

Discovery of Brain-Penetrant, Orally Bioavailable Aminothienopyridazine Inhibitors of Tau Aggregation

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Agents capable of preventing the misfolding and sequestration of the microtubule-stabilizing protein tau into insoluble fibrillar aggregates hold considerable promise for the prevention and/or treatment of neurodegenerative tauopathies such as Alzheimer's disease. Because tauopathies are characterized by amyloidosis that is restricted to the central nervous system (CNS), plausible candidate compounds for in vivo evaluation must both prevent tau fibrillization and achieve significant brain levels. Recently, we reported the discovery of the aminothienopyridazine (ATPZ) class of tau aggregation inhibitors and now describe a series of new analogues that are both effective inhibitors of tau fibrillization and display significant brain-to-plasma exposure ratios after administration to mice. Further, two of the most promising examples, **15** and **16**, were found to reach significant brain exposure levels following oral administration. Taken together, these results suggest that examples from the ATPZ class hold promise as candidates for in vivo efficacy studies in animal models of neurodegenerative tauopathies.

Introduction

The misfolding and aggregation within neurons of the microtubule (MT)⁴ associated protein (MAP) tau comprise a key pathogenic phenomenon shared by different neurodegenerative conditions, collectively known as tauopathies, which include Alzheimer's disease (AD), Pick's disease, and certain forms of frontotemporal dementia (FTD) including FTD with Parkinsonism linked to chromosome 17 (FTDP-17).¹ The protein tau, expressed predominantly in neurons, stabilizes the MT network within axons, thereby facilitating axonal transport of proteins, trophic factors, and other cellular constituents, including neurotransmitters. Tau is in dynamic equilibrium between the MT-bound and -unbound states in the cytosol. This equilibrium is believed to be controlled predominantly by the action of tau kinases and phosphatases, as the phosphorylation state of tau is known to modulate the affinity of this protein for the MTs.² Under physiological conditions, the vast majority (i.e., ~99%) of tau is associated to MTs.^{3,4} Conversely, under pathological conditions, tau is abnormally disengaged from the MTs and as such becomes considerably more prone to misfolding.⁵ Once tau is misfolded, a self-assembly reaction can ensue that

ultimately results in the formation of characteristic structures, such as neurofibrillary tangles (NFTs) and neuropil threads, that constitute the diagnostic signatures of neurodegenerative tauopathies. Although the exact mechanisms of tau-mediated neurodegeneration have not been completely defined, aggregation of tau can lead to neuropathology at least in part by causing or contributing to axonal transport deficits arising from the loss of the normal MT-stabilizing function of tau.⁵ Further, different forms of aggregated tau may also contribute to neuropathology by other less defined mechanisms.⁶ Agents capable of preventing the self-assembly of tau thus comprise an attractive strategy for the prevention and/or treatment of neurodegenerative tauopathies.^{7,8}

In recent years, several compound classes have been disclosed that can effectively inhibit the assembly of tau into filaments in vitro.^{9–19} However, with the exception of a member of the phenothiazines, methylene blue (**1**, Figure 1),

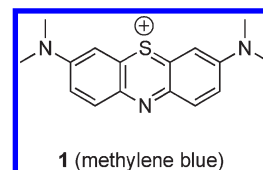


Figure 1

which has progressed to human clinical trials, no tau assembly inhibitor has been assessed for efficacy in vivo. Notably, the results from a phase 2 study, involving 321 patients with mild or moderate AD revealed a significant reduction in cognitive decline in patients that received methylene blue compared to a placebo. These promising findings, if confirmed, validate the idea that tau aggregation inhibitors can be therapeutically

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^a Abbreviations: CNS, central nervous system; ATPZ, aminothienopyridazine; MT, microtubule; AD, Alzheimer's disease; FTD, frontotemporal dementia; FTDP-17, frontotemporal dementia with Parkinsonism linked to chromosome 17; NFT, neurofibrillary tangle; BBB, blood–brain barrier; SAR, structure–activity relationship; PSA, polar surface area; HBD, hydrogen-bond donor; HBA, hydrogen-bond acceptor; ThT, thioflavine-T; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ip, intraperitoneal; iv, intravenous; PK, pharmacokinetic; AUC, area under the curve.

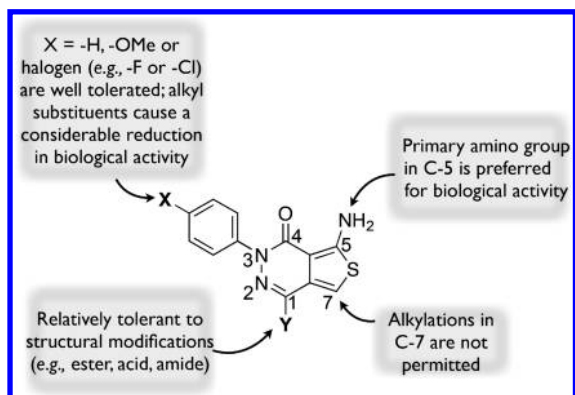


Figure 2. Summary of structure tau antifibrillization activity of ATPZs.²¹

useful for the treatment of neurodegenerative tauopathies. However, since methylene blue is known to modulate a multitude of biological targets,²⁰ whether the outcome observed in the phase 2 study can be ascribable solely to a direct inhibition of tau aggregation is not yet clear. As a result, further evaluation of the therapeutic potential of tau aggregation inhibitors is likely to require in vivo efficacy studies involving additional candidate compounds. To this end and in light of the fact that tauopathies are characterized by amyloidosis that is restricted to the central nervous system (CNS), candidate compounds for in vivo testing will have to be brain-penetrant. Although numerous classes of tau fibrillization inhibitors have been reported in recent years, including some that exhibit calculated physical–chemical properties potentially appropriate for blood–brain barrier (BBB) permeation,¹⁷ to date, there are no reports demonstrating brain penetration of any of these candidates.

Recently, we reported the discovery of a novel class of tau aggregation inhibitors, known as the aminothienopyridazines (ATPZ), which exhibit a promising combination of activity in

