]

[1]Center for Neurodegenerative Disease Research, Perelman School of Medicine, University of Pennsylvania, 3600 Spruce Street, Philadelphia, Pennsylvania, 19104-6323

[2]Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, 231 South 34th Street, Philadelphia, Pennsylvania, 19104-6323

Abstract

Microtubules (MTs)[1], cytoskeletal elements found in all mammalian cells, play a significant role in cell structure and in cell division. They are especially critical in the proper functioning of post-mitotic central nervous system neurons, where MTs serve as the structures on which key cellular constituents are trafficked in axonal projections. MTs are stabilized in axons by the MT-associated protein tau, and in several neurodegenerative diseases, including Alzheimer's disease, frontotemporal lobar degeneration, and Parkinson's disease, tau function appears to be compromised due to the protein dissociating from MTs and depositing into insoluble inclusions referred to as neurofibrillary tangles. This loss of tau function is believed to result in alterations of MT structure and function, resulting in aberrant axonal transport that likely contributes to the neurodegenerative process. There is also evidence of axonal transport deficiencies in other neurodegenerative diseases, including amyotrophic lateral sclerosis and Huntington's disease, which may result, at least in part, from MT alterations. Accordingly, a possible therapeutic strategy for such neurodegenerative conditions is to treat with MT-stabilizing agents, such as those that have been used in the treatment of cancer. Here, we review evidence of axonal transport and MT deficiencies in a number of neurodegenerative diseases, and summarize the various classes of known MT-stabilizing agents. Finally, we highlight the growing evidence that small molecule MT-stabilizing agents provide benefit in animal models of neurodegenerative disease and discuss the desired features of such molecules for the treatment of these central nervous system disorders.

[1]Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTDP-17, frontotemporal degeneration with Parkinsonism linked to chromosome 17; HD, Huntington's disease; MT, microtubules; MTOC, microtubule organizing center; NFT, neurofibrillary tangles; PD, Parkinson's disease; Tg, transgenic; 3R, 3-microtubule binding repeat tau; 4R, 4-microtubule binding repeat tau

Corresponding Author: Kurt R. Brunden, Center for Neurodegenerative Disease Research, Perelman School of Medicine, University of Pennsylvania, 3600 Spruce Street; Maloney 3, Philadelphia, Pennsylvania 19104-6323, kbrunden@upenn.edu, (215) 615-5262.

1.1 Microtubules and their role in neuronal axons

Microtubules (MTs) comprise a key cytoskeletal component of all eukaryotic cells, as they play an integral role in the process of mitosis through their involvement in the segregation of chromosomes along mitotic spindles in dividing cells[1]. In addition to their role in mitosis, MTs also provide structural and functional support in cells; this is particularly evident in the nervous system, where MTs play a fundamental role in the health of neurons[2]. The axons of neurons can extend great distances (up to 3 feet for certain motor neurons in humans), and thus vital cellular components, including nutrients, mitochondria, proteins, mRNA and growth factors, must be shuttled to and from the cell body along these axonal projections. The transport of these species is largely dependent on either fast or slow axonal transport that is mediated by molecular motors that move their associated cargo along the MTs within the axonal processes. In particular, the kinesin family of MT-associated motors are involved in anterograde transport (i.e., away from the cell body)[3], whereas the dynein motors direct retrograde transport[4] toward the cell body (Figure 1a).

MTs are typically composed of 13 aligned protofilaments, with each protofilament comprised of a polymer of repeating α- and β-tubulin heterodimers[5, 6] (Figure 1b). There are a number of α and β tubulin isoforms in mammals which may confer subtle changes to MT structure or function, although the exact significance of these differing isotypes is largely unknown[6]. The assembly of tubulin heterodimers into MTs is typically initiated at microtubule organizing centers (MTOC), with the addition of α/β heterodimers that contain one GTP each per α and β tubulin subunit to a growing MT in an outward direction, such that β-tubulin is exposed at the “plus” end, whereas the MTOC-associated “minus” end has a terminal α-tubulin. Thus, in most cells the minus end is typically near the nucleus. However, in neurons, MTs are discontinuous along the axonal and dendritic processes, such that there are multiple minus and plus ends (Figure 1a) and a traditional MTOC may not persist as neurons mature[7, 8]. The plus end of MTs in neurons thus project outward along the axon towards the terminus[9]. MTs exhibit a feature known as “dynamic instability”, in which a given MT will undergo periods of growth followed by times of disassembly[5]. This results from the hydrolysis of GTP to GDP within β-tubulin subunits, as the conversion of the terminal plus-end β-tubulin GTP to GDP, prior to the addition of another GTP-containing heterodimer, can lead to MT depolymerization. Such disassembly occurs less frequently at the minus-end, presumably because this end is typically stabilized by a MTOC, or perhaps by alternative nucleation sites in neurons[7, 8]. MTs can also undergo a process referred to as “treadmilling”, in which growth at the plus-end is accompanied by shortening at the minus-end, and this behavior may be important during mitosis[5, 6]. In neurons, MTs appear to have greater stability than in many other cell types, and thus the extent of MT “dynamicity” is reduced. This MT stability is due, at least in part, to a number of MT-associated proteins (MAPs) that interact with MTs within neurons, with tau protein playing the predominate role in stabilizing MTs in axons[6, 10-12].

