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Targeting protein kinases in central nervous system disorders

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Abstract

Protein kinases are a growing drug target class in disorders in peripheral tissues, but the development of kinase-targeted therapies for central nervous system (CNS) diseases remains a challenge, largely owing to issues associated specifically with CNS drug discovery. However, several candidate therapeutics that target CNS protein kinases are now in various stages of preclinical and clinical development. We review candidate compounds and discuss selected CNS protein kinases that are emerging as important therapeutic targets. In addition, we analyse trends in small-molecule properties that correlate with key challenges in CNS drug discovery, such as blood–brain barrier penetrance and cytochrome P450-mediated metabolism, and discuss the potential of future approaches that will integrate molecular-fragment expansion with pharmacoinformatics to address these challenges.

Protein kinases regulate diverse cellular functions through the orchestrated propagation and amplification of cellular stimuli into distinct biological responses through coordinated signal transduction cascades. With several hundred kinases encoded in the human genome, almost every signal transduction process is influenced by interconnected phosphorylation events. Deregulation of kinase activity has been implicated in various diseases, ranging from vascular disorders and inflammatory diseases to neurological disorders and cancer^{1,2}. This has generated intense interest in the pursuit of protein kinases as drug targets.

However, most kinase-targeted drugs and potential kinase targets that have been investigated are for non-central nervous system (CNS) disorders. CNS disease indications for kinase-targeted drugs seem to be lagging behind those for other disease areas, such as cancer (FIG. 1), and ~25% of publications related to CNS disorders are for CNS cancers. This pattern is also seen in pharmaceutical industry pipelines that are publicly disclosed. For example, Novartis, the manufacturer of the kinase inhibitor cancer therapeutics imatinib (Gleevec) and nilotinib (Tasigna), has various oncology candidates and CNS-targeted therapies in clinical development. Half of the oncology pipeline are kinase inhibitors, but none of the disclosed CNS candidates seems to target protein kinases. Nevertheless, there is increasing interest in the development of kinase-targeted therapeutics for CNS indications^{3–11}, and the established success in other disease indications provides encouragement for the development of small-molecule kinase modulators for CNS disorders.

This Review focuses on the special challenge of targeting protein kinases for CNS disease indications. A recent review² provides an up-to-date overview of targeting protein kinases in cancer, presents an in-depth analysis of the structural basis of protein kinase inhibitor recognition and selectivity, and discusses various general approaches to kinase inhibitor design. Therefore, the broader aspects of protein kinase inhibitor interactions in drug discovery are not a focus of this article. In addition, not all protein kinase targets can be covered in a single review. Instead, we provide an overview of promising protein kinase targets for CNS disease indications in which the disclosed state of small-molecule inhibitor development for these targets is at the *in vivo* stage of evaluation (FIG. 2; TABLES 1,2; see Supplementary information S1, S2 (box, table)), and summarize selected case studies to illustrate the issues that often arise during kinase or CNS drug discovery programmes. We conclude with a discussion of the challenges of developing small-molecule therapeutic candidates that are capable of penetrating the blood–brain barrier (BBB) and the potential of emerging approaches, such as molecular-properties-driven fragment expansion, to address these challenges.

Examples of CNS protein kinase targets

Glycogen synthase kinase 3 (GSK3)

GSK3 is a serine–threonine protein kinase family consisting of two isoforms, GSK3 α and GSK3 β , that are similar in their catalytic domain structures, substrate specificity, tissue distribution and molecular mechanisms of regulation (reviewed in REFS ^{4–7}). GSK3 regulates a wide range of cellular functions, such as glucose metabolism, gene expression, cytoskeletal organization, vesicular transport, cell growth, motility, differentiation and cell survival (FIG. 3), which are mediated through diverse substrates and signalling pathways. The activity of GSK3 can be regulated by several integrated mechanisms, such as coordinated phosphorylation of the enzyme and its substrates, dynamic regulation of the subcellular distribution of the kinase and the formation of complexes of the enzyme with scaffolding or anchoring proteins that control access to specific substrates^{5–7} (BOX 1).

The involvement of GSK3 in numerous cellular processes, coupled with the complexity of the mechanisms for its regulation, underscore the importance of appropriate functioning of GSK3-modulated signalling. Not surprisingly, upregulation of GSK3 activity has been linked to numerous human pathological conditions, including diabetes, muscle hypertrophy, cancer, Alzheimer's disease, stroke, sleep disorders, and neuropsychiatric and mood disorders (reviewed in REFS ^{4,9,12–14}). Although regulation of GSK3 is generally through modulation of enzymatic activity, there are also examples of increased GSK3 activity in Alzheimer's disease through increased levels of the protein or changes in splicing of the gene^{15,16}. The involvement of GSK3 in several human pathological conditions raised interest in the kinase as a potential drug discovery target. For example, several studies have linked GSK3 deregulation to Alzheimer's disease pathophysiology, and GSK3 seems to contribute to both the amyloid and tau pathologies that characterize the disease^{5,12,17,18}.

The potential of GSK3 as an Alzheimer's disease drug discovery target has been raised by studies showing that overexpression of GSK3 β in transgenic mouse models results in increased tau phosphorylation and deficits in spatial learning, and that inhibition of GSK3 β activity leads to neuroprotective effects, decreased amyloid- β production and a reduction in tau hyperphosphorylation^{4,12,18}. Lithium, a mood stabilizer that is widely used in the treatment of bipolar disorder, is a GSK3 inhibitor¹⁹ and is currently in an alternative-use clinical trial for Alzheimer's disease (TABLE 1). However, lithium is associated with adverse effects, especially in the elderly. For example, in an open-label clinical study²⁰ assessing the feasibility and tolerability of lithium at therapeutic doses administered for up to 1 year in patients with mild to moderate Alzheimer's disease, a significant percentage of patients had contraindications and there was a high discontinuation rate from the trial. The occurrence of

some adverse effects and the risk of serious toxicity in elderly patients could limit the potential usefulness of lithium therapy for Alzheimer's disease. Lithium apparently reduces GSK3 activity through both a direct inhibition of enzyme activity, by competing with Mg^{2+} ions, as well as by increasing the inhibitory phosphorylation of GSK3 that inactivates the enzyme^{21, 22}. Whether or not lithium exerts its therapeutic effects through GSK3 inhibition is not known, as lithium affects additional enzymes. However, studies with lithium created interest in the kinase as a CNS drug target and provided a rationale for the subsequent development of new, more selective GSK3 inhibitors. More selective maleimide-based lithium mimetics, for example, are active in a mouse model of mania²³ and in a cell-based model of Parkinson's disease²⁴.

Currently, chemically diverse small-molecule GSK3 inhibitors have shown efficacy in preclinical animal models of various CNS disorders (TABLE 2). These include indirubins, paullones, aminopyrazoles, thiazoles, and thiadiazolidinones (TDZDs). The thiazole AR-A014418 is efficacious in animal models of Alzheimer's disease⁹, Parkinson's disease²⁵, amyotrophic lateral sclerosis (ALS)²⁶, depression²⁷ and ischaemic stroke²⁸. Small heterocyclic TDZDs are being explored for their potential as therapeutics for Alzheimer's disease and other 'tauopathies'²⁹, ischaemic injury³⁰, spinal cord trauma³¹, mood disorders³² and excitotoxic conditions³³. For example, a TDZD compound, NP12, is efficacious in an Alzheimer's disease mouse model³⁴, has successfully completed a Phase I clinical trial in Europe, and is currently in a Phase II clinical trial for Alzheimer's disease (TABLE 1). Thus, several GSK3 inhibitors are well into the drug development pipeline for CNS indications³⁵, and the final validation of GSK3 as a target for CNS disorders awaits the outcomes on therapeutic efficacy in human clinical trials.