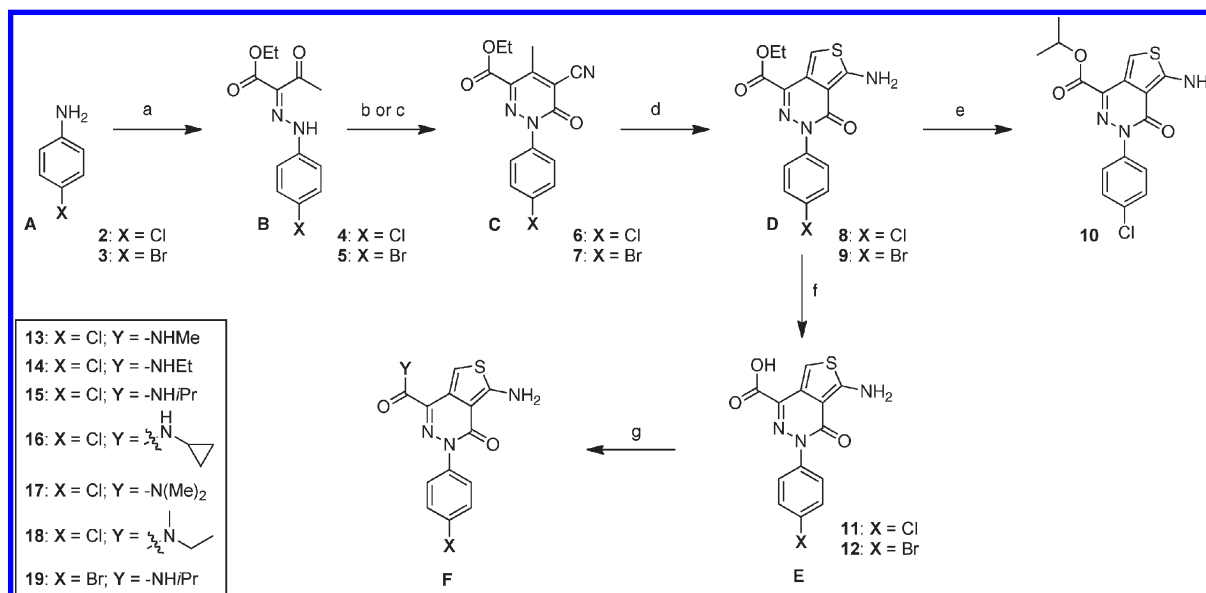
tau fibrillization assays as well as druglike physical–chemical properties (Figure 2).²¹ To evaluate better the potential of the ATPZs as possible candidates for future in vivo efficacy studies, we designed and synthesized a set of derivatives focused on possible BBB permeability. These compounds were evaluated for efficacy against tau aggregation in vitro, as well as for brain penetration in vivo. Notably, these experiments (vide infra) led to the identification of prototype compounds (i.e., **15**, and **16**; Scheme 1), which are both effective in preventing the fibrillization of tau in vitro and capable of reaching significant brain levels in mice after oral administration.

Compound Design and Synthesis

The design of the ATPZs employed in these studies took into account the structure–activity relationships (SARs) for this class,²¹ as well as key physical–chemical properties such as lipophilicity (i.e., calculated partition coefficient between *n*-octanol and water, or ClogP), polar surface area (PSA), the number of hydrogen-bond donors (HBD) and acceptors (HBA), and molecular weight, all of which are known to play an important role in determining the ability of small molecules to permeate biological membranes via passive diffusion.²² Thus, on the basis of our understanding of the SAR summarized in Figure 2, we designed analogues modified in the X and Y fragments, as these regions of the molecule were known to be relatively tolerant to a series of structural variations.



With respect to physical–chemical properties, we considered the following criteria: MW ≤ 450 Da, PSA ≤ 90 Å², HBA ≤ 7, HBD ≤ 3, and ClogP ≤ 3. On the basis of these restrictions in terms of SAR and physical–chemical properties, compounds of general structure **F** (Scheme 1), bearing a lipophilic *p*-chloro or *p*-bromophenyl (X = Cl or Br) as well as an amide moiety in the Y fragment, were identified as potentially promising (cf., Table 1 for calculated physical–chemical properties). Furthermore, as part of these studies,

Scheme 1. General Synthetic Scheme^a



^a Reagents and reaction conditions: (a) (i) NaNO₂, 37% HCl, ethanol, water, 0 °C, 20 min; (ii) ethyl acetoacetate, sodium acetate, ethanol, water, 0 °C, 2 h. (b) X = Cl: ethyl cyanoacetate, ammonium acetate, acetic acid, 170 °C (microwave irradiation), 4 min. (c) X = Br: ethyl cyanoacetate, 4-aminobutyric acid, 160 °C, 2.5 h. (d) S₈, morpholine, ethanol, 150 °C (microwave irradiation), 15 min; (e) titanium(IV) isopropoxide, isopropanol, 170 °C (microwave irradiation), 40 min; (f) LiOH·H₂O, tetrahydrofuran, water, room temp, 16 h; (g) appropriate amine, BOP reagent, *N,N*-diisopropylethylamine, dimethylsulfoxide, room temp, 4 h.

Table 1. Physical–Chemical Properties, Solubility, Activity, and Brain and Plasma Levels^g

Cpd#	X	Y	PCP ^a	Water Solubility ^b	Activity Against Tau Fibril Formation			Brain and Plasma Levels at 1 h Time Point After I.P. Administration of 5 mg/kg of Test Compound		
					K18PL (ThT) ^c	K18PL Sed. Assay ^d	Tau40 Sed. Assay ^e	Brain (ng/g)	Plasma (ng/ml)	B/P ^f
11	Cl	-OH	MW: 321.7 PSA: 95.9 ClogP: 1.8 ^g	>200 μ M	5.4 \pm 2.3 μ M ^o (84 \pm 7 %) [*]	79%	62%	121 \pm 19	5226 \pm 850	0.023 \pm 0.003
8	Cl	-OEt	MW: 349.8 PSA: 84.9 ClogP: 2.6	13 μ M	3.8 \pm 1.6 μ M ^o (71 \pm 11 %) [*]	70%	48%	67 \pm 53 (8 \pm 4.5) [†]	20 \pm 13 (3558 \pm 2000) [†]	3.3 \pm 0.72
10	Cl	-OiPr	MW: 363.8 PSA: 84.9 ClogP: 2.9	32 μ M	1.6 \pm 1.1 μ M ^o (79 \pm 1 %) [*]	44%	61%	436 \pm 170 (0) [†]	84 \pm 14 (1718 \pm 650) [†]	5.2 \pm 1.1
13	Cl	-NHMe	MW: 334.8 PSA: 87.8 ClogP: 0.9	13 μ M	21.7 \pm 16 μ M ^o (78 \pm 3 %) [*]	68%	51%	34 \pm 9.5	70 \pm 16	0.49 \pm 0.03
14	Cl	-NHEt	MW: 348.8 PSA: 87.8 ClogP: 1.4	32 μ M	6.9 \pm 3.4 μ M ^o (84 \pm 6 %) [*]	94%	57%	886 \pm 65	500 \pm 60	1.8 \pm 0.14
15	Cl	-NH <i>i</i> Pr	MW: 362.8 PSA: 87.8 ClogP: 1.8	32 μ M	5.9 \pm 4.7 μ M ^o (83 \pm 4 %) [*]	65%	45%	1100 \pm 160	499 \pm 130	2.2 \pm 0.47
16	Cl		MW: 360.8 PSA: 87.8 ClogP: 1.5	32 μ M	8.1 \pm 5.7 μ M ^o (83 \pm 3 %) [*]	69%	58%	1299 \pm 110	823 \pm 150	1.6 \pm 0.28
17	Cl	-N(Me) ₂	MW: 348.8 PSA: 79 ClogP: 0.6	32 μ M	20.5 \pm 13.6 μ M ^o (75 \pm 5 %) [*]	69%	69%	14 \pm 7.5	30 \pm 7.8	0.44 \pm 0.15
18	Cl		MW: 362.8 PSA: 79 ClogP: 1.1	80 μ M	32 \pm 4.1 μ M ^o (75 \pm 11 %) [*]	62%	58%	122 \pm 41	138 \pm 45	0.89 \pm 0.05
19	Br	-NH <i>i</i> Pr	MW: 407.3 PSA: 87.8 ClogP: 1.9	13 μ M	3.1 \pm 0.6 μ M ^o (85 \pm 1 %) [*]	58%	55%	641 \pm 210	316 \pm 56	2.0 \pm 0.37

