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Review

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Recent Advances in the Design, Synthesis, and Biological Evaluation of Selective DYRK1A Inhibitors: A New Avenue for a Disease Modifying Treatment of Alzheimer's?

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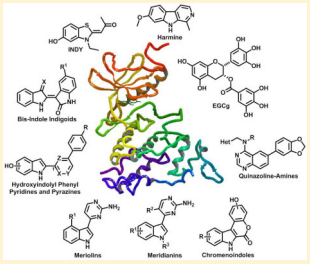
ABSTRACT: With 24.3 million people affected in 2005 and an estimated rise to 42.3 million in 2020, dementia is currently a leading unmet medical need and costly burden on public health. Seventy percent of these cases have been attributed to Alzheimer's disease (AD), a neurodegenerative pathology whose most evident symptom is a progressive decline in cognitive functions. Dual specificity tyrosine phosphorylation regulated kinase-1A (DYRK1A) is important in neuronal development and plays a variety of functional roles within the adult central nervous system. The DYRK1A gene is located within the Down syndrome critical region (DSCR) on human chromosome 21 and current research suggests that overexpression of DYRK1A may be a significant factor leading to cognitive deficits in people with Alzheimer's disease (AD) and Down syndrome (DS). Currently, treatment options for cognitive deficiencies associated with Down syndrome, as well as Alzheimer's disease, are extremely limited and represent a major unmet therapeutic need. Small molecule inhibition of DYRK1A activity in the brain may provide an avenue for pharmaceutical intervention of mental impairment associated with AD and other neurodegenerative diseases. We herein review the current state of the art in the development of DYRK1A inhibitors.



KEYWORDS: *DYRK1A, Alzheimer's disease, Down syndrome, DYRK1A inhibitors*

DYRKs are a family of eukaryotic kinases that belong to a larger super family known as the CMGC group of proline/arginine directed serine/threonine kinases. The DYRK family consists of five mammalian subtypes including 1A, 1B, 2, 3, and 4, of which only the gene for DYRK1A is located within the human chromosome 21 Down Syndrome Critical Region (DSCR).[1] These dual specificity kinases are named as such due to their ability to catalyze self-activation through autophosphorylation of a single tyrosine residue in their activation loop (Tyr321 in DYRK1A) as well as the phosphorylation of serine and threonine residues in exogenous protein substrates which, for DYRK1A and 1B, is often directed by the sequence RPX(S/T)P. Interestingly, the tyrosine self-phosphorylative activity of these kinases is believed to occur through a transiently active protein intermediate during translation, and is then lost once protein synthesis is complete. Specifically, DYRK1A is known to phosphorylate a multitude of proteins involved in a wide variety of signaling pathways *in vitro*; however, the specificity of DYRK1A *in vivo* is poorly defined.[2] The DYRK family of kinases show little sequence homology to other kinases outside of their catalytic domains, but are themselves highly conserved across species. Human DYRK1A in particular maintains over 99% sequence identity with that of the rat and mouse.[3]

DYRK1A has been shown to be expressed ubiquitously, but is abundant in the cerebellum, olfactory bulb, and hippocampus. Additionally, DYRK1A is up-regulated during the early stages of embryonic development followed by a gradual decrease to lower levels in later stages.[3] Homozygous DYRK1A knockout mice are inviable, terminating development during the period of organogenesis, due to a requirement for DYRK1A activity during differentiation.[4] Mice hemizygous for DYRK1A, while viable, show significant phenotypic effects, including decreased neonatal viability, smaller body size, reduced number of neurons in certain brain areas, alterations in motor development and function, dopaminergic deficiency in the nigrostriatal system and impairment in the development of spatial learning strategies.[4−8] This diversity of phenotypes resulting from differential DYRK1A gene dosage suggests that DYRK1A activity is tightly regulated during normal developmental processes.[2]

The β-amyloid hypothesis of Alzheimer's disease (AD) has been proposed as an overarching explanation for the adverse neurological events that occur in brains of AD patients.[9−11] AD is characterized by the presence of amyloid plaques and neurofibrillary tangles (NFTs) in certain brain regions.[12] Amyloid plaques are insoluble extracellular protein deposits