In humans, six isoforms of tau are generated via differential mRNA splicing that contain either 3 (3R) or 4 (4R) repeated (although non-identical) microtubule-binding domains[13-15] (Figure 2). Perhaps unsurprisingly, 4R tau species appear to bind with greater avidity to MTs than do 3R isoforms[16], and the balance of 4R-to-3R isoforms of tau seems to be tightly

regulated such that in human neurons there are approximately equal concentrations of 4R and 3R isoforms as a group, but not individually with respect to each of the 6 isoforms[17]. In addition to the presumed role in providing stabilization to axonal MTs, tau also appears to regulate the interaction of kinesin with MTs. For example, tau over-expression in cultured neurons reduces kinesin engagement with MTs[18, 19] and alters mitochondrial transport[20]. Likewise, physiological concentrations of tau can alter kinesin-mediated transport using a purified protein system[21]. There is also some evidence that tau may interact with dynactin[22], which plays a role in dynein-mediated transport. Thus, it would appear that tau plays a critical role in axonal transport through both the stabilization of MTs as well as through the regulation of MT motor protein interactions.

2.1 Evidence of MT and axonal transport dysfunction in neurodegenerative disease

The critical role of MTs in axonal transport suggests that any significant perturbation of MT structure or function could be highly detrimental to neurons. Similarly, alterations of normal MT motor function would compromise axonal transport and have negative consequences on neuronal physiology. In fact, transgenic (Tg) mice with altered dynactin[23, 24] display a neurodegenerative phenotype, with targeted deletion of kinesin function also inducing axonal degeneration in mice[25]. Moreover, mutations that result in altered dynein or kinesin function have been implicated in a number of neurodegenerative diseases (reviewed in [26]). These data point to the criticality of the axonal transport process to neuronal function and survival. Indeed, there is compelling evidence of MT and/or axonal transport deficiencies in a number of neurodegenerative diseases, as briefly reviewed below.

2.1.1 Alzheimer’s disease (AD) and related tauopathies

AD is the most common neurodegenerative condition in the world, with ~5 million cases in the United States. A key pathological hallmark within the AD brain is the presence of neurofibrillary tangles (NFTs) and neuropil threads comprised of fibrillar inclusions of tau protein within neuronal cell bodies and processes, respectively[13]. As noted, tau is a MAP that appears to stabilize MTs and modulate MT motor function in axons, and tau inclusions are also found in several additional “tauopathies”, such as frontotemporal lobar degenerative conditions that include Pick’s disease, corticobasal syndrome and progressive supranuclear palsy[27, 28]. Tau aggregates are thought to contribute to the neuronal loss observed in these diseases, a hypothesis substantially bolstered by the finding that mutations in tau can result in inherited frontotemporal degeneration with Parkinsonism linked to chromosome 17 (FTDP-17)[17, 29]. Many of the mutations in FTDP-17 promote tau disengagement from MTs[30, 31] and some also enhance fibrillization of tau[32, 33]. Moreover, the hyperphosphorylation of tau that is observed in AD and the other tauopathies generally decreases the avidity of tau for MTs[34-37]. Increased phosphorylation at certain residues can also enhance tau fibrillization[38, 39]. Thus, there are two potentially detrimental consequences of tau disengagement from MTs and aggregation into NFTs and neuropil threads. First, misfolded oligomeric or fibrillar tau may exert a toxic effect within neurons[40]. In addition, the dissociation of tau from MTs, with subsequent sequestration into insoluble inclusions, likely results in a loss-of-function that causes MT and axonal transport abnormalities.

This latter hypothesis is supported by a number of observations in AD brain and in Tg mouse models of tauopathy. For example, there is evidence of a reduction in both the number and length of MTs in AD brain[41], as well as a decrease in acetylated α-tubulin, which is considered a marker of stable MTs[42]. Similarly, the amount of isolated tau that is competent to bind to MTs is reduced in extracts from AD brain relative to control brain[37]. Moreover, a reduction in MT density has been observed in tau Tg mouse models in which tau inclusions form with age[43, 44]. Finally, a recent study has revealed an increase of MT dynamicity in two established Tg mouse models of tauopathy[45].

2.1.2 Parkinson's Disease (PD)

PD is a progressive neurodegenerative condition in which intracellular inclusions comprised of α-synuclein, referred to as Lewy bodies, accumulate within neurons. PD is characterized by motor deficits, with primary involvement of dopaminergic neurons within the substantia nigra, although other neuronal systems are also often affected[46]. There is evidence from both cellular and animal models of PD that neuronal function may be compromised as a result of MT and axonal transport deficits. PD is often modeled through the treatment of neuron cultures or rodents with environmental toxins such as rotenone, as these agents induce dopaminergic neuropathology that resembles certain aspects of PD[47]. Interestingly, rotenone appears to directly affect MT polymerization[48-50], as do several other toxins used in PD models, including MPTP[51, 52] and certain herbicides[53, 54].

A number of recent reports also suggest a link between α-synuclein pathology and MT dysfunction. For example, there is evidence of impaired axonal transport in Tg mice expressing a mutant form of α-synuclein (A53T) that is found in inherited PD[55]. Interestingly, the effect of α-synuclein on axonal transport may be mediated by alterations in tau, as a reduction of normal tau function has been observed in both cellular and animal models of PD. For example, increased hyperphosphorylated tau has been reported in several brain regions of aged Tg mice expressing wild-type human α-synuclein, with a consequent reduction of MT-bound tau and an increase of depolymerized tubulin[56, 57]. Similarly, increased tau phosphorylation was observed in the striatum of a Tg mouse model expressing A53T mutant human α-synuclein; again, there was a decrease of MT-associated tau[58]. These studies follow earlier observations which revealed that Tg mice expressing A30P or A53T human α-synuclein developed hyperphosphorylated tau or tau inclusions that paralleled the accumulation of α-synuclein aggregates[59, 60]. It has also been suggested that the increases of tau phosphorylation observed in the α-syn Tg mice may result from α-synuclein-mediated activation of tau kinases, including glycogen synthase kinase 3β[56, 58] and protein kinase A[61]. It is thus interesting that a recent study[62] revealed that the kinase LRRK2, which is linked to inherited PD[63], can phosphorylate MT-bound tau and reduce tau-MT interaction. Moreover, PD-associated LRRK2 mutations further increased this tau phosphorylation[62]. Notably, LRRK2 mutations that are found in PD appear to enhance the binding of LRRK2 to MTs[64], perhaps providing an explanation for the increased tau phosphorylation that was observed upon expression of mutated LRRK2[62]. Finally, cerebrospinal fluid tau levels are reduced in early PD in parallel with reduced levels of and α-synuclein and Aβ[65], and a novel cerebrospinal fluid-based biomarker methodology suggests that there is a deficit of axonal transport in PD patients relative to non-PD control subjects[55].