^a Physical–chemical properties (MW, molecular weight; PSA, polar surface area; ClogP, calculated partition coefficient between *n*-octanol and water). ^b Solubility in the sodium acetate buffer employed in the tau fibrillization assay, as determined by light scattering. ^c Tau (K18PL) fibrillization assay monitored by thioflavine (ThT) binding and fluorescence (all test compounds tested in triplicate). ^d Maximal percent inhibition of the K18PL fibrillization as determined by SDS–PAGE analyses of the soluble and insoluble fractions obtained after centrifugation of the fibrillizing K18PL mixtures. ^e Maximal percent inhibition of the tau40 fibrillization as determined by SDS–PAGE analyses of the soluble and insoluble fractions obtained after centrifugation of the fibrillizing tau40 mixtures. ^f Ratio between brain/plasma drug level, as determined by LC–MS/MS, 1 h after ip administration of 5 mg/kg test compound. ^g Symbols indicate the following: (S) ClogP of the neutral form; (o) half maximal inhibitory concentration (IC₅₀); (*) maximal percent inhibition achieved in the assay; (†) detected amount of hydrolysis product (i.e., 11).

we included two representative ester derivatives (i.e., 8 and 10) to evaluate the potential of these congeners to be brain-penetrant, possibly acting as lipophilic precursors of the corresponding acid (11). Depending on the rate of esterase-mediated hydrolysis in brain and plasma, we reasoned that such derivatives may potentially lead to an accumulation of the corresponding acid inside the CNS.

The synthesis of these compounds was achieved via the reaction sequence illustrated in Scheme 1, which entails: (a) a diazonium coupling reaction to form hydrazones of general structure B, (b) a Knoevenagel-type condensation between B and ethyl cyanoacetate to form pyridazines C,^{23,24} and (c) a Gewald aminothiophene synthesis²⁵ to furnish the desired ATPZs of general structure D. Compound 8 was then trans-esterified to the corresponding isopropyl ester 10 upon treatment with Ti(*i*PrO)₄ in isopropanol. Alternatively, compounds 8 and 9 were saponified and the resulting acids (11 and 12, respectively) employed in a series of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) coupling reactions with various primary and secondary amines to furnish compounds 13–19.

Compound Evaluation in Vitro

The solubility of all test compounds in the sodium acetate buffer used for subsequent fibrillization reactions was determined

by turbidimetric measurements (Table 1). Compounds were then evaluated in a heparin-induced tau assembly assay, in which the fibrillization of the truncated K18 tau fragment (comprising four MT-binding repeats) bearing the P301L mutation (K18PL) found in FTDP-17 was monitored by thioflavine-T (ThT) binding and fluorescence (Figure 3A).²¹

The inhibitory activity of test compounds was then confirmed with an orthogonal sedimentation assay in which compound-treated fibrillizing mixtures were centrifuged and quantitation of K18PL tau in the soluble and insoluble fraction was performed by densitometric analyses of SDS–PAGE (Figure 3B). As summarized in Table 1, the ATPZs exhibited IC₅₀ values in the 2–32 μ M range in the primary ThT assay, with maximal percent inhibition of 70–85%. The activity against tau fibrillization was confirmed by sedimentation assay, where the majority of compounds exhibited >50% reduction in pelletable material compared to the untreated control. Although many of the ATPZ compounds were found to reach a solubility limit within the upper concentration range used in the biochemical assay, maximal inhibitory effect was typically achieved at compound concentrations below 30 μ M, where the majority of compounds appeared to be fully soluble (cf., solubility data in Table 1 with representative dose–response curves shown in Figure 3A).

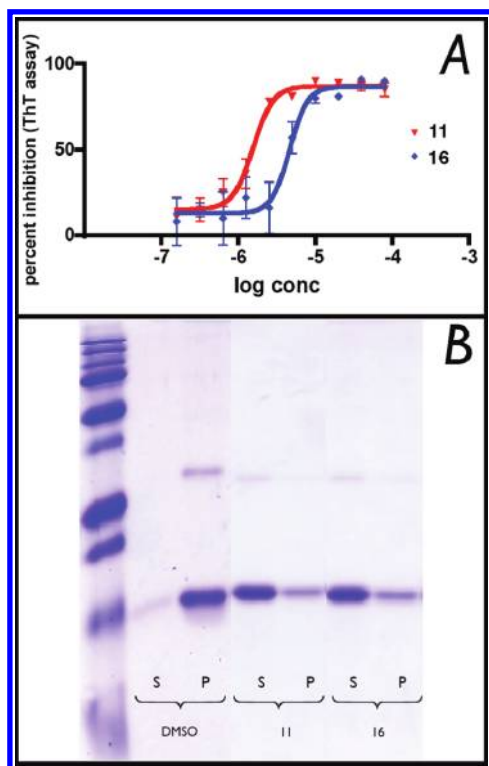


Figure 3. (A) Representative dose–response curves in the heparin-induced K18PL fibrillization assay; (B) SDS–PAGE analysis of supernatant (S) or pellet (P) samples obtained after centrifugation of fibrillizing mixtures incubated in the presence of DMSO vehicle or 100 μ M test compound (**11** and **16**).

This indicates that the inhibition of tau aggregation is likely to be caused by the ATPZs present in solution. However, to further ensure that the observed inhibitory activity of these ATPZs may not be artifactually generated by the presence of insoluble or aggregated test compound,²⁶ we conducted additional experiments in which both DMSO- and compound-treated fibrillizing mixtures were initially centrifuged for 30 min prior to incubation (i.e., at time zero of the fibrillization reaction) to remove any pelletable material. As shown in Figure 4A, no significant differences in compound-mediated inhibition of tau assembly were observed with and without the precentrifugation step. Furthermore, the IC_{50} values of the centrifuged and control samples did not appear to differ appreciably (Figure 4C). Moreover, SDS–PAGE analysis of the soluble and pelletable material after the initial centrifugation step revealed no significant difference between compound- and vehicle-treated mixtures; in all cases K18PL remained largely in the soluble fraction (Figure 4B) and thus was not precipitated by the test compound. Collectively, the activity and SDS–PAGE data confirm that the inhibition of K18PL fibrillization produced by the ATPZ analogues is not caused by insoluble or aggregated material.