Targeting GSK3 for CNS drug discovery exemplifies key considerations in kinase inhibitor drug discovery. GSK3 is a widely distributed protein kinase with many substrates and signalling pathway interactions. This raised concerns that chronic inhibition of GSK3 might lead to oncogenic side effects related to its proliferative targets, such as β -catenin or certain transcription factors. However, chronic treatment in animals and humans has not elicited these potential side effects^{36,37}, which is consistent with the prevailing view that oncogenesis is more than a 'single hit' response. GSK3-targeted therapeutic approaches show how inhibitor treatment can preferentially modulate one pathway over another through the use of low doses to attain the desired efficacy. The selective response is also related to the common issue of what proportion of the total kinase activity in a tissue is required to mediate a physiological effect or, in the case of inhibitor treatment, to mediate a pharmacological effect^{9,38}. The levels of GSK3 inhibition that are required to obtain neurological effects seem to be much lower than those required for stabilization of β -catenin, and approximately 20–25% inhibition of GSK3 is sufficient for therapeutic efficacy in CNS diseases^{7,37}. The issues relating to kinase inhibitor development discussed in this section for GSK3 as a case study are also relevant to the cyclin-dependent kinases (CDKs), protein kinase A and protein kinase C (TABLE 2).

Death-associated protein kinase 1 (DA PK1)

DA PK1 is a pro-apoptotic, calmodulin-regulated, serine–threonine protein kinase that acts early in the apoptosis pathways before the cell is committed to death^{39,40}. DAPK1 was recently identified as a CNS drug discovery target that is implicated in post-injury synaptic dysfunction, an end point of pathology progression that can be attenuated in animal models with DAPK1 inhibitor treatments within clinically relevant time frames. DAPK1, like GSK3, is a protein kinase with several biological roles. It provides an early example of the use of the co-crystal structure of an inactive small-molecule fragment bound to the target kinase as the starting point for bioavailable protein kinase inhibitor design⁴¹, allowing *in vivo* target validation studies to be performed in animal models^{41–43}.

DAPK1 was first identified as a CNS drug discovery target in animal models of CNS injury. More recent human genetics studies show a link between human disease and DAPK1 that is consistent with the animal model studies. DAPK1 is a druggable kinase target in animal models of both acute and sustained brain injury⁴¹⁻⁴³. Its enzyme activity in tissue homogenates increases after injury during a therapeutically relevant time frame⁴⁰, and small-molecule intervention during this time modifies disease progression, as assessed by subsequent neurological outcomes⁴¹. Specifically, a single treatment of rats with a small-molecule DAPK1 inhibitor (TABLE 2) 6 hours after cerebral ischaemia attenuated the loss of brain tissue, measured 1 week later⁴¹. Consistent results were obtained with the same DAPK1 inhibitor in a different cerebral ischaemia animal model⁴³. More recently, human clinical genetics studies⁴⁴ identified that the *DAPK1* gene locus is linked to late-onset Alzheimer's disease susceptibility. Specifically, two single nucleotide polymorphisms (SNPs) were associated with *DAPK1* allele-specific expression and late-onset Alzheimer's disease. It is not yet known whether the SNPs in the human *DAPK1* gene alter the expression or the activity of the kinase. A meta-analysis of genetic variation in case-control samples provided further evidence that *DAPK1* genetic mutations may affect disease susceptibility. The clinical linkage and animal pathophysiology studies are consistent with pharmacological studies in animal models⁴².

The identification of important protein substrates of DAPK1 in the CNS indicated molecular mechanisms by which DAPK1 could be involved in CNS pathophysiology progression (FIG. 3). For example, the finding that DAPK1 can phosphorylate and inactivate the neuronal survival protein Ca²⁺-calmodulin-dependent protein kinase kinase 1 (*CAMKK1*)⁴⁵, and that phosphorylation of ribosomal protein S6 by DAPK1 can alter neuronal protein biosynthesis⁴⁶, suggest that DAPK1 is involved in stress- or injury-induced phosphorylation cascades that can lead to synaptic dysfunction or neuronal death. DAPK1 has also been reported to interact with numerous other signalling proteins and pathways, such as DAPK3, protein kinase D, myosin light chains, beclin, syntaxin 1A and extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3), some of which may be related to its pro-apoptotic functions⁴⁷⁻⁵². The available evidence suggests that DAPK1 may have several physiological roles, and can promote cell death and synaptic dysfunction by inhibiting survival pathways once activated by various stress or injury stimuli. Although initial target validation evidence with bioavailable kinase inhibitors that are not drug candidates supports DAPK1 as a drug discovery target for neurological disorders, no clinically promising small-molecule DAPK1 inhibitor drugs have yet been disclosed.

The mitogen-activated protein kinase (MAPK) family

The MAPKs are serine-threonine protein kinases that integrate and process extracellular stimuli through a series of intracellular signalling complexes and phosphorylation cascades that lead to coordinated and distinct responses to many diverse stimuli (reviewed in REF. ⁵³). In its simplest form, the MAPK cascade consists of a three-tiered set of protein kinases: a MAPK (ERKs, c-Jun N-terminal kinases (JNKs) and p38s), and two upstream components (a MAPK kinase and a MAPK kinase kinase) that activate the MAPKs by a series of activating phosphorylations. The activated MAPK can phosphorylate a number of substrates, which leads to stimulus-specific responses. This description of MAPK cascades as linear, isolated pathways is oversimplified: MAPK pathways can influence and be influenced by other signalling pathways, by interactions with scaffolding proteins and by specific localizations within cells⁵⁴. In addition, each MAPK family consists of several isoforms that can have distinct functions⁵⁴. For example, the p38 α MAPK isoform (also known as MAPK14) has a crucial role in the activation of inflammatory cell signalling cascades, and its position at the interface of diverse signalling pathways allows it to modulate downstream biological responses through several regulatory mechanisms (recently reviewed in REFS ⁵⁵⁻⁵⁷). Several kinases in the MAPK signalling cascades have emerged as potential CNS therapeutic targets, including

ERK1, ERK2, JNK1–JNK3, MAPK–ERK kinase 1 (MEK1), MEK2, mixed-lineage kinase 1 (MLK1)–MLK3 and p38 α (TABLE 2).

The p38 MAPK family is being investigated as a potential therapeutic target for many CNS disorders. Diverse chemical classes of small-molecule p38 α MAPK inhibitors have been tested in preclinical animal models of CNS diseases, including Alzheimer's disease, ALS, cerebral ischaemia and neuropathic pain^{58–63}. The p38 α MAPK is an especially interesting case study for several reasons. First, the enzyme is an established therapeutic target for peripheral inflammatory disorders such as rheumatoid arthritis⁶⁴, and the clinical correlations between kinase activation and CNS disorders make p38 α MAPK a logical target for future CNS drug discovery and development programmes. For example, the p38 MAPK pathway is activated in human brain tissue in several CNS disorders, including Alzheimer's disease^{65–68}, Down's syndrome⁶⁸, Parkinson's disease^{69,70}, tauopathies^{71,72} and gliomas⁷³. Second, the use of drug-resistant p38 α knock-in mice (BOX 2) provides a rare precedent for *in vivo* target validation of a small-molecule drug⁶⁴. Third, inhibition of increased production of proinflammatory cytokines or other injurious mediators offers the potential for an extended pharmacodynamic effect, due to the modulation of a biosynthetic process⁷⁴. Fourth, the development of p38 α MAPK inhibitors is a case study of how molecular properties of small-molecule inhibitors can affect bioavailability, including uptake by the brain⁵⁸. Fifth, it provides an example of an opportunity to use complementary aspects of enzyme and small-molecule structures to generate selectivity among enzyme isoforms and the rest of the kinome (BOX 2)^{75–79}.