2.1.3 Amyotrophic Lateral Sclerosis (ALS)

ALS is a late-onset neurodegenerative disorder that affects motor neurons, typically with a rapid disease progression. Given the extreme length of motor neurons projecting to the extremities, axonal transport is particularly crucial to the functioning and health of these cells. The majority of ALS cases are sporadic, although ~10% are inherited, with multiple gene mutations linked to the disease[66]. The first mutations described in familial ALS were in the superoxide dismutase-1 (SOD1) gene, and several Tg mouse models have been created in which mutant SOD1 is expressed[67]. These mice develop motor neuron disease, with several reports revealing that axonal transport deficiencies develop relatively early in these models[68-70]. The mechanisms by which mutated SOD1 affects axonal transport are not fully understood, and there are likely multiple contributing factors, including ATP deficits, altered motor protein function, damage to transport cargo and/or MT abnormalities[71]. With regard to the latter possibility, there is evidence of increased MT dynamicity in SOD1 transgenic mice[72]. Although only ~2% of ALS patients have SOD1 mutations, axonal transport deficiencies may be a common feature found within other inherited and sporadic cases of ALS. For example, mutations in the dynactin subunit p150^{Glued} can cause inherited ALS[23, 24], directly implicating alterations in dynein/dynactin-mediated MT transport in the disease.

2.1.4 Huntington's Disease (HD)

HD is an autosomal-dominant inherited neurodegenerative condition caused by the aggregation within neurons of mutated huntingtin protein containing polyglutamine repeats at the amino-terminus, which result from expanded CAG repeats in the first exon of the huntingtin gene[73]. There is evidence of altered axonal transport in cells that express mutated huntingtin, with a diminution of mitochondrial[74, 75] and vesicular[75] transport in primary neurons. Similarly, altered axonal transport has been measured in Tg mice that express mutated hutingtin, with an onset that precedes motor symptoms in the mice[75]. Notably, a similar alteration of axonal transport has been observed both in Drosophila[76] and in mice in which expression of huntingtin was reduced[75], suggesting that the protein may normally play a role in axonal transport and that the accumulation of insoluble huntingtin aggregates results in a loss of function. This is consistent with the findings that huntingtin interacts with the Huntingtin-Associated Protein 1 (HAP1), which binds the p150^{Glued} subunit of dynactin[77, 78]. Moreover, HAP1 has also been reported to interact with kinesin light chain[79]. Interestingly, these proteins, along with tubulin, are found associated with insoluble htt in extracts from HD brain[75], consistent with a sequestration of these components within huntingtin aggregates that may result in an alteration of axonal transport and perhaps MT structure.

2.1.5 Summary

Faulty axonal transport is a recurring theme in the neurodegenerative diseases discussed above, as well as in neurodegenerative conditions not discussed here, such as the demyelinating disorders Charcot-Marie Tooth disease[71] and multiple sclerosis[80]. In general, these reductions of transport can be attributed to deficiencies in motor protein function and/or alterations of MT structure. Among the major neurodegenerative conditions

discussed here, the evidence of MT deficiencies is arguably the greatest for the tauopathies and PD, followed by ALS. There is little evidence to support a fundamental defect in MT structure in HD, although the observation that tubulin can be found associated with huntingtin deposits[75] might suggest the possibility of MT alterations in this disease.

The compelling data supporting a MT deficiency in tauopathies that results from a loss of tau function led to the hypothesis, first published nearly two decades ago[81], that MT-stabilizing drugs may have utility in the treatment of these disorders. Given the accumulating evidence of possible MT deficits in other neurodegenerative diseases, it is possible that MT-stabilizing agents may have applicability beyond the tauopathies. MT-stabilizing drugs have been used for the treatment of cancers for some time, as exemplified by the taxane family members, paclitaxel and docetaxel[82, 83]. Below, we provide an overview of the various classes of known MT-stabilizing molecules, followed by a review of the scientific evidence that supports the potential utility of such compounds for the treatment of neurodegenerative disease.

3.1 An overview of MT-stabilizing molecules

Since the discovery of paclitaxel (Taxol®, 1, Figure 3) in 1967[84, 85] and the subsequent elucidation of the MT-stabilizing properties of this natural product[86], several additional classes of molecules, primarily natural products and derivatives thereof, have been identified that are functionally similar to paclitaxel in promoting MT stabilization[87, 88]. Paclitaxel stabilizes MTs by binding within the lumen of the MT at a site in the β-tubulin subunit, which is commonly referred to as the taxane site. The interaction of 1 with β-tubulin results in conformational changes in the M-loop of β-tubulin that ultimately stabilize lateral interactions of adjacent protofilaments[89, 90]. Representative compounds from the different classes of natural products that are found to interact within or in close proximity to the taxane site on MTs, producing taxol-like MT-stabilization, are shown in Figure 3. These include members of the epothilones[91] [e.g., epothilone A (2), B (3), and D (4)], discodermolide[92, 93] (5), dictyostatin[94] (6), eleuthesides [e.g., eleutherobin[95] (7) and sarcodyctin A[96] (8)], zampanolide[97, 98] (9) and ceratamines[99] (e.g., ceratamine A, 10). For each of these classes, competition-binding experiments revealed that these compounds target binding sites that overlap with the taxane site found on β-tubulin. In addition, X-ray crystal structures of tubulin-bound 2 and 9 have confirmed that these compounds interact with the taxane binding site and promote the restructuring of the M-loop into a short helix structure[98]. Zampanolide, unlike 2, was found to bind covalently with the taxane binding site[97]. This is not the only example of a MT-stabilizing agent that covalently modifies tubulin, as cyclostreptin (11, Figure 4) was first reported as an alkylating MT-stabilizing agent. Notably, while 11 is also reported to compete with paclitaxel binding, the binding site of 11 has been localized at the surface of the MT at a site that may be important for the initial interaction of 1 with the MT, prior to its translocation to the luminal site[100].