All test compounds also inhibited the aggregation of full-length tau (tau40) as determined by sedimentation assay, which revealed efficacy values in the 50–60% range (cf., Table 1). Both K18PL and tau40 activity data are in keeping with prior SAR that suggested that the presence of halogens in the para position of the phenyl ring, as well as carboxylic acid, esters, and amides in the Y fragment, would be generally well tolerated.²¹ Also consistent with our previous studies is the observation that all ATPZs tested (i.e., **8**, **10**, **11**, **13**–**19**) did

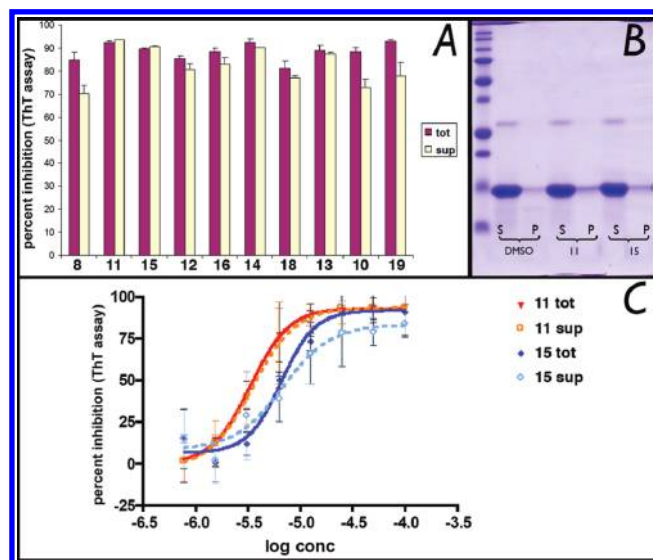


Figure 4. (A) Comparison of maximal percent inhibition of K18PL fibrillization caused by test compounds in which the fibrillizing mixtures did (sup) or did not (tot) undergo centrifugation at time zero to remove insoluble material. Compounds were added at 50 μ M to the tau fibrillization mixture and incubated for 30 min. Samples were then divided into two halves, one of which was incubated without further treatment (“tot”) and one that was centrifuged at 186000g for 30 min. After centrifugation of the latter sample, the supernatants (“sup”) were collected and incubated as per the fibrillization protocol.²¹ (B) Representative examples of SDS–PAGE analysis of supernatant (S) or pellet (P) samples obtained after centrifugation of fibrillizing mixtures prior to incubation. (C) Representative dose–response curves with (“sup”) and without (“tot”) initial centrifugation.

not appear to interfere with the normal MT-stabilizing function of tau in a MT-polymerization assay (Figure 5).

Finally, to evaluate possible major toxicities associated with the ATPZs, all test compounds were evaluated in a cytotoxicity assay that employs rapidly dividing HEK-293 cells. All compounds were found to be nontoxic at 100 μ M.

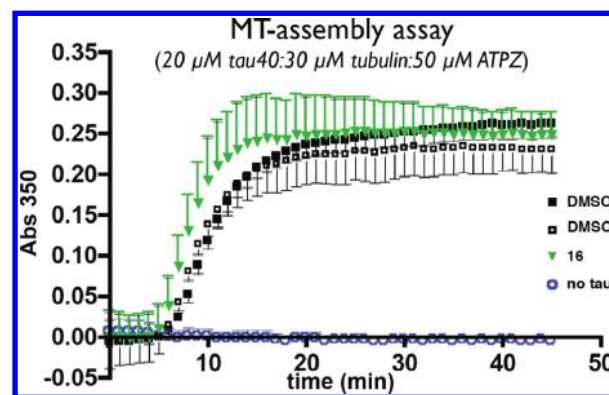


Figure 5. MT-assembly assay. Tubulin (30 μ M) and tau40 (20 μ M) were incubated with the test compound (50 μ M, e.g., **16** [green triangles]) or DMSO (open and black squares) to evaluate possible interference in the tau-promoted MT-polymerization that may be caused by the ATPZ inhibitors of tau aggregation. As control experiment, MT-polymerization assay was also conducted in the absence of tau40 (open blue circles). Additional results are presented in the Supporting Information.

Pharmacokinetic Studies

Test compounds underwent preliminary evaluations of brain penetration, in which 5 mg/kg of each compound was

administered intraperitoneally (ip) to a group of three normal mice; drug levels in brain and plasma were determined at a single time point (1 h) by LC–MS/MS using prevalidated calibration curves. The results of these experiments, summarized in Table 1, revealed that with the exception of **11**, all other test compounds displayed significant brain uptake as demonstrated by the brain-to-plasma exposure ratios (*B/P*) above 0.3. The lack of brain penetration of **11** was not unexpected given that the carboxylic acid moiety of this compound would be mostly negatively charged at physiological pH and thus likely to result in limited passive diffusion of the compound across the BBB. Conversely, the more lipophilic ester derivatives (**8** and **10**) exhibited comparatively higher *B/P* ratio. In particular, **10** was found to reach significantly higher brain concentrations compared to the corresponding acid **11**. However, both esters appeared to have relatively short half-lives in plasma, as indicated by the limited amount of parent drug detected after 1 h from administration of the compounds. Moreover, monitoring for the hydrolyzed metabolite (i.e., **11**) in both brain and plasma revealed a considerable amount of acid **11** in plasma but not in the brain 1 h after administration of either ester. This observation suggests that while lipophilic esters may be able to gain access to the CNS (e.g., **10**), these derivatives may not produce an accumulation of the corresponding ATPZ acid in the CNS at the rate observed in plasma because of significantly less esterase-mediated hydrolysis in the brain. Finally, among the amide derivatives, **14**, **15** and **16** appeared to be particularly interesting because of favorable *B/P* ratios combined with promising indications of good metabolic stability. Compound **16** was selected for full PK analysis in which brain and plasma drug levels were determined at six time points (i.e., 30 min, 1 h, 2 h, 4 h, 8 h, and 16 h) after intravenous (iv) administration of 2 mg/kg test compound to a group of 18 normal mice (3 mice per time point). The results from this experiment, illustrated in Figure 6A, confirmed that the total *B/P* exposure ratio over 16 h is ~ 1.6 . Furthermore, these data reveal that **16** exhibits good metabolic stability as indicated by an elimination half-life of ≥ 2.5 h in plasma.

Finally, in order to investigate the potential for ATPZ congeners to be orally administered, we evaluated brain and plasma level of **16** and **15** after oral administration (oral gavage) of 5 mg/kg. In both cases, significant concentrations of each test compound were achieved in the brain after oral administration (cf., Figure 6B and Figure 6C). Comparison of the integrated area under the curve (AUC) in plasma after oral and iv administration of **16** revealed an oral bioavailability (*F*) of $\sim 70\%$ (cf., Figure 6A and Figure 6B).

Discussion

Despite the hypothesis that tau aggregation inhibitors may be therapeutically useful for AD and related tauopathies, to date, only one compound of this type, methylene blue, has entered clinical trials. Numerous additional classes of compounds have been shown to inhibit tau assembly in vitro, but there are no reports of these molecules being evaluated in vivo for pharmacokinetic (PK) properties or efficacy in models of tauopathy. Because BBB permeability is known to be a major bottleneck that hampers the development of new CNS-active drugs,²⁷ an early evaluation of the brain penetration of candidate compounds is important, as such studies would permit focus on the most promising compound type. For these reasons and to assess the potential of ATPZ inhibitors as possible candidate compounds for in vivo evaluation of