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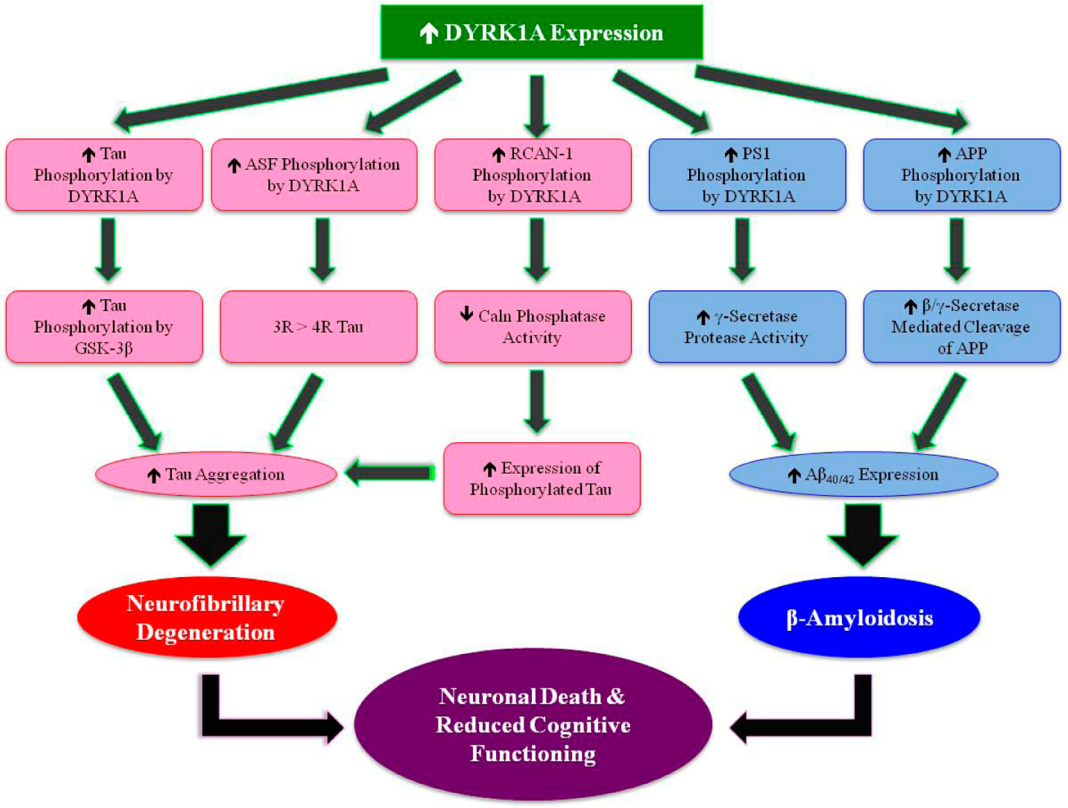

Figure 1. Scheme depicting different mechanistic pathways by which DYRK1A contributes to neurodegeneration and loss of cognitive functions.[16]

consisting primarily of β-amyloid peptides (Aβ), while NFTs are intracellular insoluble aggregates of hyperphosphorylated microtubule associated protein tau and other proteins.[13] Aβ peptides of amyloid plaques are generated by the catalytic cleavage of amyloid precursor protein (APP), the gene for which is also located on human chromosome 21. These cleavage events are mediated by the activity of β-secretase [β-site APP cleaving enzyme (BACE-1)] followed by γ-secretase which leads to Aβ fragments that are 37−42 amino acids in length ($A\beta_{37-42}$).[14,15] These Aβ fragments, mainly $A\beta_{42}$, can oligomerize to form soluble toxins that are thought to initiate signaling events that contribute to synaptic degeneration, adverse oxidative activity, and neuronal death.[16] In a process referred to as β-amyloidosis, the Aβ peptides can also fibrillize to form the insoluble β-amyloid plaques that are commonly observed in AD brains.[14,15] Aberrant phosphorylation of tau is also believed to contribute to neurodegeneration in AD. Hyperphosphorylation of tau results in loss of normal tau functioning and attenuates the stability of neuronal microtubules.[17] In addition, tau hyperphosphorylation is associated with aggregation of the protein into neurofibrillary tangles, contributing to neurofibrillary degeneration, neuronal death, and dementia severity.[16,18] The brains of Down syndrome (DS) patients are similarly characterized by these neuropathological features of AD, including increased levels of hyperphosphorylated tau protein aggregates and β-amyloid plaques, providing a connection between DS and AD that may explain the early onset of Alzheimer's associated dementia in the majority of people with DS.[16]