Among the MT-stabilizing natural products that do not interact with the taxane binding site, the most prominent examples are laulimalide (12) and peloruside (13), shown in Figure 4. Both of these compounds have been found to interact with β-tubulin at a shared site, localized at the surface of the MT[101], that does not overlap with the taxane binding site.

Synergistic effects on MT-stabilization have been described between drugs that interact with the taxane-binding site and 12 or 13[102]. An additional promising class of MT-stabilizing agents are the steroidal natural products, taccalonolides[103]. While the initially discovered taccalonolides A and E (14 and 15, respectively, Figure 4) were not highly potent MT-stabilizing agents, selected congeners have been identified which are potent both in cell-free and in cell-based assays[104, 105]. This compound class is still under investigation and the binding site for the taccalonolides has not yet been identified.

Finally, in addition to the abovementioned classes of natural products with MT-stabilizing activity, the opium alkaloid, noscapine (16, Figure 4), has been shown to modulate MT-dynamics, albeit without any significant impact on total MT mass. Although 16, which has been used for several years as an anti-tussive agent[106, 107], does not seem to alter MT polymerization over a wide range of concentrations, more potent analogues have been identified which cause vinblastine-like depolymerization of MTs[108]. These findings indicate that 16 may be a weakly active MT-destabilizing agent, which mostly affects MTs at the level of dynamics rather than MT mass. Because of these properties and the favorable pharmacokinetic and safety features of noscapine, this compound has been investigated in the context of neurodegenerative diseases (vide infra)[109].

As summarized above, the vast majority of MT-stabilizing agents are naturally occurring compounds. However, significant progress has been made in the area of synthetic small molecules with MT-stabilizing properties. Among these, particularly interesting are the triazolopyrimidines, typified by cevipabulin[110] (17, Figure 5). This compound has been reported to have a rather unique mode of action, as competition experiments revealed that 17 can displace vincristine, but not paclitaxel[111]. This observation suggests that 17 and possibly other related heterocyclic compounds[112] bind to the vinca site on β-tubulin or to an allosteric binding site that when occupied may interfere with the binding of vincristine. Other interesting examples of synthetic small-molecule MT-stabilizing agents, shown in Figure 5, are GS-164 (18)[113], phthalimide 5HPP-33 (19)[114], Synstab (20)[115], as well as the recently discovered molecules CID 4970947 (21)[116] and the Z-1-Aryl-3-arylamino-2-propen-1-ones, such as 10ae (22)[117].

4.1 The potential of MT-stabilizing agents for the treatment of neurodegenerative disease

The concept of utilizing MT-stabilizing drugs for the treatment of neurodegenerative disease was first tested in a tau Tg mouse model in which NFT-like inclusions develop with age, primarily within neurons of the brain stem and spinal cord[118]. Administration of paclitaxel (1) to these mice resulted in an improvement of axonal transport and a reduction in the motor phenotype that develops as a result of tau inclusions within motor neurons, but did not attenuate tau pathology itself[119]. This study provided an important proof-of-principle that a MT-stabilizing agent could compensate for axonal transport deficits that presumably resulted from a destabilization of MTs after tau deposition into insoluble tangles. Moreover, as therapeutic benefit was achieved without a reduction of tau pathological burden, there data suggested that correcting a loss of tau function may be more critical than eliminating tau inclusions. However, 1, like many known MT-stabilizing agents, does not cross the

blood-brain barrier effectively[120], as would be required for treatment of human neurodegenerative disease, and presumably was efficacious in the aforementioned Tg mouse model because of uptake at neuromuscular junctions.

More recently, studies have demonstrated that the epothilone family of MT-stabilizing compounds are generally brain-penetrant[120]. Work from our laboratories demonstrated that epothilone D (4) is both efficacious and safe in Tg mouse models that develop tau inclusions within the brain when used at doses that are significantly lower than had been utilized in oncology clinical trials. Notably, 3 months of once-weekly administrations of 4 at doses that are ~1/100th the amounts utilized in cancer trials were found to increase MT density, reduce axonal dystrophy and improved cognitive performance in both preventative and interventional studies with tau Tg mice that develop NFT-like inclusions[44, 121]. Importantly, the intervention study demonstrated that 4 could improve axonal transport, reduce tau pathology and prevent the hippocampal neuron and synapse loss that is observed in these animals with age[121]. Another team has recently obtained similar results with 4 in two additional tau Tg mouse models[45]. Moreover, this group demonstrated that there was MT hyperdynamicity in these aged tau Tg mice, which was normalized by treatment with 4. Thus, the results obtained with 4 in these tau Tg mouse models provide important proof-of-principle that brain-penetrant MT-stabilizing agents have the potential for the treatment of tauopathies. Importantly, epothilone D (4) has since progressed to clinical testing in AD patients (http://clinicaltrials.gov/ct2/show/NCT01492374).