The advancement of p38 MAPK inhibitors into clinical trials for diseases of peripheral tissues had early problems with unacceptable safety profiles⁸⁰. However, it is likely that the early adverse effects were due to chemical toxicity or off-target interactions that seem to be compound specific rather than target related, and the more recent success emerging from the use of new chemotypes or scaffolds is consistent with this proposal (reviewed in REF. 55). Insights into the mechanisms by which p38 α MAPK contributes to proinflammatory cytokine overproduction in the CNS suggest that this kinase could also be a valid target for neurological disorders (reviewed in REF. 3). For example, the p38 MAPK pathway is activated in neurons and glia in the brains of patients with early-stage Alzheimer's disease and in rodent models of neurodegenerative disease, as assessed by staining for phosphorylated (activated) p38 MAPK. Furthermore, increased p38 MAPK expression and activity have been linked to glial proinflammatory cytokine production and neuronal tau phosphorylation and synaptic dysfunction^{65–67,81}. Initial causative links between p38 α MAPK activation and CNS pathophysiology have been provided by preclinical studies using small-molecule p38 α MAPK inhibitors in animal models of various CNS disorders, as discussed above (TABLE 2). The available data make a compelling argument for further exploring p38 α MAPK as a therapeutic target for the treatment of CNS diseases.

Other branches of the MAPK pathways are also being investigated as potential CNS targets. For example, compounds such as PD-98059, U0126 and PD198306, which block ERK1 and ERK2 activation through inhibition of upstream kinases MEK1 and MEK2 have shown efficacy in preclinical animal models of cerebral ischaemia, traumatic brain injury and neuropathic pain^{82–85}. Similarly, inhibitors of the JNK pathway are efficacious in various animal models of neurodegenerative disorders, including cerebral ischaemia, subarachnoid haemorrhage, motor neuron disease, excitotoxic cell death, age-dependent hearing loss, Huntington's disease and Parkinson's disease^{86–94}. However, an inhibitor of MLK that blocks JNK activation (CEP-1347) failed to show efficacy in the recent Parkinson Research Evaluation of CEP-1347 Trial (PRECEPT) of early Parkinson's disease⁹⁵. It is unclear why the drug was ineffective in delaying disability. Various explanations have been proposed, such as failure of the inhibitor to reach therapeutic levels in the CNS, an insufficient therapeutic window for rescue of neuronal apoptosis or the targeting of a less optimal step or kinase

pathway than the intended target. Another possible explanation was that additional signalling pathways need to be targeted to effectively prevent dopaminergic neuron death^{96,97}. An interesting suggestion has been made in relation to this⁷⁶, that therapy with an MLK inhibitor in combination with a GSK3 inhibitor (to activate the phosphoinositide 3-kinase pathway) may be a more effective therapy for neurodegenerative diseases than using a single kinase inhibitor approach. The clinical effectiveness of imatinib, which is a multi-target kinase inhibitor, suggests that combining single-target kinase inhibitors could be a reasonable approach.

Rho-associated protein kinase 1 (ROCK1) and myosin light chain kinase (MLCK)

ROCK1 and MLCK are actomyosin-associated serine–threonine protein kinases that play a part in tissue barrier dysfunction by increasing myosin light chain phosphorylation and by mediating downstream cytoskeletal changes^{98,99}. Both kinases are drug discovery targets in disorders that have microvascular dysfunction as their pathological basis^{100,101}. Microvascular dysfunction is a co-morbid variable in many CNS disorders¹⁰².

Box 1 | Regulation of GSK3 activity

Glycogen synthase kinase 3 (GSK3) activity can be regulated by several integrated mechanisms, such as coordinated phosphorylation of the enzyme and its substrates, dynamic regulation of its subcellular distribution and the formation of complexes of the enzyme with scaffolding or anchoring proteins that control access to specific substrates (reviewed in REFS⁵⁻⁷). For example, exposure of cells to growth factors, insulin, receptor ligands and other stimuli that activate intracellular kinases — such as phosphoinositide 3-kinase–AKT, protein kinase A and protein kinase C — leads to inhibition of the constitutively active GSK3 through an inhibitory phosphorylation of an amino-terminal serine in the enzyme (ser21 in GSK3 α and ser9 in GSK3 β). In contrast to inhibition of enzyme activity by serine phosphorylation, GSK3 enzymatic activity is enhanced by tyrosine phosphorylation (tyr279 in GSK3 α and tyr216 in GSK3 β). The mechanisms regulating tyrosine phosphorylation of GSK3 are not well defined, but may occur through autophosphorylation and/or by other kinases⁶.

In addition to the regulation of GSK3 pathways by phosphorylation of the kinase, physiological responses can also be regulated by the phosphorylation state of GSK3 substrates. The mechanism of action for this mode of regulation is the requirement that most GSK3 substrates must be pre-phosphorylated, or ‘primed’, to be phosphorylated effectively by GSK3. Another mechanism of GSK3 pathway regulation is to limit the substrates upon which GSK3 acts by dynamic regulation of the subcellular localization of the enzyme, such as in the nucleus and mitochondria. The association of GSK3 with distinct multiprotein complexes in the cell, including in subcellular organelles, ensures that the action of GSK3 in response to particular stimuli is directed towards specific substrates. These integrated mechanisms allow coordinated and restricted substrate-specific phosphorylation, thereby enabling GSK3 to act on a diverse array of protein substrates while producing selective biological responses to discrete stimuli.

The Rho–ROCK1 pathway has been implicated in numerous disorders, including stroke and asthma, and ROCK1 inhibitors have shown efficacy in animal models and in human disease (reviewed in REFS^{8,10}). ROCK1 is the target of the first kinase inhibitor drug to be approved, fasudil (AT-877; Asahi Kasei)¹⁰³. The approved indication was for cerebral vasospasm, a microvascular disorder. Currently, fasudil is in clinical trials in the United States for Raynaud’s phenomenon (TABLE 1). At higher concentrations (in the micromolar range), fasudil can function as an MLCK inhibitor¹⁰⁴.

Studies with knockout mice and with MLCK inhibitors in wild-type mice in several animal disease models have provided extensive preclinical target validation evidence for this kinase in disorders in which tissue barrier dysfunction is part of the pathology^{101,105,106}. For example, studies^{106,107} using both an isoform-selective MLCK-knockout mouse and a selective inhibitor in wild-type mice showed that MLCK inhibition can provide protection against tissue injury. Diverse animal model studies, using either genetic or *in vivo* chemical biology approaches, showed that MLCK inhibition provided protection from disease-relevant stressors in diseases that have tissue barrier dysfunction as part of pathology progression. These include ventilator and inflammation-induced lung injury^{101,106,107}, immune-mediated intestinal diarrhoea¹⁰⁸, severe-burn-induced microvascular barrier injury¹⁰⁵ and endotoxic shock¹⁰⁹. The accumulating data that MLCK inhibitors can protect against tissue injury provide the target validation impetus to extend the investigation to CNS disorders that have tissue barrier dysfunction as a component of pathology, such as multiple sclerosis, stroke and traumatic brain injury.

In summary, both ROCK1 and MLCK are promising kinase targets for CNS disorders involving BBB dysfunction or microvascular pathologies. Like GSK3, they are examples of widely distributed protein kinases. The successful clinical use of fasudil provides a case study showing that ubiquitous kinases, like many widely distributed targets, can be therapeutically modulated if they are validated as targets for the disorder and appropriate dosing regimes are employed. In addition, distinct kinase isoforms of ROCK1 and MLCK seem to be crucial for drug action^{100,106}, which is reminiscent of the p38 MAPK isoforms.

Selected protein kinase targets that lack in vivo pre-clinical proof of concept validation

Evidence relating to the neurobiology of disease has highlighted various CNS protein kinases as candidate therapeutic targets, but *in vivo* evidence of outcome modulation by small-molecule intervention has not yet been disclosed. Furthermore, the clinical evidence is often an analysis of post-mortem human tissue, which has its limitations. However, it is anticipated that current research will soon provide initial target validation evidence, adding to the rapidly growing body of protein kinase targets for CNS drug discovery. Several of these are worth mentioning specifically as they are examples of possible future targets in the disease areas discussed above. For example, leucine-rich repeat kinase 2 (LRRK2) can localize to Lewy bodies in human Parkinson's disease brain tissue, and *LRRK2* genetic variance is linked to familial autosomal-dominant, late-onset Parkinson's disease and some forms of sporadic Parkinson's disease^{110,111}. The gene encoding dual-specificity tyrosine phosphorylation-regulated kinase 1A is located in the Down's syndrome region of chromosome 21, and overexpression is thought to contribute to abnormal brain development and disease pathogenesis¹¹². Members of the p21-activated kinase (PAK) family of kinases, especially PAK1, have altered expression in neurodegenerative diseases and are upregulated in gliomas¹¹³. Expression of the casein kinase 1 (CK1) family of serine–threonine protein kinases, such as CK1 δ , is increased in Alzheimer's disease brain samples, and these kinases can phosphorylate the Alzheimer's disease -related protein presenilin¹¹⁴. Furthermore, CK1 is a priming kinase for GSK3 β and is an upstream regulator of CDK5 (REF. ¹¹⁴) — two other protein kinases that are implicated in Alzheimer's disease¹¹⁵.