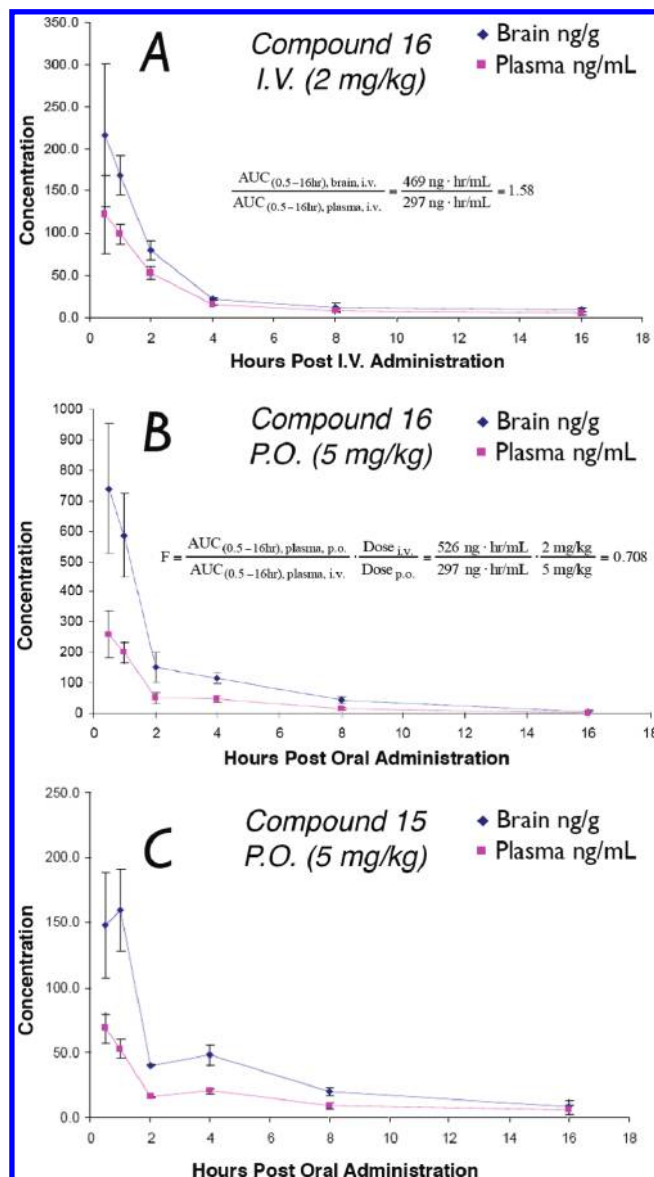


Figure 6. (A) Brain and plasma level of **16** after iv administration of 2 mg/kg; (B) brain and plasma level of **16** after oral (po) administration of 5 mg/kg; (C) brain and plasma level of **15** after oral administration of 5 mg/kg.

efficacy, we conducted a study in which selected analogues, designed for improved BBB-permeability, were evaluated for in vitro activity as well as for brain penetration. Results from the in vitro efficacy studies appeared to be fully consistent with our previous results and confirmed that the ATPZs are most effective in preventing tau fibrillization when present in $\sim 1:1$ molar ratio with tau (i.e., 15 μ M in the fibrillization assay).²¹ Assuming that similar stoichiometric requirements also exist in vivo, any ATPZ candidate for in vivo efficacy study will have to reach free brain concentrations that are comparable to that of the unbound fraction of tau. The total intraneuronal tau concentration (i.e., MT-bound and MT-unbound tau) is estimated to be in the low micromolar range,³ and as previously noted, $>99\%$ of the protein is bound to MTs under physiological conditions.^{3,4} Although the concentration of the MT-unbound fraction of tau is likely to increase in diseased neurons, this may be in the submicromolar range. Thus, only compounds that readily cross the BBB would be viable candidates for in vivo evaluations of efficacy. Interestingly,

preliminary evaluation of brain exposures of the ATPZ test compounds revealed that with the exception of the acid derivative **11**, all other ATPZ congeners exhibited *B/P* ratios above 0.3. Considering that most CNS-active drugs typically exhibit *B/P* > 0.3–0.5,²⁸ these results indicate that ATPZs have the potential to achieve appreciable brain concentrations. Furthermore, selected amide derivatives, such as **14**, **15**, and **16**, were found to reach brain concentrations above 800 ng/g (i.e., >2 μ M) 1 h after ip administration of 5 mg/kg. These results were further confirmed by complete PK studies on **16**, which displayed an average brain-to-plasma AUC exposure ratio of \sim 1.6. Equally important, significant brain concentrations were also observed after oral administration of **15** or **16**, with the oral bioavailability of the latter compound being \sim 70%. Collectively, the results from the PK studies combined with the promising in vitro activity and safety data suggest that the ATPZ class of tau aggregation inhibitors holds considerable promise as candidate compounds for efficacy testing in transgenic mouse models of tauopathies.

Conclusions

Although the preliminary phase 2 clinical data obtained with methylene blue suggest that tau aggregation inhibitors may be therapeutically useful, further validation of the therapeutic potential of compounds of this type for the treatment and/or prevention of neurodegenerative tauopathies is likely to require additional in vivo efficacy studies involving different candidate compounds. To this end, the ATPZs presented here appear to be very promising candidates because of a favorable combination of biological activity in vitro and desirable PK properties, including excellent brain penetration and oral bioavailability.

Experimental Section

Materials and Methods. All solvents were reagent grade. All reagents were purchased from Aldrich or Acros and used as received. Thin layer chromatography (TLC) was performed with 0.25 mm E. Merck precoated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040–0.062 mm) supplied by Silicycle and Sorbent Technologies. TLC spots were detected by viewing under a UV light. Infrared (IR) spectra were recorded on a Jasco model FT/IR-480 Plus spectrometer. All melting points were obtained on a Thomas-Hoover apparatus. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AMX-500 spectrometer. Chemical shifts were reported relative to solvents. High-resolution mass spectra were measured at the University of Pennsylvania Mass Spectrometry Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Single-crystal X-ray structure determinations were performed at the University of Pennsylvania with an Enraf Nonius CAD-4 automated diffractometer. Analytical reverse-phased (Sunfire C18; 4.6 mm \times 50 mm, 5 mL) high-performance liquid chromatography (HPLC) was performed with a Waters binary gradient module 2525 equipped with Waters 2996 PDA and Waters micromass ZQ. All samples were analyzed employing a linear gradient from 10% to 90% of acetonitrile in water over 8 min and a flow rate of 1 mL/min, and unless otherwise stated, the purity level was >95%. Preparative reverse phase HPLC purifications were performed on a Gilson instrument (i.e., Gilson 333 pumps, a 215 liquid handler, 845Z injection module, and PDA detector) employing Waters SunFire preparative C₁₈ OBD columns (5 μ m; 19 mm \times 50 mm, or 19 mm \times 100 mm). Purifications were carried out employing a linear gradient from 10% to 90% of acetonitrile in water for 15 min with a flow rate of 20 mL/min. Yields refer to chromatographically and spectroscopically pure compounds.

Ethyl 2-(2-(4-Chlorophenyl)hydrazono)-3-oxobutanoate (4). **4** was prepared as previously reported.²⁹ Yield: 83%. Mp: 84–85 °C (from ethanol, lit.²⁹ 81–84 °C). ^1H NMR (CDCl_3): δ 1.41 (t, J = 7.0 Hz, 3H), 2.60 (s, 3H), 4.34 (q, J = 7.2 Hz, 2H), 7.34–7.36 ppm (m, 4H). ^{13}C NMR (CDCl_3): δ 14.5, 30.9, 61.2, 117.6, 126.5, 129.8, 131.0, 140.4, 164.9, 197.4 ppm. IR: ν 3359, 1706, 1617 cm^{-1} . HRMS (ESI^+): calculated for $\text{C}_{12}\text{H}_{13}\text{ClN}_2\text{NaO}_3^+$ 291.0518, found 291.0512.