Epothilone D (4) has also recently undergone evaluation in an MPTP-induced mouse model of PD[122]. As noted previously, toxins such as rotenone and MPTP have been shown to affect MT dynamics[48, 49, 51, 123], and rotenone-induced toxicity in dopaminergic midbrain neuron cultures can be abrogated by treatment with 1[123]. In this recent study, MPTP-treated mice showed an impairment of axonal transport in dopaminergic axons and changes in post-translational MT modifications that were normalized by treatment with 4[122]. Interestingly, MPTP treatment appeared to have a somewhat complicated effect on MTs, increasing the depolymerization of dynamic MTs while also causing enrichment of stable MTs, with a decrease in dynamicity [52, 122]. Importantly, treatment with 4 partially prevented the decrease in dopamine levels and the loss of nigral dopaminergic neurons that was observed after MPTP treatment[122]. These data further extend the potential utility of 4 in neurodegenerative disease, and provide evidence of MT dysfunction in an animal model of PD. However, it will be important to further validate brain-penetrant MT-stabilizing agents in additional PD animal models, including Tg mice that express genes that are mutated in familial PD, such as α-synuclein and LRRK2.

There is also evidence that the MT-modifying agent, noscapine (16), can improve MT and axonal transport deficits in a mutant SOD1 Tg mouse model of ALS[72]. Interestingly, this study revealed that the MTs in axons from the spinal cord and sciatic nerve, as well as from the cortex, showed hyperdynamicity that appeared to manifest at an early age and increase with time. Moreover, 16 decreased the observed MT hyperdynamicity, improved axonal transport and delayed disease onset with an improvement of motor performance. As noted, 16 is reported to differ from typical MT-stabilizing compounds in that it does not promote MT polymerization, but rather modulates MT dynamics[107]. The change in MT dynamicity

observed in the SOD1 Tg model bears resemblance to that found in Tg models of tauopathy[45], where the MT-stabilizing agent 4 has proven effective[44, 45, 121]. This raises the question of whether the mechanism by which 4 improves outcomes in the various tauopathy models may in fact be more akin to the reported action of 16, with increases of MT-stabilization[107, 108] perhaps being less important than a reduction of MT hyperdynamics. In this regard, doses of MT-modulating agents that are below those which promote MT assembly or disassembly are known to affect MT dynamics[124]. Thus, as discussed further below, an important feature of drugs to treat MT alterations in neurodegenerative disease may be to normalize dynamicity without over-stabilizing MTs.

5.1 Desired features of MT-stabilizing agents for neurodegenerative disease

As summarized above, there is growing evidence of the potential of MT-stabilizing compounds for the treatment of neurodegenerative disease; however, only a very limited number of example compounds have been tested in animal models of these diseases. As with nearly all therapeutics, MT-stabilizing agents are unlikely to fully compensate for the deficits observed in neurodegenerative disease. Rather, the hope is that drugs of this type will at least partially restore MT function, with meaningful improvements in patient outcomes. Ideal candidate compounds to treat MT alterations in neurodegenerative disease would be expected to: (A) cross the blood-brain barrier; (B) normalize MT dynamicity and perhaps also increase the stability of the MT system so as to restore effective axonal transport in diseased neurons; and (C) have little or no systemic toxicity at effective doses. In addition, it would be advantageous if MT-stabilizing agents with these properties could be administered orally for ease of administration to the generally elderly patients affected by neurodegenerative disease.

MT-stabilizing agents used to treat neurodegenerative diseases of the brain must be able to readily cross the blood-brain barrier so as to maintain a sufficient brain exposure to provide effective modulation of axonal MTs. Unfortunately, the majority of MT-stabilizing agents exhibit low or negligible brain exposure, due to unfavorable physical chemical properties that can hamper passive diffusion and/or to the molecules being P-glycoprotein substrates, such that they are actively transported back into the bloodstream. Nonetheless, several examples of brain-penetrant MT-stabilizing agents have been identified. In addition to different members of the epothilone class, such as 3, 4, and sagopilone (23, Figure 6)[125, 126], selected paclitaxel derivatives have been developed which exhibit improved brain penetration. These include IDN-5109 (24)[127], cabazitaxel (25)[128], and TPI-287 (26)[129] (Figure 6). Moreover, recent studies from our laboratories demonstrate that dictyostatin (6) crosses the blood-brain barrier in mice and maintains prolonged brain exposure and pharmacodynamic activity in a manner similar to epothilone D (4)[130]. In addition to good blood-brain barrier permeability, another potential desirable feature of MT-stabilizing drugs for the treatment of neurodegenerative disease is prolonged brain exposure relative to that in blood. For many CNS drugs, the pharmacokinetic profiles in the plasma and brain are similar, with comparable half-lives and clearance values. However, our studies with 4[44, 120] and more recently 6[130] have demonstrated that these compounds show a brain retention that far exceeds their duration in the plasma. This differential between brain and plasma exposure for MT-stabilizing drugs may be advantageous, particularly in the context of

neurodegenerative disease treatment. First, this property allows for less frequent drug administration, as exemplified by the once-weekly dosing of 4 in the studies conducted in tau Tg mice[44, 121]. In addition, extended brain exposure of MT-stabilizing drugs allows for lower overall doses and clearance of the drug from the blood and periphery, where dose-limiting side-effects are observed in cancer patients receiving drugs of this class.