Challenges in targeting kinases in CNS disorders

BBB penetrance

The greatest challenge facing any CNS-targeted drug discovery programme is effective penetration of the BBB. It is estimated¹¹⁶ that only ~2% of small-molecule drugs are able to effectively cross the BBB. The physicochemical properties of a drug considerably influence passive diffusion across biological membranes^{117–119} and the potential to serve as a substrate

for the P-glycoprotein (PGP) efflux transporter¹²⁰. Molecular weight, polar surface area (PSA) and lipophilicity (LogP) are key molecular properties that correlate with and may have an important role in influencing the BBB penetrance of a molecule. For example, an analysis (FIG. 4) of the mean LogP, molecular weight, and PSA values of CNS-penetrant small molecules (see Supplementary information S1,S3 (box, table)) compared with those of kinase inhibitor drugs (see Supplementary information S1,S4 (box, table)) or drugs approved and marketed for all disease indications¹²¹ reveals that kinase inhibitor drugs tend to have higher mean values for these parameters than CNS-penetrant compounds. PGP substrates also tend to have higher average molecular weight, PSA and LogP values than molecules which are not PGP substrates (see Supplementary information S3 (table)).

The trend for discrepancy between the molecular properties of CNS-penetrant compounds and those of current kinase inhibitor drugs raises concerns about the outcome of pending clinical trials and the prevailing approach of trying to use drugs that were developed and approved for peripheral-tissue diseases to treat CNS disorders. It is not known how the characteristics of small molecules affect the targeting of protein kinases in CNS disorders, but case studies provide some insights into emerging trends. For example, imatinib is an effective non-CNS cancer therapeutic¹²² that has now entered trials for glioma (TABLE 1), but such CNS clinical trials have generally failed^{123,124}. This may be related to the observations that the molecular weight and PSA of imatinib (493.60 and 86.28 Å², respectively) are greater than those of other CNS-penetrant compounds (FIG. 4a,b,c), and that imatinib is a PGP substrate¹²⁵. Imatinib has a LogBB of -1.50, showing that it has poor brain uptake. LogBB values greater than -1 are considered to have reasonable brain penetration¹²⁶. The follow-on drug dasatinib (Sprycel; Bristol–myers Squibb) (TABLE 1) has a greater molecular weight and PSA than imatinib, but does not seem to be a good substrate for the PGP efflux transporter¹²⁷. Dasatinib has an incrementally improved LogBB of -1.30, which will hopefully be sufficient for CNS efficacy¹²⁸. These borderline cases are difficult to evaluate, but the more extreme cases of higher molecular weight and PSA of many kinase inhibitor drugs (FIG. 4) suggest that future efforts at targeting protein kinases in CNS disorders might benefit from a primary focus on CNS disorders at the outset of the discovery programme.

Box 2 | Generation of small-molecule p38 MAPK inhibitors

The p38α mitogen-activated protein kinase (MAPK) is a serine–threonine protein kinase that plays a crucial role in the regulation of inflammatory cell signalling cascades. Its position at the interface of diverse signalling pathways allows it to modulate downstream biological responses through several regulatory mechanisms (reviewed recently in REFS 55–57). The p38α and p38β MAPK isoforms have key structural differences in their active sites compared with most other protein kinases and other p38 MAPK isoforms. This can be exploited to generate selective small-molecule inhibitors that bind within the ATP fold^{58, 77}. Specifically, p38α and p38β have a hydrophobic pocket with a ‘gatekeeper’ threonine, an amino acid with a small side chain, whereas most other kinases have a larger amino acid, such as methionine, at this locus⁷⁸. The small side chain allows bulky substituents on diverse small molecules to occupy the pocket, which would be restricted in kinases with the larger gatekeeper amino acid. This structural feature can be combined with a nearby interaction that is potentially common to many kinases, such as H-bond interactions available close-by in three-dimensional space, to generate selective small-molecule inhibitors^{76,79}.

The three-dimensional selectivity filter has been used⁶⁴ to generate knock-in mouse strains that express only a mutant p38(T106M) kinase that had normal p38 MAPK enzyme activity but could no longer bind small-molecule p38 MAPK inhibitors that exploit this structural feature (this is an example of designed drug resistance). These p38-knock-in mice provided

valuable tools to show that the *in vivo* efficacy of an experimental therapeutic agent was through inhibition of its target kinase — in this case, p38 α MAPK⁶⁴.

Forecasting the potential uptake of small molecules by the brain in specific CNS drug discovery programmes remains an elusive goal. The consideration of several physicochemical characteristics of protein kinase-targeted drugs represents a promising strategy to achieve this. However, this is a context-dependent approach owing to the complex nature of *in vivo* absorption, tissue distribution, diffusion across the BBB and PGP substrate status. Only tenuous conclusions can be drawn from a single medicinal chemistry refinement programme. For example, early studies of benzodiazepines¹²⁹ found correlations among brain uptake, efficacy and blood levels of the drugs with high lipophilicity. However, the authors were careful to note the complicating factors of increased metabolism to active metabolites of some analogues and a potential bias in the data set towards some physical properties such as lipophilicity. As more results became available, it was clear that there was not a simple correlation between LogBB and LogP. For example, flunitrazepam, diazepam and midazolam have LogP values that increase linearly with respect to their corresponding LogBB values (see Supplementary information S3 (table)). However, the benzodiazepine oxazepam has a lower LogP value of 1.35 and a higher LogBB value of 0.61 than the three other benzodiazepine compounds. Similarly, the elevated LogP of alprazolam (3.13) does not give a corresponding increase in LogBB. The refinement of antihistamines provides another example. Diphenhydramine, a first-generation antihistamine, generates sedative effects, which are attributed to its ability to penetrate the BBB¹³⁰. However, these effects are absent with the second-generation antihistamine fexofenadine (Allegra; Sanofi–Aventis). A possible explanation is that efflux of fexofenadine by PGP^{130,131} limits its brain uptake and sedative effects.

The trend analysis of molecular properties presented in FIG. 4 shows that CNS-penetrant compounds have much lower PSA values than other drug classes (for example, marketed drugs and kinase inhibitors). Certain molecular drug target classes, such as kinase inhibitors, are characterized by high mean PSA values¹³². This raises the question of whether the CNS kinase target class is not amenable to a lower PSA, or whether there has not been sufficient focus on such properties during medicinal chemistry refinement. There is the concern that decreasing key properties such as PSA to improve BBB penetrance could produce a better substrate for cytochrome P450 2D6 (CYP2D6)¹³³. This is an undesired outcome as the polymorphic nature of this drug-metabolizing enzyme results in differences in metabolism among individuals. Like CNS drugs, CYP2D6 substrates are characterized by lower mean PSA values (35.25 Å²) than non-CYP2D6 substrates (57.49 Å²)¹³³. This is an interesting correlation, as a higher proportion of CNS drugs are CYP2D6 substrates than non-CNS drugs¹³³.

Box 3 | Fragment-based discovery strategies for CNS kinase inhibitors

Molecular-fragment-based approaches start with weakly active or inactive chemical scaffolds that are amenable to chemical diversification at multiple points¹⁴¹. Structure-assisted, fragment-based drug discovery uses small, usually weakly binding, molecular fragments or scaffolds as a starting point for finding novel hits and lead compounds. Studies usually begin with a high-resolution X-ray crystal structure of the kinase domain of a potential central nervous system (CNS) target containing an inactive molecular fragment bound to the active site of the kinase. The assumption is that the fragment makes specific interactions with the protein that are typically retained through the process of fragment expansion and optimization.