We highlight below (1) the literature suggesting that DYRK1A may provide a therapeutically exploitable link between aberrant amyloid and tau pathology in AD that makes the development of DYRK1A antagonists a promising approach to treat neurodegeneration associated with these pathologies and (2) the current state of the research toward the identification of potent and selective DYRK1A inhibitors, for which the majority of efforts have evolved over the last 5 years. In this review, reported active molecules have been divided into three main categories: natural products and their synthetic derivatives, synthetic inhibitors, and promiscuous kinase inhibitors subsequently found to possess DYRK1A activity. In addition, calculated topological polar surface area (tPSA), clogP, and molecular weights (MW) of key reported inhibitors are included, key determinants of a molecule's ability to passively diffuse across the blood-brain barrier (BBB).[19] When available, levels of ATP used in competitive assays are also described. During the review, discussion is occasionally directed toward key, conserved residues in the kinase domain, often engaged by ATP and inhibitors alike. The reader is directed to excellent articles by Traxler which describe the molecular architecture common to all kinases in more depth.[20] In regard to modern kinase inhibitor design, many efforts are focusing on the discovery of so-called Type II inhibitors that stabilize a new DFG-out conformation in the kinase domain, resulting in less conserved "active site" molecular architecture and consequently the ability to produce more selective small molecules. To the best of our knowledge, no small molecules that stabilize this conformation have been identified that significantly inhibit DYRK1A and reported inhibitors herein are assumed to be classical Type I ATP-competitive inhibitors.[21]

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DYRK1A: ROLE IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

The location of the DYRK1A gene in the DSCR of human chromosome 21 leads to overexpression of DYRK1A 1.5 times that of normal upon trisomy of chromosome 21. Increased activity of DYRK1A has been reported in various brain compartments in subjects that suffer from DS and neurodegenerative diseases including sporadic and familiar Alzheimer's disease as well as Parkinson's, Huntington's, and Pick's disease although much less is known about the function of DYRK1A in the latter three diseases. In particular, the percentage of DYRK1A positive nuclei in the frontal cortex of AD brains is about 10% compared to 0.5% in normal brains.[16,22]

Three main murine models for Down syndrome have been developed (Ts65Dn,[23] Ts1Cje,[24] and Ts1Rhr[25]) that exhibit partial trisomy of chromosome 16, the murine ortholog to human chromosome 21. In all three models, the trisomic region of MMU16 contains the gene for DYRK1A. These mice show characteristic symptoms of DS including learning and behavioral deficits[23−25] and alterations in their dendritic spines within the hippocampus and cortical regions of the brain.[25−29] Transgenic mice have also been prepared using a yeast artificial chromosome YAC 152F7 bearing extra copies of five different genes found in the DSCR of human chromosome 21 including DYRK1A, PIGP, TTC3, DSCR9, and DSCR3. These mice demonstrate significantly impaired learning ability and brain abnormalities.[30−32] In comparison, murine models transgenic for the yeast artificial chromosome YAC 141G6 which contains all genes encompassed in YAC 152F7 except DYRK1A do not exhibit noticeable cognitive impairment. In addition, mouse models that are transgenic for the human BAC gene (DYRK1A BAC Tg) or a murine BAC clone (TgDYRK1A) have been generated that specifically overexpress either human or murine DYRK1A, respectively. These mice similarly exhibit Down syndrome phenotypes including hippocampal-dependent spatial learning and motor deficits and developmental delays, which is highly suggestive of a central function for DYRK1A in mental retardation associated with Down syndrome.[30,33,34]

DYRK1A has been shown to phosphorylate numerous protein substrates including other kinases, as well as splicing, transcription, and translation factors residing in various cellular compartments that are involved in a wide range of cellular functions (Figure 1). Evidence is accumulating which suggests that the activity of DYRK1A is involved in regulating a multitude of cellular pathways, and thus, its abnormal functioning likely elicits a cascade of signaling events leading to multiple phenotypes including the loss of cognitive functioning and neuronal death. Hence, DYRK1A could support the pathogenesis of Alzheimer's disease by ultimately promoting NFT and Aβ formation.

