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Pharmacophore-based models for therapeutic drugs against phosphorylated tau in Alzheimer's disease

Jangampalli Adi Pradeepkiran[1], **Arubala P. Reddy**[4], and **P. Hemachandra Reddy**[1,2,3,4,5,6,7,*]

[1]Garrison Institute on Aging, Texas Tech University Health Sciences Center, 3601 4th Street, MS 9424, Lubbock, TX 79430, USA

[2]Cell Biology Department, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, 109 Hunterian, Baltimore, MD 21205, USA

[3]Cell Biology & Biochemistry Department, Texas Tech University Health Sciences Center, 3601 4th Street, MS 9424, Lubbock, TX 79430, USA

[4]Pharmacology & Neuroscience Department, Texas Tech University Health Sciences Center, 3601 4th Street, MS 9424, Lubbock, TX 79430, USA

[5]Neurology Department, Texas Tech University Health Sciences Center, 3601 4th Street, MS 9424, Lubbock, TX 79430, USA

[6]Speech, Language and Hearing Sciences Departments, Texas Tech University Health Sciences Center, 3601 4th Street, MS 9424, Lubbock, TX 79430, USA

[7]Garrison Institute on Aging, South West Campus, Texas Tech University Health Sciences Center, 6630 S. Quaker Suite E, MS 7495, Lubbock, TX 79413, USA

Teaser:

This review focuses on the latest developments in tau research for Alzheimer's disease and other tauopathies, highlighting therapeutic approaches against antiphosphorylated tau using pharmacophore-based molecular and bioinformatics methods.

Phosphorylated tau (P-tau) has received much attention in the field of Alzheimer's disease (AD), as a potential therapeutic target owing to its involvement with synaptic damage and neuronal dysfunction. The continuous failure of amyloid β (Aβ)-targeted therapeutics highlights the urgency to consider alternative therapeutic strategies for AD. The present review describes the latest developments in tau biology and function. It also explains abnormal interactions between P-tau with Aβ and the mitochondrial fission protein Drp1, leading to excessive mitochondrial fragmentation and synaptic damage in AD neurons. This article also addresses 3D pharmacophore-based drug models designed to treat patients with AD and other tauopathies.

[*]*Corresponding author*: Reddy, P.H. (hemachandra.reddy@ttuhsc.edu).

Keywords

Alzheimer's disease; tau protein; hyperphosphorylation; 3D pharmacophore; dynamin-related protein 1; tauopathies

Alzheimer's disease and tau

Alzheimer's disease (AD) is characterized by a collection of multiple, age-related mental conditions that include memory loss and other cognitive impairments. Currently, 50 million people, including 5.4 million Americans, suffer from AD [1]. With increased longevity in the general population, AD is a major health concern for society. Several years of intense research using postmortem human AD brains and brain specimens from AD mouse models have revealed physiological deregulation associated with AD disease progression, including loss of synapses and synaptic function, mitochondrial structural and functional abnormalities, inflammatory responses and neuronal loss, in addition to the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles [2,3]. Although amyloid β (Aβ) and phosphorylated tau (P-tau) are involved in disease progression [4], mounting evidence suggests that the role of P-tau in the progression and pathogenesis of AD is largely through the impairment of axonal transport of subcellular organelles, including mitochondria, lysosomes, vesicles and proteins, to nerve terminals from cell soma [5]. In addition to its involvement in AD, P-tau is also reported to be involved in frontotemporal dementia and other tauopathies [6]. Cellular changes that are involved in disease progression of AD and other tauopathies are not completely understood. The purpose of this review is to highlight the recently discovered involvement of P-tau in AD and other tauopathies and to summarize the results from the latest studies of structure and function in healthy persons and in persons in diseased states. Our article also explains abnormal interactions between P-tau with Aβ and the mitochondrial fission protein dynamin-related protein (Drp)1, leading to excessive mitochondrial fragmentation and synaptic damage in AD neurons and how reduced P-tau and/or Drp1 protects from P-tau-induced toxicities in AD. This article also outlines pharmacophore-based models as therapeutic approaches in AD and other tauopathies.