Part **a** of the figure shows a typical protein kinase ATP fold containing a bound ATP analogue, and examples of fragments or scaffolds that are used in the discovery of kinase inhibitors. The ATP fold is the region of protein kinases that has been successfully targeted

to generate drugs and candidate compounds. Part **b** shows two widely used fragment-based discovery strategies: fragment expansion and fragment linkage. The fragment expansion variation of the approach uses chemical diversification of the core fragment to incrementally grow the starting fragment in multiple directions, thereby sampling regions of ‘chemical space’ that could result in favourable interactions with the protein. The assumption is that the custom set of interactions between a minimally expanded ligand and its target protein will optimize activity and selectivity. Linking two fragments that bind in different locations is an alternative strategy. Both approaches are compatible with inclusion of a selection filter that is based on small-molecule physical property considerations. Computed molecular properties of the proposed products are examined to select candidates for synthesis that are similar to known CNS-penetrant compounds. The aim is to increase the probability of producing kinase inhibitors that are more CNS drug-like than existing compounds.

The consideration of CNS drug characteristics for designed compounds complements the inhibitor design method that maximizes target interactions. The combination thereby reduces the total number of compounds to be made initially in the discovery phase. Once an initial set of compounds is synthesized based on the drug-like ligand design and hits are obtained, selected pharmacology-driven *in vitro* screens can be used as an initial experimental filter. The approach provides a medicinal chemistry refinement strategy that is driven by a recursive design–synthesis–evaluation–design cycle.

In summary, the complexity and challenge of penetrating the BBB make it difficult to apply existing drugs for peripheral-tissue disorders to the CNS, even when the drugs are safe and efficacious. Studying the molecular basis of CNS drug failures for well validated targets might allow additional BBB-related issues to be elucidated beyond physicochemical properties that affect drug uptake or PGP substrate status. The technology now available for protein kinase inhibitor design allows for the introduction of CNS-relevant considerations at the early ligand design stage concerning key physicochemical properties, especially lower molecular weight and PSA.

Cytochrome P450 (CYP) substrate status

The impact of CYP-mediated metabolism on drug safety and efficacy makes it a key consideration in drug discovery, irrespective of the target or tissue site of action. Together, CYP2D6 and CYP3A4 account for the metabolism of ~70% of marketed drugs¹³⁴. However, the prevalence of CYP2D6-mediated metabolism of existing CNS drugs and the variable patient responses due to functional genetic polymorphisms in the *CYP* gene^{119,135} emphasize the importance of avoiding CYP2D6 metabolism in the discovery of new CNS therapeutics. The CYP2D6-mediated conversion of codeine to its active drug form, morphine, provides an example of unwanted, variable patient responses. Patients with a ‘slow metabolizer’ phenotype can experience reduced analgesic effects resulting from diminished morphine production, whereas a ‘rapid metabolizer’ phenotype increases toxicity risks as excessive levels of morphine can be produced¹³⁶. Increasing LogP and minimizing PSA are sometimes used to improve brain uptake of small molecules, but this can also increase the likelihood that the small molecule will serve as a CYP2D6 substrate¹³³. Therefore, medicinal chemistry optimization to improve brain uptake must be done carefully to minimize the probability of creating good CYP2D6 substrates. A promising trend is that early testing and avoiding the generation of good CYP2D6 substrates have become standard practices in medicinal chemistry refinement.

CYP3A4-mediated metabolism remains a pervasive issue in kinase inhibitor drug discovery. For example, nearly half of the kinase inhibitor drugs in TABLE 1 and a high percentage of all kinase inhibitor drugs, regardless of disease area, are CYP3A4 substrates (see Supplementary information S4 (table)). One example is imatinib¹³⁷, which is often used clinically in conjunction with anti-convulsant therapy¹²³ — a therapy that can increase CYP3A4 activity. As a result, the pharmacokinetics of imatinib can be altered. Although it is difficult to alter CYP substrate status by changing a single molecular property, CYP3A4 substrates have a higher average LogP than other CYP substrates, providing another reason to minimize lipophilicity and molecular weight during medicinal chemistry refinement. Although treatment of patients can be guided by genotyping or the adjustment of dosing for each drug, a preferable strategy is to reduce variability and safety issues at the medicinal chemistry refinement stage by a consideration of the features that determine whether a drug will be a good substrate for CYP2D6 or CYP3A4.

The kinase ATP site — selectivity and affinity

The kinase catalytic core structure (FIG. 5) has a bi-lobe architecture comprising a smaller N-terminal domain consisting mainly of β -sheets and a larger C-terminal domain consisting mainly of α -helices. Between the two domains is the ATP-binding fold and the linker hinge region that connects the two domains. The adenine ring of ATP interacts with the hinge region, and the ribose and triphosphate groups of ATP bind in a channel that extends to the peptide-substrate-binding region. The ATP fold contains a set of key conserved regions that are essential for substrate recognition and catalysis. For example, an activation loop containing a conserved DFG motif is important in regulating kinase activity, and a P-loop containing a glycine-rich motif forms the ‘roof’ of the ATP-binding site. Although the canonical protein kinase ATP-binding site contains many conserved regions and features, there are also distinctive pockets and residues that differentiate the structural landscapes of the various kinase ATP folds. Consequently, a mixture of conserved and unique features makes targeting the protein kinase ATP fold an attractive strategy for designing selective kinase inhibitors.

Most kinase inhibitor drugs and drug candidates occupy the ATP fold, but gain selectivity by exploiting space and contacts with the enzyme that are not used by ATP. In general, inhibitor selectivity is achieved through sets of contacts and binding modes that are unique for individual kinases or small groups of kinases². Crystal structures of kinases in complex with an inhibitor (see FIG. 5 for the crystal structure of imatinib in complex with SYK tyrosine kinase) show

that the compounds can occupy a similar three-dimensional space as ATP, but interact with distinct amino-acid residues. Kinase inhibitors can bind to conserved enzyme structural features that are used by ATP, and to nearby three-dimensional topologies that are unique or restricted to a subclass of kinases, such as a hydrophobic pocket with restricted accessibility. This steric array of binding modes allows a selective and high-affinity interaction of the small molecule with only a small subset of an organism's proteome. One complementary inhibitor design strategy involves targeting regions outside the ATP-binding site using non-competitive or allosteric inhibitors². For example, non-competitive inhibitors have been identified for MEK1 and MEK2 (REFS^{138,139}) that exploit a region adjacent to the canonical ATP-binding site and show high target selectivity. Although the development of allosteric inhibitors has so far not had the clinical success of ATP-competitive inhibitors, this may be due in part to the lack of knowledge about small-molecule allosteric pockets, such as that of MEK1 and MEK2, in other kinases.

A primary drug discovery goal is to design an inhibitor with sufficient affinity to allow competition with the two kinase substrates, ATP and the substrate protein, the apparent affinities of which are well above 1 μ M. The aim is to inhibit the phosphorylation reaction, the rate-limiting step of which is the release of the products ADP and phosphorylated protein¹⁴⁰. Kinase targets are usually components of complex, interconnected signal transduction cascades comprising many protein kinases, with pathway redundancy and crosstalk between pathways (reviewed in REF.⁹¹). The presence of such complex intracellular networks raises the possibility that broad specificity of a kinase inhibitor for a cluster of kinase targets, rather than high affinity for a single target, may provide the desired pharmacological effectiveness *in vivo*. Imatinib is an example of this paradigm: its apparent inhibition constant for the target kinases is just below the operational goal of less than 1 μ M and its pharmacological targets are a discrete set of protein kinases¹²². The key challenge to the development of protein kinase inhibitors for CNS disorders is the design of inhibitors that exhibit sufficient affinity and selectivity for a molecular target, or set of molecular targets, and have the appropriate molecular property profiles for CNS penetration.