Tau hyperphosphorylation and aggregation in AD is a complex process mediated by the activity of various enzymes of which DYRK1A seems to exhibit a central function. First, studies have shown that DYRK1A directly phosphorylates tau protein on at least 11 serine and threonine residues, some of which have been detected to be phosphorylated in tau aggregates of neurofibrillary tangles in DS associated AD and sporadic AD brains.[35−39] Precisely how many of these sites are drivers of NFT pathology in AD remains an issue for clarification. However, these results nevertheless suggest that DYRK1A inhibition may result in reduction of tau protein that is hyper-phosphorylated on numerous sites. DYRK1A mediated tau phosphorylation has also been shown to promote further tau phosphorylation at multiple sites by GSK-3β which increases the accumulation of DYRK1A positive neurofibrillary tangles. Second, DYRK1A has been demonstrated to phosphorylate the alternative splicing factor (ASF) specifically on three different serine residues (Ser227, Ser234, and Ser238).[40] ASF functions normally to promote the inclusion of exon 10 during tau splicing, leading to the synthesis of tau protein containing four microtubule binding motifs (4R-tau). A delicate balance of 3R- and 4R-tau is necessary to maintain proper neuronal function.[41] This phosphorylation of ASF by DYRK1A results in its deactivation and storage, sequestering its ability to regulate tau splicing, leading to an increased amount of 3R-tau relative to 4R-tau, and facilitating tau aggregation.[40]

DYRK1A also phosphorylates regulator of calcineurin-1 (RCAN1), the gene for which is also located in the DSCR. Phosphorylation of RCAN1 by DYRK1A at Thr192 slows its degradation and tightens the binding interaction between RCAN1 and calcineurin (Caln), thereby enhancing the ability of RCAN1 to inhibit Caln phosphatase activity and contributing to a resulting increase in phosphorylated tau protein levels as well as a reduction in Caln/NFAT transcriptional activity.[42] Indeed, increased expression of pThr192 RCAN1 has been detected in the brains of DYRK1A Tg mice. In addition, phosphorylation of RCAN1 by DYRK1A at Ser112 primes RCAN1 for GSK-3β mediated phosphorylation at Ser108, the downstream effects of which have not yet been fully elucidated, but DYRK1A promoted GSK-3β activity has been implicated in AD progression.[42]

Overactivity of DYRK1A has also been implicated in increased proteolytic cleavage of amyloid precursor protein (APP), contributing to increased production of neurotoxic Aβ peptides. DYRK1A phosphorylates Thr688 of APP in mammalian cells, which promotes β/γ-secretase mediated cleavage of APP, resulting in elevated production of neurotoxic Aβ peptides.[43] Furthermore, DYRK1A also phosphorylates presenilin 1 (PS1), a subunit of γ-secretase, and this phosphorylation event increases γ-secretase protease activity, further elevating Aβ peptide production.[44] Moreover, research suggests a positive feedback mechanism through which Aβ stimulates the expression of DYRK1A, thereby further accelerating the synthesis of neurotoxic Aβ peptides.[45] These combined observations implicate DYRK1A intimately in the generation of both amyloid and tau pathologies associated with DS and AD and suggest the possibility that efficient pharmacologic inhibition of DYRK1A activity may help to ameliorate simultaneously the production of both pathologies.