Ethyl 2-(2-(4-Bromophenyl)hydrazono)-3-oxobutanoate (5). 4-Bromoaniline (2.0 g, 11.63 mmol) was dissolved in 37% hydrochloric acid (2.94 mL), ethanol (1.50 mL), and water (1.50 mL). The reaction mixture was cooled to 0 °C in an ice–water bath before a solution of sodium nitrite (0.88 g, 12.75 mmol) in water (2.0 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 20 min. Sodium acetate (3.72 g, 45.3 mmol) in water (5.9 mL) and ethyl acetoacetate (1.51 g, 1.43 mL, 11.63 mmol) were added, and the reaction mixture was stirred at 0 °C for 2 h. The precipitated solid was then filtered, washed with water, and dried under high vacuum for 16 h to provide **5** as yellow solid that was crystallized in ethanol and used without further purification in the next step. Yield: 98%.

Ethyl 1-(4-Chlorophenyl)-5-cyano-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxylate (6). **6** was prepared as previously reported.³⁰ Yield: 47%. Mp: 160–161 °C (from ethanol, lit.³⁰ 190 °C). ^1H NMR (CDCl_3): δ 1.41 (t, J = 7.0 Hz, 3H), 2.76 (s, 3H), 4.43 (q, J = 7.2 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.62 ppm (d, J = 8.5 Hz, 2H). ^{13}C NMR (CDCl_3): δ 14.2, 19.4, 63.0, 112.4, 116.0, 126.3, 129.4, 135.5, 137.5, 138.5, 150.8, 155.8, 162.0 ppm. IR: ν 2235, 1726, 1685 cm^{-1} . MS (ESI^+): calculated for $\text{C}_{15}\text{H}_{13}\text{ClN}_3\text{O}_3^+$ 318.06, found 318.07.

Ethyl 1-(4-Bromophenyl)-5-cyano-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxylate (7). A mixture of **5** (2.0 g, 6.39 mmol), 4-aminobutyric acid (1.33 g, 12.99 mmol), and ethyl cyanoacetate (1.1 g, 1.0 mL, 9.44 mmol) was stirred neat at 160 °C for 2.5 h. After the mixture was cooled, the residue was purified by silica gel flash chromatography (using an ethyl acetate/*n*-hexane gradient from 5% to 80% of ethyl acetate) to provide **7** as a white solid. Yield: 76%. Mp: 174–176 °C (from ethanol). ^1H NMR (CDCl_3): δ 1.41 (t, J = 7.0 Hz, 3H), 2.76 (s, 3H), 4.43 (q, J = 4.4 Hz, 7.2 Hz, 2H), 7.56 (d, J = 9.0 Hz, 2H), 7.64 ppm (d, J = 9.0 Hz, 2H). ^{13}C NMR (CDCl_3): δ 14.2, 19.4, 62.9, 112.4, 116.0, 123.5, 126.6, 132.3, 137.6, 139.1, 150.8, 155.7, 162.0 ppm. IR: ν 2230, 1727, 1681 cm^{-1} . MS (ESI^+): calculated for $\text{C}_{15}\text{H}_{13}\text{BrN}_3\text{O}_3^+$ 362.02, found 362.01.

Synthesis of Thienyl Derivatives with General Structure D (Gewald Reaction). Representative Example: Ethyl 5-Amino-3-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxylate (8). A mixture of **6** (0.100 g, 0.31 mmol), sulfur (0.015 g, 0.47 mmol), and morpholine (0.55 g, 0.55 mL, 0.63 mmol) was heated to 150 °C using microwave irradiation for 15 min. After the mixture was cooled, the precipitate that formed was collected and purified by preparative HPLC to give **8** as a yellow solid. HPLC–MS retention time: 8.20 min. Yield: 64%. Mp: 189–191 °C (from ethanol). ^1H NMR (CDCl_3): δ 1.43 (t, J = 7.3 Hz, 3H), 4.44 (q, J = 7.2 Hz, 2H), 6.20 (broad s, 2H), 7.25 (s, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.56 ppm (d, J = 8.5 Hz, 2H). ^{13}C NMR (CDCl_3): δ 14.4, 62.2, 104.9, 106.9, 127.1, 127.2, 128.9, 133.3, 134.0, 139.2, 159.4, 161.8, 163.0 ppm. IR: ν 3411, 3303, 1724, 1709, 1660 cm^{-1} . HRMS (ESI^+): calculated for $\text{C}_{15}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S}^+$ 350.0366, found 350.0355.

Ethyl 5-Amino-3-(4-bromophenyl)-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxylate (9). **9** was synthesized in the same manner as **8** starting with **7**. Yield: 55%. Mp: 198–200 °C (from ethanol). ^1H NMR (CDCl_3): δ 1.42 (t, J = 7.0 Hz, 3H), 4.44 (q, J = 7.2 Hz, 2H), 6.12 (broad s, 2H), 7.25 (s, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.58 ppm (d, J = 8.5 Hz, 2H). ^{13}C NMR (CDCl_3): δ 14.1, 62.1, 105.9, 106.7, 121.3, 127.1, 127.5, 131.8, 134.0, 139.7, 159.4, 161.9, 163.0 ppm. IR: ν 3407, 3310, 1709, 1657 cm^{-1} . HRMS (ESI^+): calculated for $\text{C}_{15}\text{H}_{13}\text{BrN}_3\text{O}_3\text{S}^+$ 393.9861, found 393.9879.

Isopropyl 5-Amino-3-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxylate (10). A mixture of **8** (0.050 g, 0.143 mmol) and titanium(IV) isopropoxide (0.002 g, 2.1 μ L, 7 μ mol) in isopropanol (0.5 mL) was heated to 170 °C using microwave irradiation for 40 min. After the mixture was cooled, the solvent was evaporated and the residue was purified by preparative HPLC to provide **10** as yellow solid. HPLC–MS retention time: 8.73 min. Yield: 77%. Mp: 118–120 °C (from ethanol). ¹H NMR (CDCl₃): δ 1.41 (d, *J* = 6.5 Hz, 6H), 5.26–5.33 (m, 1H), 6.25 (broad s, 2H), 7.17 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.57 ppm (d, *J* = 8.5 Hz, 2H). ¹³C NMR (CDCl₃): δ 22.0, 70.1, 104.7, 106.8, 127.1, 127.2, 128.8, 133.1, 134.4, 139.2, 159.4, 161.9, 162.5 ppm. IR: ν 3408, 3294, 3145, 1716, 1663, 1585 cm^{−1}. HRMS (ESI⁺): calculated for C₁₆H₁₄ClN₃O₃NaS⁺ 386.0342, found 386.0331.

Synthesis of Compounds with General Structure E (Esters Hydrolysis). Representative Example: 5-Amino-3-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxylic Acid (11). Lithium hydroxide monohydrate (0.063 g, 1.29 mmol) was added to a solution of **8** (0.150 g, 0.43 mmol) in tetrahydrofuran (3 mL) and water (2 mL). The reaction mixture was stirred at room temperature for 16 h. Then 1 N HCl was added (pH ~2) and the formed precipitate was filtered and purified by preparative HPLC to afford **11** as a yellow solid. HPLC–MS retention time: 6.63 min. Yield: 67%. Mp: >300 °C (from ethanol). ¹H NMR (CD₃OD): δ 7.19 (s, 1H), 7.44 (d, *J* = 9.0 Hz, 2H), 7.58 ppm (d, *J* = 9.0 Hz, 2H). ¹³C NMR (DMSO-*d*₆): δ 104.4, 104.5, 126.8, 128.3, 128.9, 132.0, 134.5, 140.2, 158.9, 163.9, 164.6 ppm. IR: ν 3439, 3322, 3065, 1712, 1646, 1590 cm^{−1}. HRMS (ESI⁺): calculated for C₁₃H₉ClN₃O₃S⁺ 322.0053, found 322.0062.