Conclusions and trends

The targeting of protein kinases in CNS disorders is a promising field at an early stage of development. Although there are challenging barriers to overcome, these have the potential to be addressed with current approaches. Analysis of current protein kinase inhibitor drugs and CNS-penetrant small molecules reveals addressable challenges related to the bioavailability and pharmacokinetics of kinase inhibitor drug candidates. It is crucial to consider the physicochemical properties of small molecules that influence the interplay of pharmacokinetics and pharmacodynamics at an early stage in CNS drug discovery. Current technologies increase the potential for success by allowing the generation of compounds that share molecular and pharmacological properties with compounds that were previously successful, yet are unique chemical structures.

Greater emphasis is increasingly being placed on finding approaches that allow early assessment of a candidate drug's potential for long-term success. This is based on the realization that, across several molecular-target classes, the percentage change in compound properties made during medicinal chemistry refinement of initial hits to leads is substantially higher than during refinement of lead compounds to clinical candidates. The trend indicates the impact of the starting hit on the drug discovery and development process. Such challenges are now addressed earlier in CNS drug discovery programmes than they were previously. The strategy represents a major challenge in early-stage screening and substantially reduces the medicinal chemistry effort, especially in terms of the refinement of less desirable hits. The new approaches also offer the potential to improve the integration of ligand discovery with drug

discovery. For example, activity-based high-throughput screening (HTS) has been a primary source of new hits for drug discovery. However, as noted by Lipinski¹¹⁷, hits and leads that are derived from such programmes tend to be far from optimal in their drug-like physicochemical properties. The outcome requires that considerable effort be expended on detailed, quantitative structure–activity relationship analyses on hits from HTS activity screens to define the minimal structure that is required for activity. The analyses are needed at this stage owing to the usual changes in molecular properties, such as increased molecular weight and lipophilicity, that occur as a drug development programme progresses.

The significant difference in mean values for physicochemical properties of CNS-penetrant small molecules compared with those for current protein kinase inhibitor drugs indicates an even greater divide between hits and CNS drug candidates than for other molecular targets and disease areas. Based on analyses of the molecular properties of the CNS-penetrant small-molecules database (see Supplementary information S3 (table)) and kinase inhibitor drugs and drug candidates (see Supplementary information S2,S3,S4 (tables)), it seems that kinase inhibitor drugs for CNS indications will require a more restricted profile of molecular properties than the Lipinski ‘Rule of Five’ — a widely used filter to prioritize medicinal chemistry refinement efforts¹¹⁷. Most of the brain-penetrant small molecules (see Supplementary information S3 (table)) have a molecular weight < 400, LogP < 4 and PSA < 80 Å². Compounds outside this cluster of values have a higher likelihood of having undesired CYP metabolism and PGP efflux and a lower mean LogBB. Therefore, the likelihood of success of a CNS-focused kinase inhibitor discovery programme might be increased by a careful approach to the screening and selection of hit compounds, and by the monitoring of key molecular properties during recursive medicinal chemistry refinement.

One emerging and promising approach with the potential to address the challenges in targeting protein kinases for CNS disorders is a form of fragment-based drug discovery (BOX 3), referred to as fragment expansion. Recent reviews highlight the success in developing fragments into clinical candidates (for example, see REFS^{141–143}). Fragment expansion approaches tend to yield fewer but higher quality compounds than other drug discovery approaches, and result in less time being spent on non-essential structure–activity studies. This ‘smart chemistry’ approach also allows *in vivo* proof of concept testing using animal models of preclinical safety, pharmacology and efficacy at an early stage of the drug discovery timeline.

A compound discovery and refinement paradigm that allows early testing *in vivo* is attractive for targeting protein kinases in CNS disorders owing to the inherent molecular and tissue-related challenges. The complexity of protein kinase-mediated signal transduction cascades and the limited knowledge about these highly integrated intracellular pathways necessitate *in vivo* testing of hypotheses early in the discovery process. Early testing of the potential for a given protein kinase to be druggable requires *in vivo* experimental probing with bioavailable compounds. The initial design of molecules with the appropriate characteristics and the use of pharmacological filters as an adjunct to target activity screens increase the probability that the fewer compounds emerging from the early stages will be more drug-like and stable and will have BBB penetrance (see FIG. 6 for an example flowsheet). Implementing procedures to reduce the risk of compound failure at an early stage will result in a ‘failing fast *in vivo*’ paradigm, but the recursive nature of the process will allow lessons to be learned from well designed failures, as well as from successes, which should rapidly enhance the process as the project progresses. The net result is that fewer compounds are synthesized and less time is required to produce clinical candidates.

In summary, protein kinases hold tremendous promise as therapeutic targets for CNS disorders, with an increasing number of protein kinases emerging as viable targets for major neurological diseases that lack effective therapies. The development of kinase-targeted therapeutics for CNS

diseases must address the usual key drug discovery hurdles, such as first-pass metabolism, target affinity and selectivity, and therapeutic index. Although kinase inhibitor drug discovery in general has successfully overcome many of the challenges and progressed considerably over the past decade, there are special challenges related to CNS drug discovery that must be addressed at the start of the drug discovery process. Consideration of important CNS challenges in molecular design and the early-stage integration of key feasibility screens should enhance the likelihood of success in developing new kinase-targeted therapeutics for CNS disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Blood–brain barrier	The barrier formed by tight junctions of brain endothelial cells that restrict the passage of drugs between the bloodstream and the brain.
Pharmacodynamics	The biochemical and physiological effects of an administered drug on the body, and the relationships between drug dose and efficacy or toxicity.
Kinome	The subset of genes that encode protein kinases in the genome of an organism.
P-glycoprotein	An efflux transporter at the blood–brain barrier that can prevent drug substrate accumulation in the brain.
Polar surface area	The total surface area of all polar atoms (usually oxygen and nitrogen), including attached hydrogen ions.
LogP	A measure of a drug's lipophilicity, it is the logarithm of the octanol–water partition coefficient. Higher values are associated with greater lipophilicity, whereas lower values are characteristic of more water-soluble compounds.
LogBB	The steady-state ratio of drug concentration in the brain versus drug concentration in the blood.
Pharmacokinetics	'What the body does to the drug', including the extent and rate of drug absorption, distribution, metabolism and excretion.

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Figure 1. Trends in drug discovery and development efforts targeting protein kinases

The number of publications since 1990 on small-molecule protein kinase inhibitors that have been evaluated in animal efficacy models and clinical trials for oncology indications was determined by a literature search with SciFinder Scholar. The number steadily increased until the recent plateau in 2007. The orange bar denotes the number of publications on central nervous system (CNS) disease indications in 2008. This activity level is comparable to that for the cancer field in 1999. The curve shows several small inflection points that correlate with milestones related to the kinase inhibitor imatinib (Gleevec; Novartis). For example, the first reports of *in vivo* efficacy of imatinib appeared in 1995 and 1996, and shortly thereafter the slope of the curve showed a small increase. An increased slope is also seen after the first clinical trials with imatinib were initiated in 1998 and after drug approval in 2001. Before 2001, only one kinase inhibitor was approved as a drug (fasudil; approved in Japan in 1995 for cerebral vasospasm). Since 2001, eight additional kinase inhibitors have received approval for oncology indications: gefitinib (Iressa; AstraZeneca) was approved in the United States in 2003; erlotinib (Tarceva; OSI/Genentech/Roche) was approved in the United States and Europe in 2004; sorafenib (Nexavar; Bayer/Onyx) was approved in the United States and Europe in 2005; sunitinib (Sutent; Pfizer) and dasatinib (Sprycel; Bristol–Myers Squibb) were approved in the United States and Europe in 2006; and temsirolimus (Torisel; Wyeth) was approved in the United States, and lapatinib (Tykerb; GlaxoSmithKline) and nilotinib (Tasigna; Novartis) were approved in the United States and Europe in 2007.

Figure 2. Selected chemical structures of small-molecule kinase inhibitors in clinical trials for CNS indications

See TABLE 1 for further information.