In this regard, another important consideration is that the therapeutic regimens of MT-stabilizing agents for the treatment of neurodegenerative diseases are likely to be substantially different from those typically employed to treat cancer. Indeed, the objective would be to avoid triggering apoptosis of rapidly dividing cells, as in cancer treatment, so as to minimize side-effects and rather to normalize MTs and axonal transport in the axons of diseased neurons. As a result, optimal treatment of neurodegenerative diseases will likely require long-term administration of low doses of a MT-stabilizing drug. Although the safety and tolerability of chronic administration of low doses of MT-stabilizing agents has not yet been reported in humans, the absence of toxicities after multiple months of dosing in mice suggest that such treatments may not be associated with the severe side-effects caused by such drugs in cancer chemotherapy[82, 131, 132]. Nonetheless, it will be imperative to monitor the tolerability of low doses of MT-stabilizing drugs in patients upon long-term dosing, as there are still many unknown aspects of such a therapeutic strategy. This includes the effects that MT modulation might have on non-diseased cells within the brain, including glia and unaffected neurons. As with all therapeutics, the benefits of such treatments will have to be weighed against any observed side-effects. Finally, an interesting unresolved question is the relative importance of MT stabilization and increased MT mass vs. normalization of MT dynamics in the treatment of neurodegenerative disease. As noted, there is evidence of decreased MT mass in AD[41, 42] and in a Tg mouse model of tauopathy[44], as well as increased MT hyperdynamicity in similar tau Tg models[45]. Treatment with 4 resulted in both an improvement of MT density[44, 121] and suppression of MT hyperdynamicity[45] in these models. Similarly, there appears to be increased MT dynamicity in a mutant SOD1 model of ALS, with a normalization of MT dynamics after treatment with 16[72]. In contrast, neuron-like cells[52] and mice[122] treated with MPTP to model PD have been reported to have an increase in markers of stable MTs, and perhaps a decrease of MT dynamics. Notably, 4 seemed to normalize MTs in MPTP-treated mice, such that there was an attenuation of the toxin-induced neurodegeneration[122]. Taken together, these data might suggest that a disruption of MT dynamics is the common feature of these models of neurodegenerative disease. In cancer, it is believed that the suppression of MT dynamics, and not an overall change in MT mass, is the important therapeutic feature of both MT-stabilizing and MT-depolymerizing drugs[124]. If this is also true for the treatment of MT deficits in neurodegenerative conditions, it is possible that MT-directed molecules need not have dramatic effects on overall MT mass, and that normalization of dynamicity may be sufficient to improve outcomes in these diseases.

In conclusion, an increasing body of literature is pointing to axonal transport deficiencies as being a critical feature of a number of neurodegenerative diseases, and these transport problems may arise in several of these diseases through an alteration of MT stabilization and/or dynamicity. Accordingly, brain-penetrant MT-directed agents that can stabilize MTs and/or normalize MT dynamics hold considerable promise as therapeutics for these

devastating conditions, and there is thus a need for the further characterization and development of such agents.

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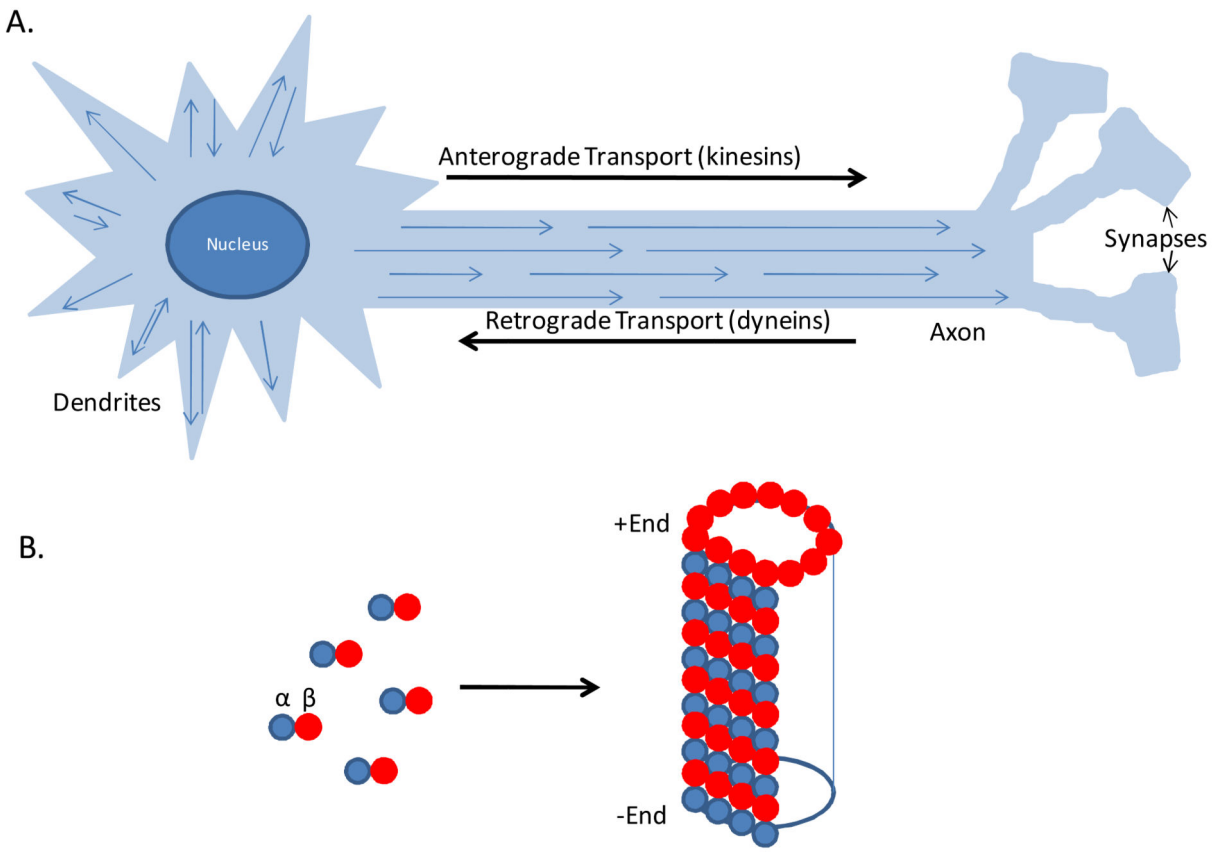

Figure 1.