5-Amino-3-(4-bromophenyl)-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxylic Acid (12). **12** was prepared in the same manner as **11** starting with **9**. HPLC–MS retention time: 6.76 min. Yield: 77%. Mp: >300 °C (from ethanol). ¹H NMR (DMSO-*d*₆): δ 7.12 (s, 1H), 7.52 (d, *J* = 9.0 Hz, 2H), 7.63 (broad s, 2H), 7.66 ppm (d, *J* = 8.5 Hz, 2H). ¹³C NMR (DMSO-*d*₆): δ 104.4, 104.5, 120.4, 126.8, 128.6, 131.9, 134.5, 140.6, 158.8, 163.9, 164.5 ppm. IR: ν 3439, 3322, 3065, 1712, 1646 cm^{−1}. HRMS (ESI⁺): calculated for C₁₃H₇BrN₃NaO₃S⁺ 387.9367, found 387.9378.

Synthesis of Compounds with General Structure F. Representative Example: 5-Amino-3-(4-chlorophenyl)-*N*-methyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (13). DIPEA (0.028 g, 38 μ L, 0.23 mmol) was added to a mixture of **11** (0.050 g, 0.15 mmol), methylamine (2.0 M solution in THF, 110 μ L, 0.22 mmol), and BOP reagent (0.097 g, 0.22 mmol) in anhydrous DMSO (2 mL). The reaction mixture was stirred at room temperature for 4 h. Water was added, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO₄, filtered, and evaporated. The residue was purified by preparative HPLC to give **13** as yellow solid. HPLC–MS retention time: 6.83 min. Yield: 52%. Mp: 192–194 °C (from ethanol). ¹H NMR (DMSO-*d*₆): δ 2.75 (d, *J* = 5.0 Hz, 3H), 7.24 (s, 1H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.60 (broad s, 2H), 7.69 (d, *J* = 9 Hz, 2H), 8.27 ppm (broad s, 1H). ¹³C NMR (DMSO-*d*₆): δ 25.7, 103.9, 104.6, 126.0, 127.5, 128.1, 131.0, 131.6, 136.1, 139.5, 158.3, 162.9, 163.0 ppm. IR: ν 3418, 3302, 1656, 1593 cm^{−1}. HRMS (ESI⁺): calculated for C₁₄H₁₀ClN₄O₂S⁺ 333.0213, found 333.0210.

5-Amino-3-(4-chlorophenyl)-*N*-ethyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (14). **14** was prepared in the same manner as **13** starting with **11** using ethylamine hydrochloride. HPLC–MS retention time: 7.43 min. Yield: 58%. Mp: 203–205 °C (from ethanol). ¹H NMR (CDCl₃): δ 1.23 (t, *J* = 7.3 Hz, 3H), 3.41–3.47 (m, 2H), 6.18 (broad s, 2H), 7.07 (s, 1H), 7.45 (d, *J* = 9.0 Hz, 2H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.60 ppm (broad s, 1H). ¹³C NMR (CDCl₃): δ 14.9, 34.3, 106.8, 106.9, 126.7, 127.3, 129.0, 133.3, 135.6, 139.2, 159.5, 161.4, 162.6 ppm. IR: ν 3405, 3297, 1652, 1600 cm^{−1}. HRMS (ESI⁺): calculated for C₁₅H₁₃ClN₄NaO₂S⁺ 371.0345, found 371.0341.

5-Amino-3-(4-chlorophenyl)-*N*-isopropyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (15). **15** was prepared in

the same manner as **13** starting with **11** using isopropylamine. HPLC–MS retention time: 6.58 min. Yield: 89%. Mp: 197–199 °C (from ethanol). ¹H NMR (CD₃OD): δ 1.22 (d, *J* = 6.5 Hz, 6H), 4.09–4.19 (m, 1H), 7.25 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.59 ppm (d, *J* = 8.5 Hz, 2H). ¹³C NMR (CD₃OD): δ 19.6, 39.9, 102.9, 103.3, 124.9, 126.1, 126.9, 131.2, 135.1, 138.1, 158.0, 161.4, 162.1 ppm. IR: ν 3404, 3294, 3178, 1651, 1590 cm^{−1}. HRMS (ESI⁺): calculated for C₁₆H₁₅ClN₄O₂NaS⁺ 385.0502, found 385.0492.

5-Amino-3-(4-chlorophenyl)-*N*-cyclopropyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (16). **16** was prepared in the same manner as **13** starting with **11** using cyclopropylamine. HPLC–MS retention time: 7.43 min. Yield: 41%. Mp: 188–190 °C (from ethanol). ¹H NMR (DMSO-*d*₆): δ 0.56–0.60 (m, 2H), 0.66–0.70 (m, 2H), 2.76–2.81 (m, 1H), 7.19 (s, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.67 (d, *J* = 9.0 Hz, 2H), 8.28 ppm (broad s, 1H). ¹³C NMR (DMSO-*d*₆): δ 5.7, 22.6, 103.8, 104.4, 125.9, 127.5, 128.1, 131.0, 136.3, 139.4, 158.3, 163.0, 163.7 ppm. IR: ν 3408, 3302, 1654, 1593 cm^{−1}. HRMS (ESI⁺): calculated for C₁₆H₁₃Cl₄O₂N₄NaS⁺ 383.0345, found 383.0333.

5-Amino-3-(4-chlorophenyl)-*N,N*-dimethyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (17). **17** was prepared in the same manner as **13** starting with **11** using dimethylamine hydrochloride. HPLC–MS retention time: 6.43 min. Yield: 62%. Mp: 242–244 °C (from ethanol). ¹H NMR (CDCl₃): δ 3.14 (s, 3H), 3.16 (s, 3H), 6.18 (broad s, 2H), 6.73 (s, 1H), 7.41 (d, *J* = 9.0 Hz, 2H), 7.52 ppm (d, *J* = 9.0 Hz, 2H). ¹³C NMR (CDCl₃): δ 35.7, 39.0, 104.0, 106.9, 127.0, 128.1, 128.8, 132.9, 139.2, 139.3, 159.1, 161.7, 164.3 ppm. IR: ν 3414, 3300, 1646, 1598 cm^{−1}. HRMS (ESI⁺): calculated for C₁₄H₁₃ClN₄NaO₂S⁺ 371.0345, found 371.0366.