Tau stabilizes microtubules

Tau is a cytoskeletal protein that stabilizes neuronal microtubules. These microtubules are abundant in nerve cells and present to a much lesser degree in oligodendrocytes and astrocytes [7]. The importance of tau in the assembly of microtubules was first elucidated in the 1970s [8–12]. Neuronal microtubules are the major component of nerve cells and are involved in nutrient transport and signal neurotransmission [13–15]. When tau becomes defective and fails to adequately stabilize microtubules, impaired axonal transport in neurons results [16]. Tau is an essential protein for signaling molecules that regulate gene transcription and cell cycle activity [17]. The cytoskeleton and plasma membrane, both of which act as scaffolding for a variety of signaling molecules, modulate the axonal transport process [18]. αβ-Tubulin proteins are the cytoskeleton proteins that are essential for the formation of the microtubule structure [19,20]. The microtubule-associated protein tau

(MAPT) interacts with the αβ-tubulin dimers during structure formation and maintenance of the microtubules [21–23]. However, in neurodegenerative diseases, such as AD and frontotemporal dementia, excessive tau deposition disassembles and the structure of microtubules and neurofibrillary tangles forms [24,25]. Tau, in a nondiseased state, functions as a valuable protein in intracellular transport and in the development and maintenance of the cellular cytoskeleton. However, in AD, tau excessively aggregates in neurons, leading to abnormal tau phosphorylation and microtubule disintegration [26–28]. It has been hypothesized that, in disease states such as AD, aggregates of excessive tau disrupt microtubule formation and interfere with maintenance, impairing axonal transportation in the neuronal cells, resulting in synaptic starvation and neuronal death [29–31]. The human tau protein is encoded by a single gene known as *MAPT*, located on chromosome 17q21.

The structure of this gene has been hard to characterize owing to its alternative splicing and immature mRNA sequences [32]. *MAPT* comprises 16 exons. Although exons −1 and 14 are transcribed, they are not translated [33]. Six tau isoforms are produced with the full-length tau protein, which is 441 amino acid residues long. The major domains of the tau protein are the N-terminal acidic domain, the proline-rich domain and the C-terminal microtubule-binding repeat domain. The N-terminal acidic domain is composed of two inserts (exon 2 and exon 3), and the microtubule-binding domain is composed of the four inserts R1–R4, which are encoded by exons 9–12 in *MAPT*. In combination, these six tau isoforms contain either three (3R) or all four (4R) of the repeat inserts of the microtubule-binding domains [34,35]. The N-terminal part interacts with the neuronal plasma membrane and cytoskeletal elements, and the C-terminal microtubule-binding domain regulates the rate of microtubule polymerization and stabilization [36,37]. Either three (3R) or four (4R) repeat domains are responsible for tau interacting with microtubules [38]. There is a significant difference in the ratio of 4R to 3R in nondiseased and diseased brains. In microtubule-binding repeats of nondiseased human brains, 4R and 3R ratios are approximately equal (1:1) in contrast to their ratio in diseased states, such as in AD and tau pathologies, where the ratio is 2:1 (4R: 3R) [39]. The tau protein promotes and regulates the assembly of microtubules and the stability of the cellular assembly [40] through the propagation of dendritic signals to particular axons.

Phosphorylated tau

Tau contains nearly 80 potential serine (Ser) and threonine (Thr) phosphorylation sites on the longest tau isoform [41]. Tau is hyperphosphorylated at specific sites in AD neurons [42] by kinases and phosphatases. In turn, P-tau plays a significant part in disrupting tau–microtubule interactions, impairing axonal transport, leading to defective neurite outgrowth [43]. Recent research also revealed that tau phosphorylation occurs owing to several factors, including Aβ-mediated caspase activation, Aβ-mediated oxidative stress, chronic oxidative stress, reduced insulin-like growth factor (IGF)1-mediated oxidative stress and mutations in the tau gene [44].

Interaction between tau and microtubules

Tau uses microtubule polymerization to regulate axonal stability and cell morphology, maintain cell structure, and regulate the transport of cargo across neurons. In the neuron, tau

promotes the mobility of secretory vesicles, organelles and the organization of macromolecules, such as kinesin and dynein [44]. In several neurodegenerative diseases, including AD, P-tau has been abnormally associated with microtubules, leading to defective assembly of microtubules (Figure 1) [45]. This defective microtubule association can lead to impaired axonal transport of organelles and synaptic impairment; and could ultimately lead to neuronal dysfunction. In addition, in a diseased state, P-tau cannot bind to microtubules for stabilization, owing to binding vicinities of microtubule-associated proteins (MAPs) with P-tau. Studies have found that, in a diseased state, defective binding of P-tau with microtubules is associated with neuronal death in tauopathies [46–49]. In neurodegenerative diseases and tauopathies, the normal functioning of tau is disrupted, tau undergoes phosphorylation and tau is aberrantly cleaved. In AD, tau undergoes several aberrant changes that lead to neuronal damage including: (i) tau mislocalization; (ii) hyperphosphorylation [50,51]; and (iii) tau–Aβ interactions causing aggregation and/or fibrillation [52].