Figure 3. Protein kinase targets as components of intracellular signal transduction networks

Glycogen synthase kinase 3 (GSK3) is an example of a widely distributed target that modulates diverse cellular functions. Death-associated protein kinase 1 (DAPK1) has a more restricted cellular role, but both are components of an integrated web of pathways. Selective biological responses are possible because such networks can transduce various cellular stimuli into an integrated response, through mechanisms that are incompletely understood. The target kinases represent network nodes, the quantitative importance of which is altered under conditions of deregulation. The activity of GSK3 is regulated by several mechanisms that allow selective phosphorylation of substrates in response to distinct input signals. The regulatory mechanisms include modulation of GSK3 activity by inhibitory serine phosphorylation and activating tyrosine phosphorylation of the enzyme, coordinated phosphorylation of its substrates, dynamic regulation of the subcellular distribution of GSK3 and the assembly of GSK3 into distinct multi-protein complexes for selective substrate phosphorylation. DAPK1 regulates signalling pathways that are important to cell survival and translational control through phosphorylations of a highly selective set of substrate proteins in response to its activation by distinct cellular stimuli, such as increased Ca^{2+} levels, which are sensed through its calmodulin (CAM) subunit. Examples of central nervous system functions that have been proposed for DAPK1 and GSK3 are shown in orange. A β , amyloid- β ; BAX, BCL2-associated X protein; CAMK1, Ca^{2+} -calmodulin-dependent protein kinase 1; CAMKK, CAMK kinase; C/EBP, CCAAT/enhancer-binding protein; CREB, cyclic AMP response element binding protein; ECM, extracellular matrix; EIF2B, eukaryotic initiation factor 2B; ERK, extracellular signal-regulated kinase; HSF1, heat shock factor 1; IRS1, insulin receptor substrate 1; JNK, Jun I-terminal kinase; MAP1B, microtubule-associated protein 1B; MAPK, mitogen-activated protein kinase; MLC, myosin light chain; MRPL13, mitochondrial ribosomal protein L13; MRPS6, mitochondrial ribosomal protein S6; MYC, myelocytomatosis viral oncogene homologue; NFAT, nuclear factor of activated T cells; NF- κ B, nuclear factor κ B; phospho-S,

phosphorylated serine; phospho-Y, phosphorylated tyrosine; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PYK2, proline-rich tyrosine kinase 2; TP53, tumour protein p53; RPS6KA1, ribosomal protein S6 kinase, 90kDa, polypeptide 1.

Figure 4. Key molecular-property trends for small molecules

Comparisons of mean values of three key molecular properties, lipophilicity (LogP) (a), molecular weight (MW) (b) and polar surface areas (PSA) (c), are shown for CNS-penetrant small molecules (CNS; see Supplementary information S3 (table)), approved and marketed drugs for any indication (MKT; data from Vieth¹²¹) and kinase inhibitor drugs for all indications (KID; see Supplementary information S4 (table)). One-way ANOVA was used to evaluate significant differences to CNS mean values. Mean values for LogP (d), MW (e) and PSA (f) were also calculated for compounds in the CNS dataset (see Supplementary information S3 (table)) that are P-glycoprotein substrates (PGP⁺) compared with compounds that have been experimentally validated as non-P-glycoprotein substrates (PGP⁻). Student's t-test was used to compare mean values. * $p < 0.05$. ** $p < 0.001$.

Figure 5. Structure of the protein kinase catalytic core and bound inhibitor in the ATP fold
 Protein kinases are characterized by a bi-lobe architecture consisting of an amino-terminal β -sheet lobe and a larger α -helical carboxy-terminal domain, with a canonical fold that contains the nucleotide-binding site located between the two domains. Other conserved protein kinase features include the P-loop (shown in magenta), which contains a glycine-rich motif (GXGXXG), and the activation loop (shown in purple), which is characterized by an initial DFG and terminal APE motif. Upon ATP binding, a reorientation of the C-helix (shown in light blue) and the glycine-rich loop is often observed. This structure shows SYK tyrosine kinase in complex with the therapeutic kinase inhibitor imatinib (Protein Data Bank identification number: 1XBB).

Figure 6. Example of a potential compound discovery paradigm for CNS kinase targets

Focusing on small-molecule kinase inhibitors with key molecular property characteristics and promising profiles in early-stage pharmacological screens increases the potential for positive outcomes in the subsequent preclinical development of investigational new drugs. This substantially reduces early discovery demands on synthetic chemistry and biology efforts and the later attrition rate in development. Medicinal chemistry refinement takes into consideration molecular properties, suitability for central nervous system (CNS) penetration and ligand activity. Pharmacology-related screens are used to measure brain uptake, assess microsomal stability, determine whether the compound is a cytochrome P450 2D6 (CYP2D6) or CYP3A4 substrate and ensure that there are no adverse effects. HTS, high-throughput screening; IC₅₀, half-maximal inhibitory concentration; SAR, structure–activity relationship.

Table 1
Protein kinase targets for CNS indications: small-molecule compounds in clinical trials

Kinase	Type	Disease indication	Clinical trial identifier (Phase)	Example compound (company)	
BCR-ABL	Y	Glioma	NCT00021229 (I-II)	Imatinib (Novartis)	
			NCT00010049 (I-II)*		
			NCT00423735 (II)		
KIT	Y	Glioma	NCT00021229 (I-II)	Imatinib (Novartis)	
			NCT00010049 (I-II)*		
			NCT00329719 (I-II)		
			Neurofibromatosis type 1		
			NCT00727233 (I)		
		Glioma	NCT00459381 (II)	Pazopanib (GlaxoSmithKline)	
			NCT00423735 (II)		
			NCT00423735 (II)		
			NCT00589784 (II)		
			NCT00387933 (I)		
		Glioma	NCT00387933 (I)	Vatalanib (Novartis)	
			NCT00052013 (II)		
			Von Hippel-Lindau-related haemangioblastoma		
			NCT00348790 (II)		
EGFR1	Y	Glioma	NCT00025675 (II)	Gefitinib (AstraZeneca)	
			NCT00054496 (II)	Erlotinib (Genentech)	
			NCT00107237 (I-II)		
EGFR2	Y	Glioma	NCT00589784 (II)	AEE-788 (Novartis)	
FLT3	Y	Meningioma	NCT00589784 (II)	Sunitinib (Pfizer)	
GSK3	S-T	Alzheimer's disease	NCT00088387 (II)	Lithium (several companies)	
			No identifier (II)	NP-12 (Noscira)	
		Depression	No identifier (Phase unreported)	NP031115 (Noscira)	
			NCT00040404 (II-III) [‡]	CEP-1347 (Cephalon)	
MLK1, MLK2 and MLK3	S-T	Parkinson's disease	NCT00040404 (II-III) [‡]		
mTOR	S-T	Glioma	NCT00831324 (II)	Everolimus (Novartis)	
			NCT00047073 (I-II)	Sirolimus (Wyeth)	
			NCT00457808 (I/II)		
p38α	S-T	Neuropathic pain	NCT00022724 (I-II)	Tensirolimus (Wyeth)	
			NCT00390845 (II)	SB-681323 (GlaxoSmithKline)	

Kinase	Type	Disease indication	Clinical trial identifier (Phase)	Example compound (company)
PDGFRβ	Y	Glioma	NCT00021229 (I-II)	Imatinib (Novartis)
			NCT00010049 (I-II)*	
			NCT00329719 (I-II)	Sorafenib (Bayer)
			NCT00727233 (I)	
		Neurofibromatosis type 1	NCT00459381 (II)	Pazopanib (GlaxoSmithKline)
		Glioma	NCT00423735 (II)	Dasatinib (Bristol-Myers Squibb)
		Meningioma	NCT00589784 (II)	Sunitinib (Pfizer)
		Glioma	NCT00387933 (I)	Vatalanib (Novartis)
		Von Hippel-Lindau-related haemangioblastoma	NCT00052013 (II)	
		Meningioma	NCT00348790 (II)	
PDHK	S-T	Glioma	NCT00703859 (I)	Dichloroacetate (several companies)
PKC	S-T	Bipolar disorder	NCT00026585 (II)	Tamoxifen (AstraZeneca)
			NCT00411203 (III)	
RAF	S-T	Glioma	NCT00329719 (I/II)	Sorafenib (Bayer)
			NCT00727233 (I)	
ROCK	S-T	Raynaud's phenomenon	NCT00498615 (III)	Fasudil (Asahi Kasei)
SRC	Y	Glioma	NCT00423735 (II)	Dasatinib (Bristol-Myers Squibb)
VEGFR1	Y	Glioma	NCT00459381 (II)	Pazopanib (GlaxoSmithKline)
			NCT00006247 (I)	Semaxanib/SU5416 (Pharmacia)§
			NCT00387933 (I)	Vatalanib (Novartis)
			NCT00052013 (II)	
		Von Hippel-Lindau-related haemangioblastoma	NCT00348790 (II)	
VEGFR2	Y	Glioma	NCT00107237 (I-II)	AEE-788 (Novartis)
			NCT00329719 (I-II)	
			NCT00727233 (I)	Sorafenib (Bayer)
			NCT00459381 (II)	
			NCT00006247 (I)	Semaxanib/SU5416 (Pharmacia)§
			NCT00589784 (II)	Sunitinib (Pfizer)