5-Amino-3-(4-chlorophenyl)-*N*-ethyl-*N*-methyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (18). **18** was prepared in the same manner as **13** starting with **11** using ethylmethylamine. HPLC–MS retention time: 6.85 min. Yield: 47%. Mp: 148–150 °C (from ethanol). ¹H NMR (CDCl₃): δ 1.19–1.26 (m, 3H), 3.46–3.63 (m, 2H), 6.16 (broad s, 2H), 6.70 (s, 1H), 7.40–7.42 (m, 2H), 7.51–7.54 ppm (m, 2H). ¹³C NMR (CDCl₃): δ 12.2, 14.0, 32.9, 36.3, 42.9, 46.1, 103.5, 106.4, 111.3, 117.0, 126.7, 126.8, 127.0, 127.1, 127.8, 127.9, 128.8 (×2), 132.8, 132.9, 159.1 (×2), 162.2, 164.0, 164.5 ppm. IR: ν 3409, 3290, 3175, 1635 cm^{−1}. HRMS (ESI⁺): calculated for C₁₆H₁₅ClN₄NaO₂S⁺ 385.0502, found 385.0491.

5-Amino-3-(4-bromophenyl)-*N*-isopropyl-4-oxo-3,4-dihydrothieno[3,4-*d*]pyridazine-1-carboxamide (19). **19** was prepared in the same manner as **13** starting with **12**. HPLC–MS retention time: 8.20 min. Yield: 68%. Mp: 222 °C dec (from ethanol). ¹H NMR (DMSO-*d*₆): δ 1.15 (d, *J* = 6.6 Hz, 6H), 4.02–4.10 (m, 1H), 7.20 (s, 1H), 7.60 (broad s, 2H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.99 ppm (broad d, 1H). ¹³C NMR (DMSO-*d*₆): δ 22.0, 40.1, 103.8, 104.5, 119.4, 125.9, 127.9, 131.1, 136.5, 139.9, 158.3, 161.6, 162.9 ppm. IR: ν 3412, 3305, 1655, 1598 cm^{−1}. HRMS (ESI⁺): calculated for C₁₆H₁₅BrN₄NaO₂S⁺ 428.9997, found 428.9986.

Solubility Studies. Compounds were serially diluted in DMSO to achieve a 2.5-fold dilution series from 20 mM down 8 points to 3 μ M on a 384-well master plate (Costar 3672). Aliquots (2 μ L) were transferred to a clear 96-well assay plate (Fisher 12-565-501) containing 198 μ L of fibrillization buffer (100 mM NaOAc, pH 7.0) for a 100-fold dilution (200 μ M down to 0.03 μ M). The plate was incubated with agitation for 2.5 h, and the absorbance at 550, 600, 650, and 700 nm was measured on a Spectramax M5 spectrophotometer. DMSO (1%) in fibrillization buffer served as a control that was subtracted from each data point. The absorbances were averaged, and the concentration at which the average absorbance rose above 0.03 AU was an indication of insoluble compound, with solubility limits reported as the previous concentration in the dilution series. Absorbance scans of compounds in DMSO indicated that the compounds tested here did not have any absorbance

peaks in the above range; therefore, all absorbance was due to light scattering by insoluble material.

Tau K18PL Fibrillization Assay. Activity evaluations of test compounds in the K18PL fibrillization assay were performed as previously described.²¹

Secondary Sedimentation Assay (K18PL and tau40). The determination of compound-induced effects on the amount of tau (K18PL or tau40) that remains soluble upon centrifugation following incubation with test compounds was conducted as previously described.⁹

MT-Assembly Assay. The MT assembly assay was conducted in a 384-well plate essentially as described in Hong et al.³¹ Wild-type full-length human brain tau (tau40, 2N4R) was employed in this assay. Lyophilized bovine brain tubulin (Cytoskeleton Inc., TL238) was reconstituted in 1 × RAB (100 mM MES, pH 6.9, 1 mM EDTA, 0.5 mM MgSO₄), pH 6.9, to a concentration of 10 mg/mL. ATPZ compounds (62.5 μM) were added to tau (25 μM) in RAB, pH 6.9, and preincubated at room temperature for 120 min. To initiate the MT assembly reaction, 8.25 μL per well of 10 mg/mL tubulin was dispensed on a UV-clear 384-well plate (NUNC 265196) followed by 1.0 μL of 100 mM GTP (1 × RAB pH 6.9) and 40 μL of the ATPZ/tau mixture. The final reaction mixture was 30 μM tubulin, 20 μM tau, 50 μM ATPZ, and 2 mM GTP. The plate was then incubated in a Spectramax M5 plate reader at 37 °C, and the absorbance at 340 nm was measured every minute for 45 min.

Cytotoxicity Assay. Cells were plated in each well of a 96-well plate in 100 μL of media (20 000 cells; DMEM plus 10% fetal calf serum with antibiotics). After 24 h, half-log serial dilutions of the test compounds, starting at 200 μM, were added to an additional 100 μL of media. The cells were cultured for an additional 72 h, and then Alamar blue (Biosource, Camarillo, CA) was added, with cell viability at each drug concentration measured using a SpectraMax M5 fluorescence plate reader (Molecular Devices, Sunnyvale, CA). CC₅₀ values were generated using the Prism software.

Determination of Plasma and Brain Drug Concentrations. Test compounds were administered to 2 month old female B6C3F1 mice (20 g average body weight). Whole brain hemispheres, obtained from mice euthanized according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, were homogenized in 10 mM ammonium acetate, pH 5.7 (1:2, w/v), using a hand-held sonic homogenizer. Plasma was obtained from blood that was collected into a 1.5 mL tube containing 0.5 M EDTA solution and subjected to centrifugation for 10 min at 4500g at 4 °C. Aliquots (50 μL) of brain homogenate or plasma were mixed with 0.2 mL of acetonitrile and centrifuged at 15000g, and the resulting supernatants were used for subsequent LC–MS/MS analysis. The LC–MS/MS system comprised an Aquity UPLC and a TQ MS that was controlled using MassLynx software (Waters Corporation, Milford, MA). Compounds were detected using multiple reaction monitoring (MRM) of their specific collision-induced ion transitions. Samples (5 μL) were separated on an Aquity BEH C18 column (1.7 μm, 2.1 mm × 50 mm) at 35 °C. Operation was in positive electrospray ionization mode, with mobile phase A of 0.1% (v/v) formic acid and mobile phase B of either acetonitrile or methanol with 0.1% (v/v) formic acid at a flow rate of 0.6 mL/min using a gradient from 5% to 95% B over 2 min, followed by wash and re-equilibration steps. The mass spectrometer was operated with a desolvation temperature of 450 °C and a source temperature of 150 °C. Desolvation and source nitrogen gas flows were 900 and 50 L/h, respectively. Source and MS/MS voltages were optimized for each compound using the MassLynx autotune utility. Standard curves were generated for each compound from brain homogenate and plasma samples that had compound added at 4, 40, 400, and 4000 ng/mL and extracted as above. Peak areas were plotted against concentration, and a 1/x weighted linear regression curve was used to quantify the tissue-derived samples using

the average peak area from triplicate injections. Drug half-life was calculated using the elimination rate constant determined from the slope of a line plotted through natural log of the plasma concentration versus time for the 4, 8, and 16 h time points using Microsoft Excel. Area under the brain or plasma concentration curve from 0.5 to 16 h was calculated using the trapezoid rule with GraphPad Prism.

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Supporting Information Available: HPLC chromatograms and mass, IR, ¹H NMR, and ¹³C NMR spectra for compounds **8**, **10**, **11**, **13**–**19**; X-ray crystal structures for compounds **8**, and **16**; MT-assembly assay results for compounds **8**, **10**, **11**, **13**–**19**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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