Mislocalization of tau

Aberrant tau, in particular P-tau, plays a crucial part in mediating tau mislocalization and subsequent synaptic impairments in AD. P-tau can cause abnormal interactions with other cytosolic proteins, such as MAPs, resulting in the inhibition and disruption of microtubules [53]. The mislocalization of P-tau in neurons in diseased states can also disrupt axonal transport, leading to the degeneration of synapses [54], in contrast to axonal transport in nondiseased states, where healthy neurons maintain the proper special gradient of tau higher in axons and where somato-dentrites exhibit comparatively low levels of healthy tau [55].

In the studies of tau using wild-type and AD mouse models, tau-mediated neurodegeneration was found to be widespread in the AD-affected brains and has been found to exhibit P-tau with an inverted gradient reported [56]. The mislocalization of P-tau in diseased states has been found to increase the proteasome components of tau oligomers, leading to increased synaptic damage in AD and tauopathies [57].

Tau phosphorylation and kinase regulations

Many kinases, such as proline-directed proteins, mitogen-activated proteins and cyclin-dependent kinases, are involved in the AD-associated phosphorylation of tau [58,59]. There is a counterbalance between kinase and phosphatase functions associated with stability and axonal growth maintained by normal tau. The phosphorylation of tau at different sites in tau protein impacts its function and pathogenic role differently [60]. When tau phosphorylation occurs at the sites of kinases, such as the cyclin-dependent kinases (Cdks), protein kinase A (PKA), protein kinase C (PKC) and calmodulin-dependent protein kinase (CaMK), tau functions abnormally. At Cdk sites, hyperphosphorylation of tau occurs at Ser235, Ser202 and Ser404, and tau promotes hyperphosphorylation and promotes self-aggregation of tau filaments. When tau phosphorylation occurs at Ser and Thr sites (e.g., Ser214, Ser324, Ser356, Ser409 and Ser416), tau targets PKA, resulting in the pathological state (Figure 2) [61]. All the Ser or Thr sites have been reported to participate in kinase-dependent tau hyperphosphorylation, leading to paired helical filaments (PHFs) of tau [62].

Phosphorylation sites of tau, whether at Ser, Thr or kinase sites, are affected by several proline-directed kinases, such as mitogen-activated protein kinase (MAPK), Cdk5 and glycogen synthase 3 (GSk3) [63]. The tau phosphorylation at kinase-dependent sites could have a role in the identification of diagnostic markers in AD and tauopathies.

Phosphorylated tau and Aβ interactions

Aβ is an important pathological protein found in the brains of individuals with AD [64]. Aβ is formed by successive cleavage of the Aβ precursor protein (APP), and the successive cascade of cellular events in the AD process [65]. Using postmortem brains from control subjects and AD patients at different stages of disease progression, and from AβPP, AβPPxPS1 and 3xTg-AD transgenic mouse models, researchers found that monomeric Aβ and oligomeric Aβ interact with P-tau and that these interactions progressively increased as AD progresses [66]. This increase in the interaction between P-tau and Aβ interaction was found to correlate with damaged neuronal structure, a condition that could lead to cognitive decline in AD patients. Tau aggregation and interactions, such as in hyperphosphorylation and mislocalization of tau, contribute to P-tau pathology, and apparent molecular mechanisms underlining P-tau-mediated neuronal loss acknowledged a potential therapeutic target in AD and tauopathies.

Phosphorylated tau and mitochondrial fission protein Drp1 interactions

Recent co-immunoprecipitation and co-localization studies from our laboratory revealed that the mitochondrial fission protein Drp1 interacted with P-tau, and this interaction increased as AD progressed [44]. Based on these findings, we hypothesize that a partial deficiency of Drp1 inhibits Drp1-phosphorylated tau interactions and protects neurons from P-tau-induced mitochondrial and synaptic toxicities; and maintains mitochondrial and neuronal functions in AD neurons. To test this hypothesis, Drp1 heterozygote knockout ($^{+/-}$) mice were crossed with transgenic tau mice (P301L line) creating double-mutant (TauXDrp1$^{+/-}$) mice [67]. Mitochondrial fission, fusion biogenesis and synaptic genes in brain tissues from 6-month-old Drp1$^{+/-}$, tau, TauXDrp1$^{+/-}$ and wild-type mice were studied. Results showed mRNA and protein levels of fission genes and increased levels of fusion, biogenesis and synaptic genes in 6-month-old double-mutant (TauXDrp1$^{+/-}$) mice relative to tau mice. P-tau was found to be reduced in double-mutant mice relative to tau mice. These findings suggest that a partial reduction of Drp1 decreases the production of P-tau, reduces mitochondrial dysfunction and maintains mitochondrial dynamics, enhancing mitochondrial biogenesis and synaptic activity in tau mice.