Kinase	Type	Disease indication	Clinical trial identifier (Phase)	Example compound (company)
VEGFR3	Y	Glioma	NCT00387933 (I)	Vatalanib (Novartis)
		Von Hippel–Lindau-related haemangioblastoma	NCT00052013 (II)	
		Meningioma	NCT00348790 (II)	
		Glioma	NCT00459381 (II)	Sorafenib (Bayer)
		Neurofibromatosis type 1	NCT00727233 (I)	
		Glioma	NCT00459381 (II)	Pazopanib (GlaxoSmithKline)
			NCT00387933 (I)	Vatalanib (Novartis)
		Von Hippel–Lindau-related haemangioblastoma	NCT00052013 (II)	
		Meningioma	NCT00348790 (II)	

* The trial has been completed but the therapy was not efficacious.

† The trial was terminated early owing to a failure to delay disability.

§ Development has been discontinued owing to a lack of clinical efficacy in colorectal cancer trials. EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FLT, FMS-related tyrosine kinase; GSK, glycogen synthase kinase; JAK3, Janus kinase 3; JNK, c-Jun N-terminal protein kinase; MEK, mitogen-activated protein kinase (MAPK)–ERK kinase; MLK, mixed lineage kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PDHK, pyruvate dehydrogenase kinase; PKC, protein kinase C; ROCK, Rho-associated protein kinase; S–T, serine–threonine; VEGFR, vascular endothelial growth factor receptor; Y, tyrosine.

Table 2
Kinase targets for CNS indications: small molecules with preclinical *in vivo* efficacy data

Kinase	Type	Disease indication	Example compounds	Refs
AMPK	S-T	Multiple sclerosis	Arasine	144
		Cerebral ischaemia	Compound C	145
CDK	S-T	Traumatic brain injury	Roscovitine	147
		Cerebral ischaemia	Flavopiridol and indolinone A	148,149
DAPK1	S-T	Acute brain injury	11-(3-imino-5,6-dihydrobenzo[h]cinnolin-2(3 <i>H</i>)-yl)- <i>N</i> -(6-phenylpyridazin-3-yl)undecanamide	41,43
		Alzheimer's disease	11-(3-imino-5,6-dihydrobenzo[h]cinnolin-2(3 <i>H</i>)-yl)- <i>N</i> -(6-phenylpyridazin-3-yl)undecanamide	42
GSK3	S-T	Alzheimer's disease	SB-216763	150
			AR-AOI4418	150
			Lithium	150,151
			Indirubin-3'-monoxime	150
			Alsterpaullone	150
			Chir98014	150
			NP-12*	34
		Amyotrophic lateral sclerosis	AR-AOI4418	26
		Bipolar disorder	Multiple 3-benzofuranyl-4-indolylmaleimide	152
Cerebral ischaemia			Compound 1	153
			Chir025*	154
			TDZD-8	30
			AR-AOI4418	28
Depression			NP031115*	32
HIV-associated dementia			Valproic acid	155
Parkinson's disease			AR-AOI4418	156
			Indirubin-3'-oxime ((<i>E</i>)-3-(hydroxyimino)-2,3'-biindolin-2'-one)	156
Neurocognitive deficits after cranial irradiation			SB-415286	157
			SB-216763	157

Kinase	Type	Disease indication	Example compounds	Refs
		Shock	TDZD-8	30
		Spinal cord injury	TDZD-8	31
		Traumatic brain injury	Lithium	158
JAK3	S-T	Amyotrophic lateral sclerosis	WHL-P131	159
JNK1, JNK2 and JNK3	S-T	Subarachnoid haemorrhage	SP600125	87
		Parkinson's disease	SP600125	160
		Cerebral ischaemia	AS601245	86
MEK1 and MEK2	S-T	Behavioral disorders and drug abuse	SL 327	161
		Cerebral ischaemia	U0126	82
		Traumatic brain injury	PD-98059	162,163
		Neuropathic pain	PD 198306	85
		Excitotoxic injury	CEP-1347	164
MLK1, MLK2 and MLK3	S-T	Hearing loss	CEP-1347	165
		Huntington's disease	CEP-1347	89
			CEP-11004	90,166
		Motor neuron disease	CEP-1347	90
		Parkinson's disease	CEP-5104, CEP-6331 and CEP-1347	167
MLCK	S-T	Cerebral vasospasm	ML-9	94
mTOR	Y	Autism	Rapamycin	168
		Huntington's disease	Temsirolimus	169
		Tuberous sclerosis	Rapamycin	170
p38 α MAPK	S-T	Alzheimer's disease	MW01-2-069ASRM	169
		Amyotrophic lateral sclerosis	SB203580	58
		Cerebral ischaemia	SB203580	59
			SB239063	60
		Neuropathic pain	FR167653	61; 62
		Parkinson's disease	SB239063	171
		Spinal cord injury	SB203580	70
				172

Kinase	Type	Disease indication	Example compounds	Refs
PKA	S-T	Cerebral ischaemia	VX-745	173
		Traumatic brain injury	SB203580	85
		Alzheimer's disease	H89	174
		Memory impairment	Halofantrine	175
PKC	S-T	Morphine tolerance	KT 5270	176
		Neuropathic pain	Triterpene mixture	177
		Alzheimer's disease	GF-109203X	178
		Bipolar disorder	Tamoxifen	179
		Brain tumours	LY 333531	180
		Cerebral ischaemia	Staurosporine	181
			GF-109203X	178
			Ebselen	182
		Cerebral vasospasm	H-7 and calphostin C	183
			Cheliritrine and rottlerin	184
		Neuropathic pain	Staurosporine and calphostin C	181
		Opioid dependence	H-7	185
PLK2	S-T	Parkinson's disease	Rottlerin	186
		Parkinson's disease	B12536	187
		Alzheimer's disease	Y-27632	188
		Cerebral ischaemia	Fasudil and hydroxyfasudil	189, 190
ROCK	S-T	Multiple sclerosis	Fasudil	191
		Epilepsy	Y-27632	192
		Spinal canal stenosis	Fasudil	171
		Pain	Y-27632	193
SNRPE	S-T	Spinal cord injury	Y-27632	194
		Cerebral ischaemia	SB-699393	146
		Brain injury	PP-1	195
		Intracerebral haemorrhage	PP-2	196
TGFβR	Y	Glioblastoma	SX-007*	197

* The structure has not been disclosed. AMPK, AMP-activated kinase; CDK, cyclin-dependent kinase; DAPK1, death-associated protein kinase 1; GSK3, glycogen synthase kinase 3; JAK3, Janus kinase 3; MAPK, mitogen-activated protein kinase; MEK, MAPK-ERK kinase; MLCK, myosin light chain kinase; MLK, mixed lineage kinase; mTOR, mammalian target of rapamycin; PKA, protein kinase A; PKC, protein kinase C; PLK2, polo-like kinase 2; ROCK, Rho-associated protein kinase; SNRPE, small nuclear ribonucleoprotein polypeptide E (also known as B-RAF); TGF β R, transforming growth factor- β receptor.