A. Schematic of a neuron with microtubules (MTs) within axonal and dendritic processes. Arrowheads represent the (+) end of MTs, with dendrites containing both (+)-end distal and (−)-end distal MTs. Distinct molecular motors transport cellular cargo in the anterograde (kinesins) and retrograde (dyneins) directions along MTs. **B.** MTs are comprised of aligned protofilaments comprised of α- and β-tubulin heterodimers, with exposed β-tubulin at the (+) end and α-tubulin at the (−) end.

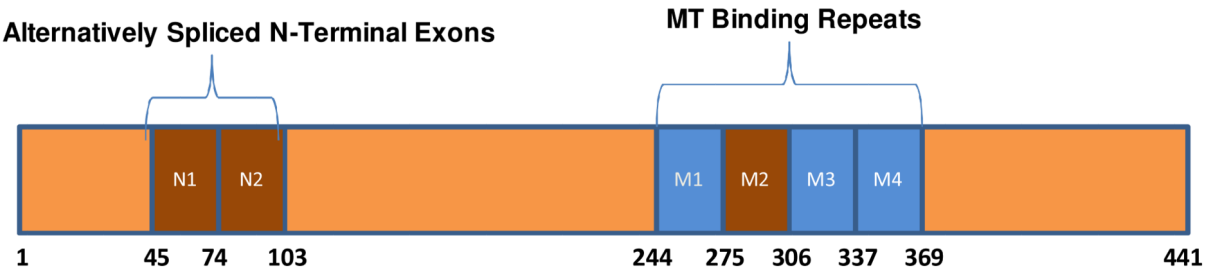

Figure 2.

Schematic of human tau. The inclusion or exclusion of the second MT-binding repeat (M2) encoded by exon 10 of the tau gene results in 4R or 3R tau species. Additional isoforms are created by the inclusion or exclusion of two coding exons (N1 and N2) in the amino-terminal region of tau. Amino acid numbers refer to the longest tau isoform.

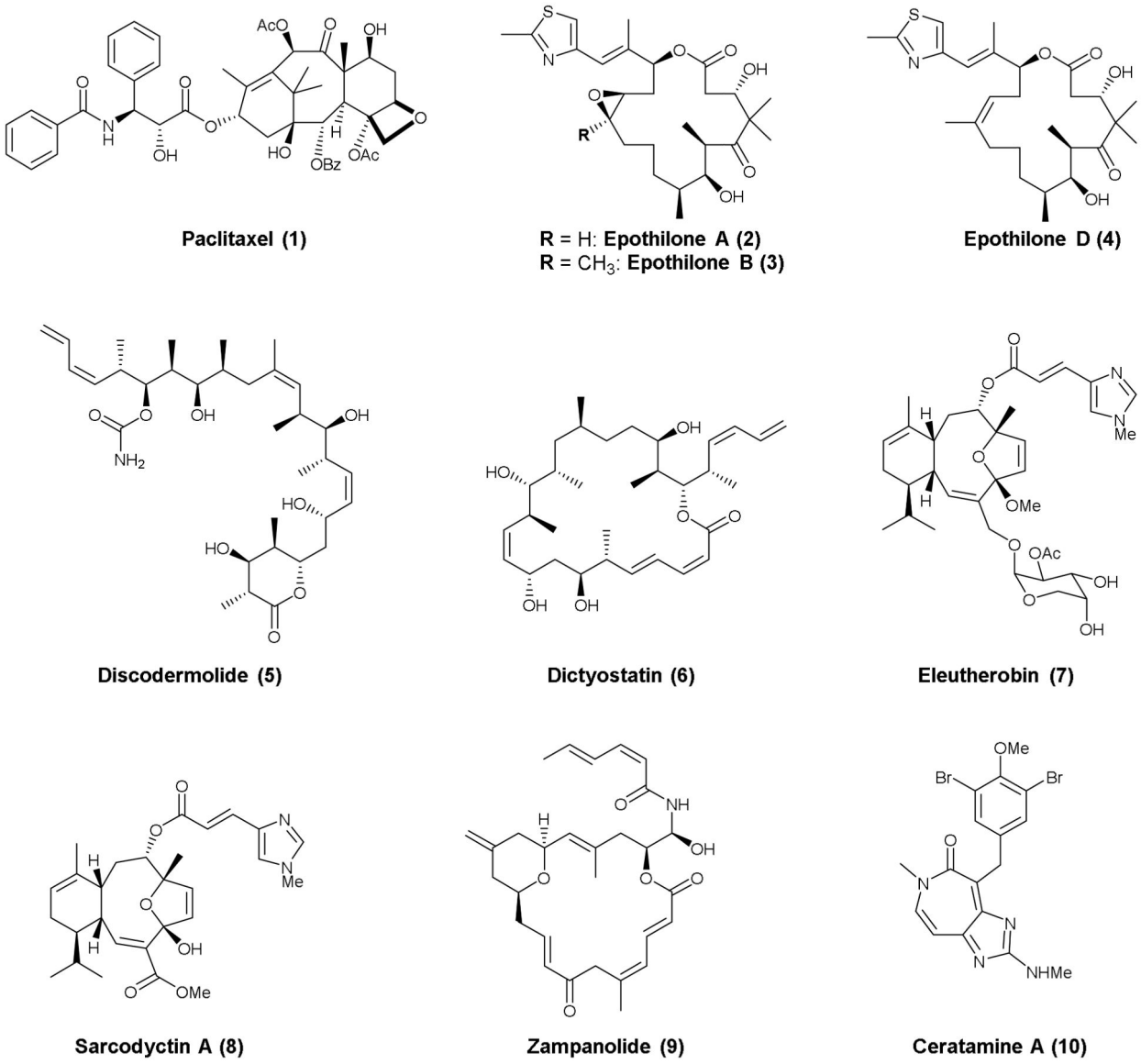

Figure 3.