Loss of tau function

Increasing evidence suggests that tau is also involved in synaptic activity, and complete loss of tau triggers synaptic dysfunction and neuronal damage. To understand the links between loss of tau and synaptic and cognitive functions, Velazquez and colleagues [68] generated an adeno-associated virus (AAV) expressing a doxycycline-inducible short-hairpin (Sh)RNA targeted to tau, referred to as AAV-ShRNATau. Using bilateral stereotaxic injections in 7-month-old C57Bl6/SJL wild-type mice with either the AAV-ShRNATau or a control AAV,

they acutely knocked-down tau in the adult hippocampus and found significantly impaired motor coordination and spatial memory. Blocking the expression of the AAV-ShRNATau, thereby allowing tau levels to return to control levels, restored motor coordination and spatial memory. Mechanistically, the reduced tau levels were associated with lower brain-derived neurotrophic factor (BDNF) levels, reduced levels of synaptic proteins associated with learning and decreased spine density. Their study findings suggest that tau is necessary for motor and cognitive function in the adult brain, indicating that tau loss-of-function could contribute to the clinical manifestations of many tauopathies [68].

Therapeutic strategies targeting P-tau

Our current understanding of tau function and dysfunction has been informed by pharmacophore-based strategies to discover new molecules that can target P-tau. P-tau-mediated therapeutic drug molecules can increase microtubule stability, inhibit P-tau production, as well as oligomerization and fibrillization, and activate phosphatase functions [69]. The inhibition of P-tau kinases and the activation of phosphatases in AD and other tauopathies could play a part in new molecular drugs that can target these kinases and phosphatases [70].

Tau reduction

Several tau mouse knockout studies have found that reduced neurotoxic functions of P-tau in tauopathies were linked to Aβ-dependent toxicity [70]. Other studies have found that an important therapeutic strategy in the treatment of neurodegenerative diseases might be to reduce tau expression [71].

Microtubule stabilization

Stabilizers of the microtubule structure have been found to be drug resistant and subject to toxicities that necessarily limit drug doses. These stabilizers include the antitumor drug taxol, which stabilizes the microtubule structure by stalling the cell cycle in its G1 or M phases [72]. However, adverse side effects have been associated with taxol and its second-generation analog docetaxel [73]. Some drugs, such as davunetide and epothilone D microtubule-stabilizing drugs, were tested in human clinical trials to determine their adverse and well-tolerated side effects [74–78]. All microtubule-stabilizing drugs showed no therapeutic benefits, and they did not significantly stabilize the microtubules.

Phosphatase activation and regulation

Tau is dephosphorylated by protein phosphatase (PP)-2A and, to a minor extent, by PP-1 and PP-2B (calcineurin). Recent studies revealed that the mRNA levels of PP-2A and PP-1 are reduced in AD patients compared with controls, indicating that P-tau influences the extent of phosphatase activation [79]. A decrease in phosphatase activity might result in impaired tau dephosphorylation, as well as enhanced tau phosphorylation because various tau-directed kinases are activated by hyperphosphorylation [80]. To date, there are no published papers that suggest pharmacological approaches have successfully activated PP-2A. Okadaic acid is an inhibitor of the Ser and Thr phosphatases PP-1 and PP-2A, each of which blocks the activation of extracellular, signal-regulated protein kinases [81]. However, phosphatase

therapeutics might not serve as productive drugs in AD owing to their adverse side effects in clinical trials.

Drug development

Phosphorylation of tau could play a crucial part in the formation of neurofibrillary tangles. However, researchers have not yet been able to develop drugs to prevent P-tau propagation and aggregation.

Drug discovery

There have been significant advances in the development of drugs to treat AD and other tauopathies. Several small molecules have been identified that target tau-mediated AD and tauopathies. The current structure-based pharmacophore approach has accelerated pharmacokinetic studies and has focused on discovering small molecules capable of reducing various diseases, including AD and other tauopathies [82]. From the past decade to the present day, current therapeutics of AD and other tauopathies have been unsuccessful [83]. Such a lack of success could be caused, at least in part, by the lack of computational and bioinformatics methodologies in studies aiming to discover effective treatments for neurodegenerative diseases. One such computational methodology is pharmacophore molecular docking.

Molecular docking

Molecular docking is an effective methodology used to characterize the behavior of small molecules targeting binding sites of P-tau. In the ligand-based pharmacophore approach, molecular docking is designed to predict the primary binding modes of ligands within a 3D receptor molecule. Molecular docking explains how the ligands inhibit the target in a measurable manner. The interaction of residues and binding modes of ligands could help explain fundamental biochemical processes of a ligand. This molecular docking elucidates the binding behavior of lead molecules and the targets and is essential for rational drug discovery. In light of the significance of P-tau, 3D molecular docking interactions of ligands within the active core site are expected to provide the most effective inhibition of ligands for drug development. In studies of drug development for AD patients, small molecules have been reported to target and to bind at specific P-tau sites, resulting in a change in how P-tau functions [84].

Tau pharmacophore models

P-tau-based pharmacophore modeling procedures are expected to elucidate characteristics of tau aggregates and to identity hyperphosphorylated sites where binding occurs in AD and tauopathies. Structural-based pharmacophore models of P-tau have increased our knowledge of steric and electrostatic features of ligand atoms, which appear to be optimal for molecular docking and interacting with P-tau. 3D features of P-tau models are based on the actual biological features of molecular modeling. It is these actual features of pharmacophores that are responsible for the molecular behavior of the P-tau protein [85,86]. Further, based on these features, molecular modeling could effectively inhibit or reduce the phosphorylation of tau and reduce the interaction of P-tau with ligands. The P-tau pharmacophore models

strengthen the phenomena with the key functional groups of atoms and pertained volume associated with the functional scaffold. As a pharmacophore, P-tau could target the supramolecular interactions between tau and ligands within the confined 3D structure of P-tau (Figure 3) [87].

Researchers of pharmacophore models are trying to develop drugs capable of inhibiting P-tau aggregation in AD and other tauopathies. These models are expected to mimic the features of tau kinases and to promote the interaction of tau and ligands [88] but their adverse side effects must prove to be minimal. They are also expected to facilitate HTS of drug targets. Ultimately, these models are expected to become the gold standard in drug delivery (Figure 4).

Concluding remarks and future perspectives

Tau plays an important part in the effective functioning of microtubules by promoting the structural integrity of microtubules and facilitating axonal transport. Results from studies of tau phosphorylation and neurofibrillary tangles have pointed to the importance of tau receiving more attention as a therapeutic target in AD and other tauopathies. Currently, Aβ-based therapeutics have failed patients with AD, forcing researchers and clinicians to consider other types of drugs, including P-tau-based therapeutics. Reduced P-tau is also considered as a therapeutic approach in AD. There is increasing interest in developing 3D, pharmacophore-based drug discovery systems that target P-tau for AD and related tauopathies.

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Highlights:

- Tau plays an important part in the formation of axonal microtubules
- Phosphorylated tau (P-tau) plays an aberrant part in causing Alzheimer's disease
- P-tau is a potential therapeutic target in Alzheimer's disease and tauopathies
- P-tau-targeted 3D pharmacophore drug models are important for Alzheimer's disease and tauopathies