Representative compounds from different classes of MT-stabilizing natural products that interact with the taxane binding site.

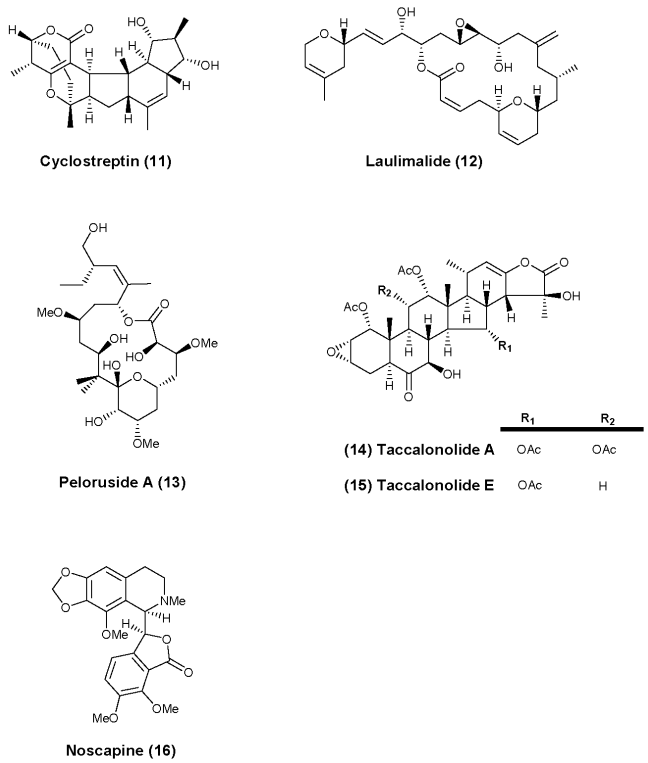

Figure 4.

Representative compounds from different classes of naturally occurring MT-stabilizing (**11**-**15**) or MT-modulating (**16**) agents that do not interact with the taxane binding site.

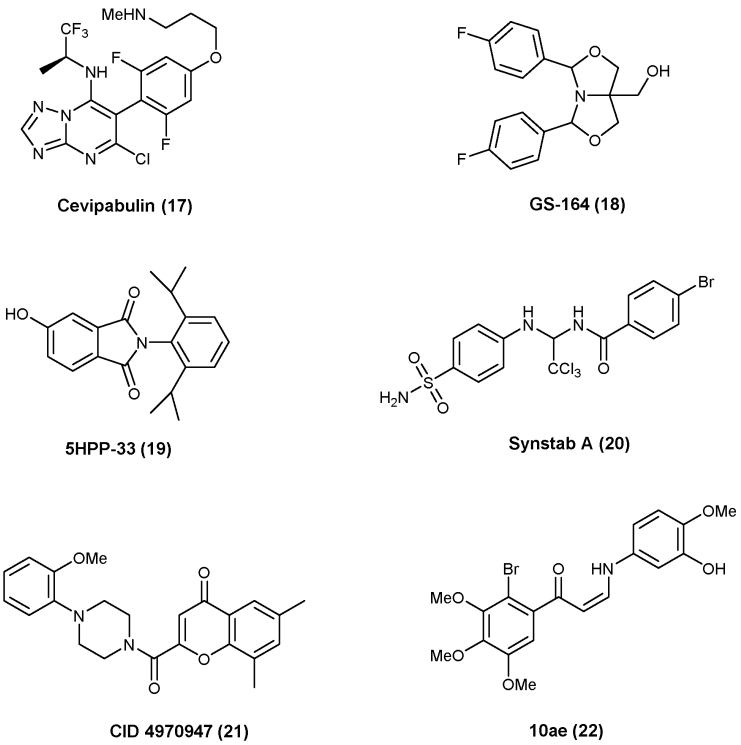

Figure 5.
Representative examples of synthetic small molecule MT-stabilizing agents.

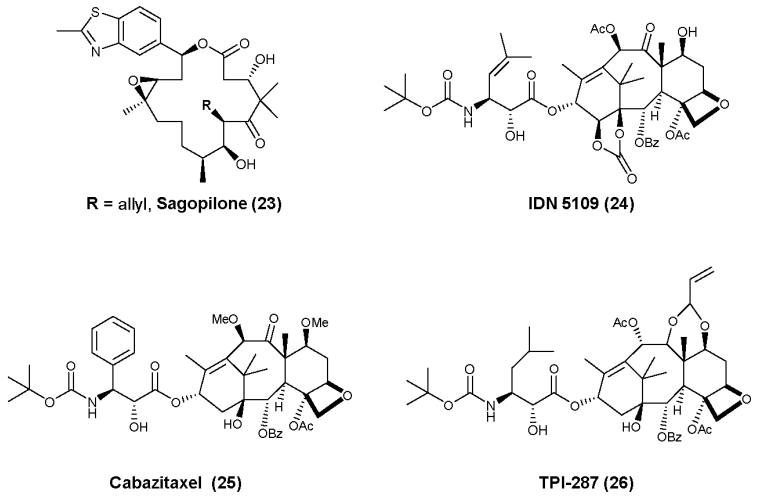

Figure 6.

Selected brain-penetrant MT-stabilizing agents. These examples, like **3**, **4** and **6** shown in Figure 3, have been reported to enter the brain.