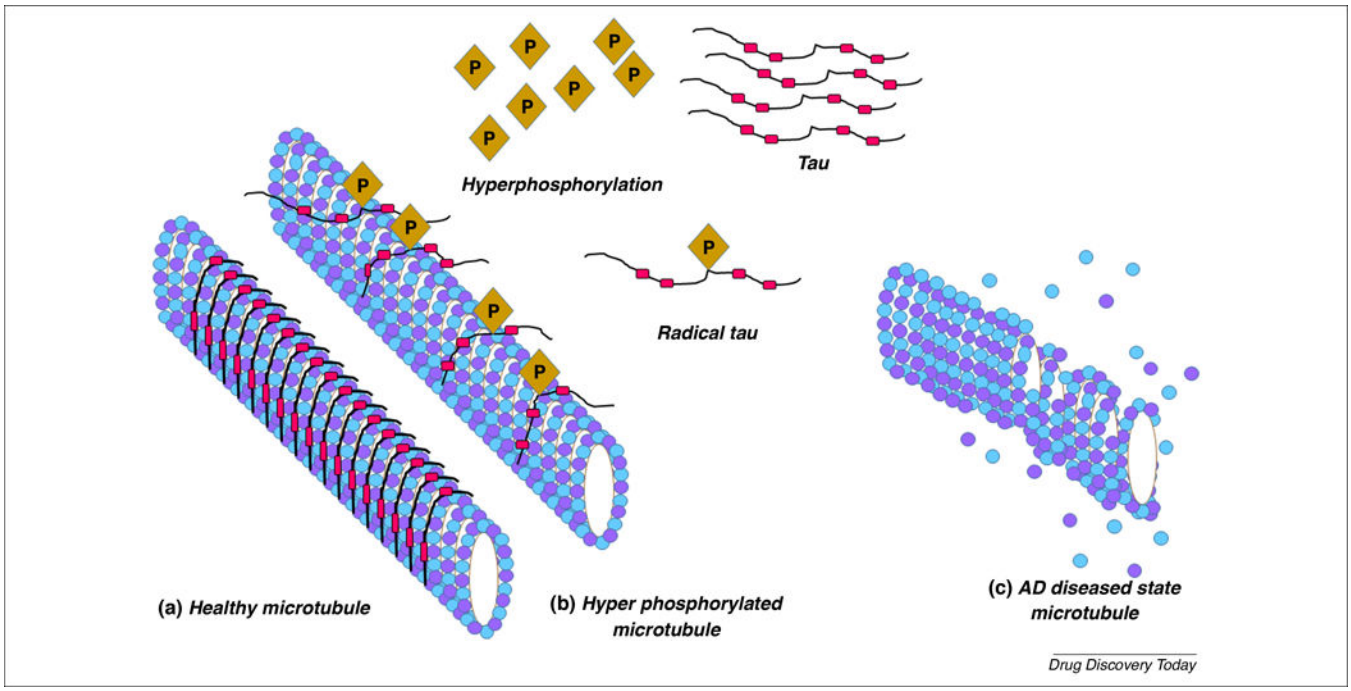

Figure 1.

Possible cascade of tau events. Normal tau maintains the stability of microtubules (**a**). Hyperphosphorylated tau can trigger the dissociation of radical tau from the microtubule surface, potentially destabilizing the microtubule structure (**b**). Changing the properties of the microtubule surface with dissociated microtubules could affect the transport of organelles and lead to the formation of paired helical filaments and (**c**) paired helical filaments in the Alzheimer's disease (AD) state.

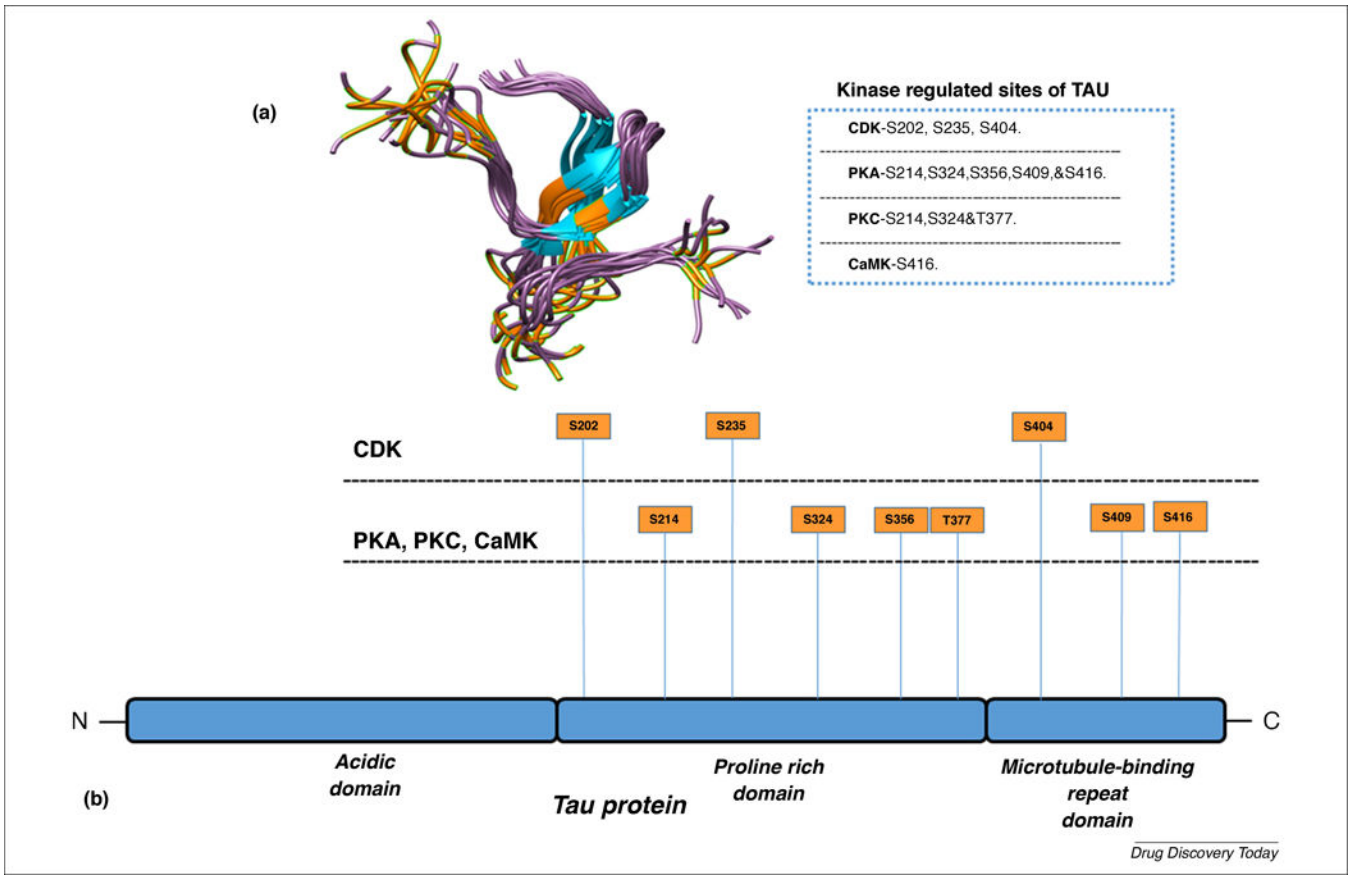

Figure 2.

3D structure of the tau protein at serine and threonine sites. Tau regulated by four main kinase enzymes: cyclin-dependent kinase, protein kinase A, protein kinase C and calmodulin-dependent protein kinase. (**a**) The kinase-dependent regulated site of serine and threonine residue locations on a 3D structure of tau (highlighted in orange), and (**b**) the full-length tau protein consists of an acidic, proline-rich domain and a microtubule-binding repeat domain. See the four kinase-targeting locations of tau (highlighted on the protein model of tau).

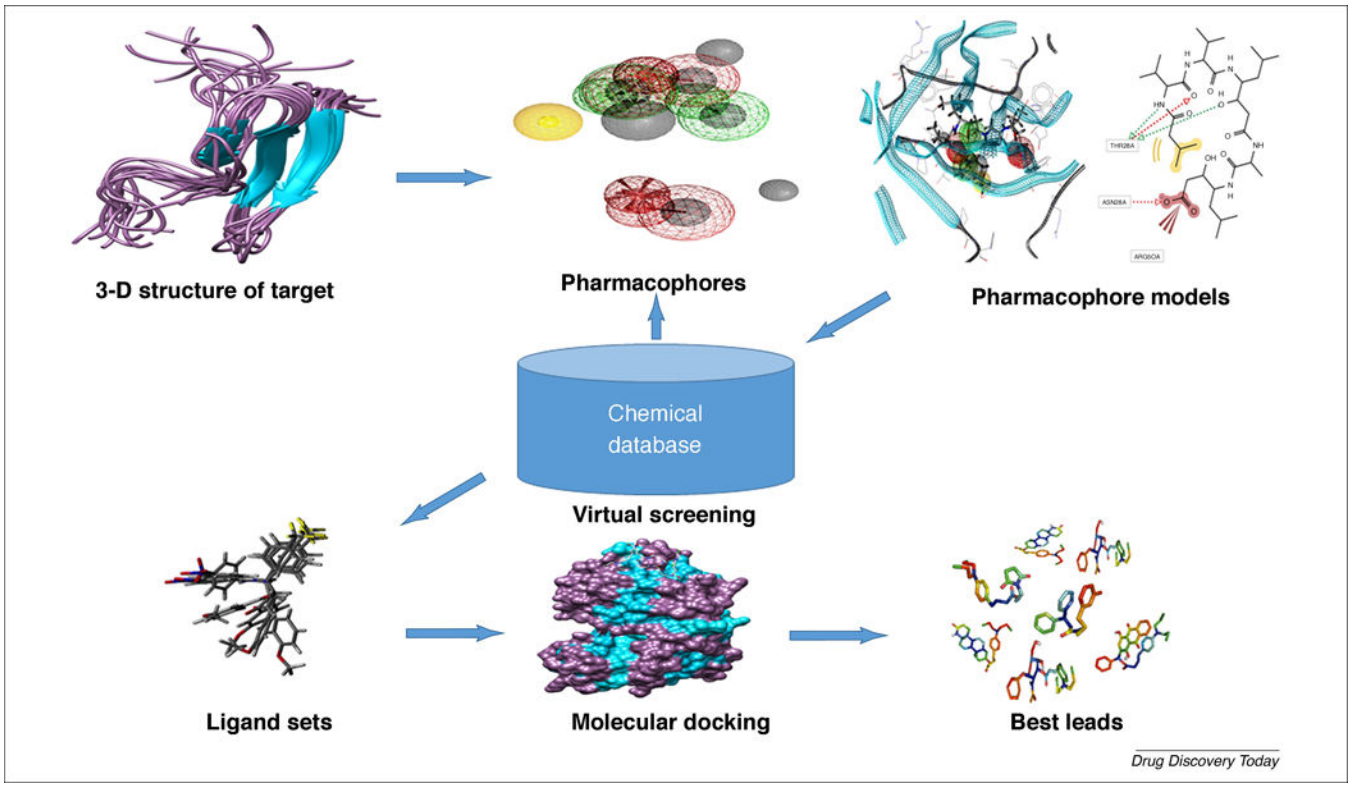

Figure 3.

Pharmacophore models of P-tau. 3D structural architecture of P-tau and the drug discovery workflow with 3D structure of target (tau), pharmacophores, pharmacophore models, virtual screening, ligand sets, molecular docking and best leads.

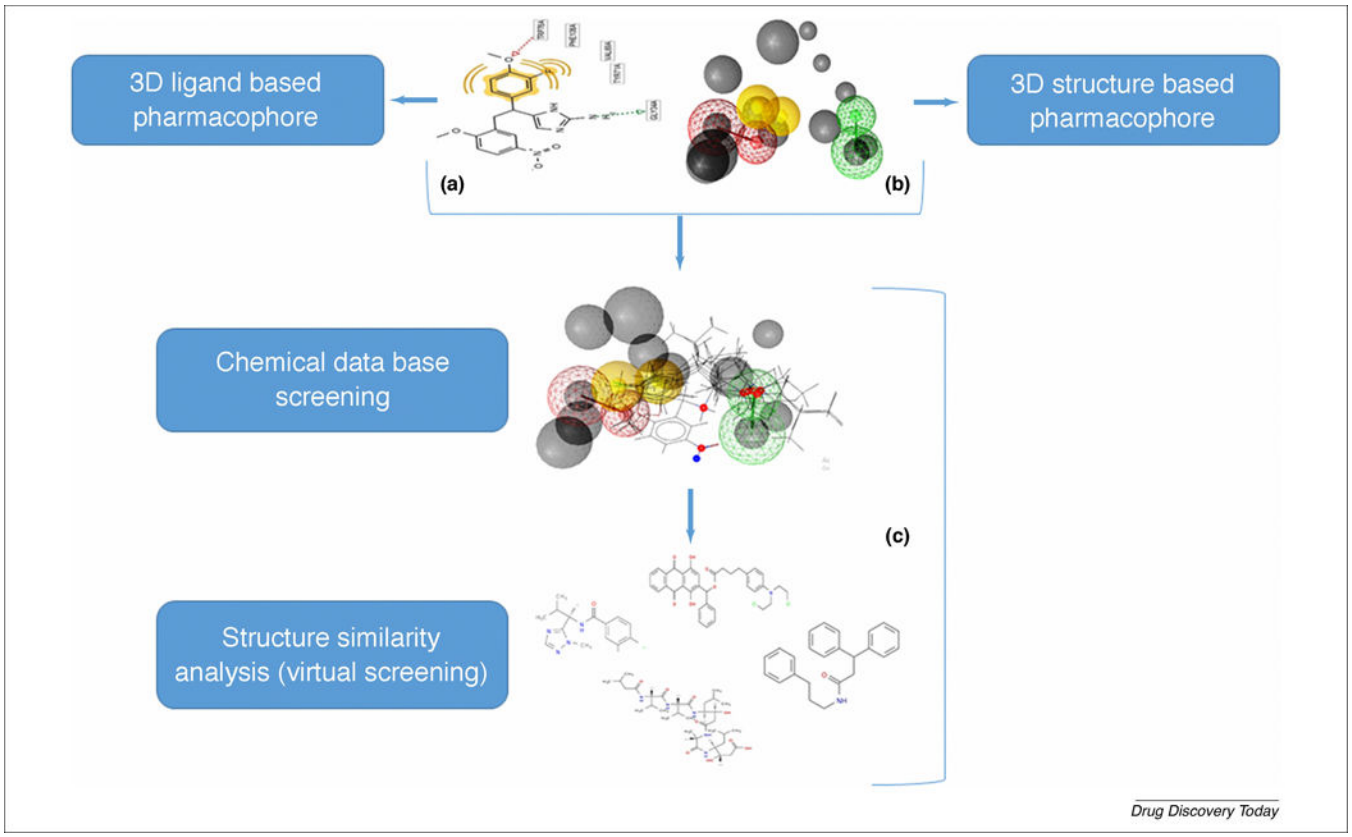

Figure 4.

Tau pharmacophore-based screening of drug targets. Shown are two procedures of pharmacophore development that could be encountered when starting a pharmacophore-based virtual screening of tau: (**a**) a ligand-based 3D pharmacophore with a known ligand structure and ligand fingerprints; and (**b**) a 3D pharmacophore model with a known protein structure. In both cases, pharmacophore query can be used to identify chemical features, such as H-bond acceptors and H-bond donors, and anionic (−), cationic (+), hydrophobic (H) and aromatic (R) groups for virtual screening. (**c**) Results are shown from virtual searches of chemical databases using preferential software algorithms to find the best reliable ligand sets with structure similarity targets for docking.