In addition to roles in the production of pathologies associated with AD-related neurodegeneration, DYRK1A has been suggested to affect other cellular pathways that may be involved in mental impairment and neurodegenerative dementia. For example, DYRK1A can activate PI3K/Akt signaling, a pathway largely involved in neuronal development, growth, and survival.[46] DYRK1A also stimulates the activity of ASK1/JNK1, possibly inducing neuronal death and apoptosis.[47] In addition, DYRK1A phosphorylates p53 during embryonic brain development, altering neuronal proliferation.[48] Synaptic proteins Amph 1, Dynamin 1, and Synaptojanin, involved in the regulation of endocytosis, are also subject to DYRK1A mediated phosphorylation, which may alter the number, size, and morphology of dendritic spines, thereby reducing synaptic plasticity.[49] Furthermore, research suggests a role for DYRK1A

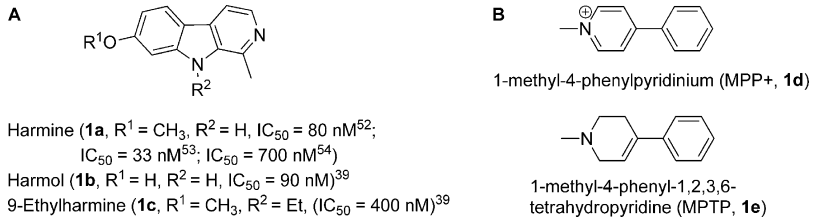

Figure 2. (a) Structures of β-carbolines **1a**–**c**. *In vitro* IC_{50} values against DYRK1A are indicated in parentheses. [ATP] = 50 mM;[52] [ATP] = 100 μM;[53] [ATP] = 1 mM.[39] (b) Structures of MPP+ **1d** and MPTP **1e**.

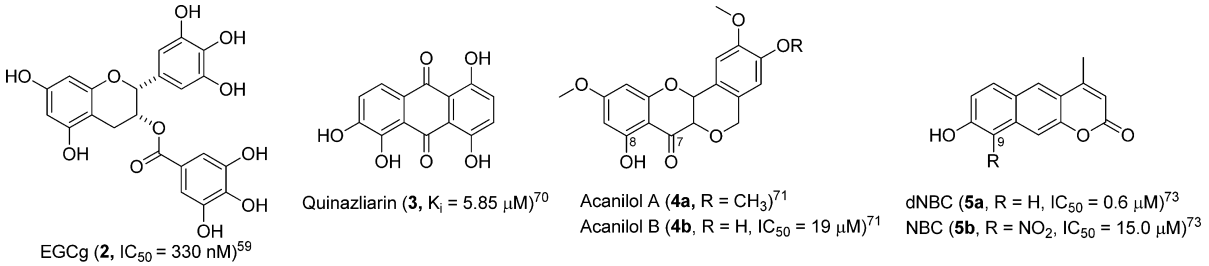

Figure 3. Structure of DYRK1A inhibiting natural and synthetic phenols and polyphenols. **2**, [ATP] = 100 μM;[59] **3**, [ATP] = 20 μM;[70] **4a**–**b**, [ATP] = 15 μM;[71] **5a**–**b**, [ATP] = 500–1000 c.p.m./pmol; [73] [ATP] = K_M for each kinase).[74]

in other neurodegenerative diseases. For example, α-synuclein, the major component of Lewy bodies that are associated with Parkinson's disease and other synucleinopathies, has recently been reported to be a substrate of DYRK1A.[50] DYRK1A also phsophorylates HIP-1 an enzyme involved in the death and differentiation of hippocampal neuroprogenitor cells implicated in Huntington's disease.[51]

Collectively, a large body of evidence now exists to support critical roles for DYRK1A in facilitating neurodegeneration and dementia through various pathways. Inhibition of DYRK1A functioning should theoretically mitigate multiple processes underlying the progression of neurodegeneration, particularly in Alzheimer's disease for which key DYRK1A targets (tau protein, amyloid precursor protein, presenilin 1) point to clear mechanisms through which elevated DYRK1A activity may be promoting disease progression.[16] Additionally, despite the nonviability of DYRK1A null mice and the growing consensus supporting DYRK1A as a regulatory protein controlling multiple signaling pathways, inhibition of its activity is quickly gaining interest as a possible mode of treatment for AD and DS. Currently, little is known about the possibility of adverse effects caused from DYRK1A inhibition; however, as medicinal research in this area matures into *in vivo* studies and preclinical testing, certainly we will learn more about additional on target consequences resulting from reduced DYRK1A activity. However, because the goal of this approach is not to deplete DYRK1A activity completely, but rather to a level comparable to that of healthy individuals not affected by these pathologies, the risk of target based side effects, although not inconceivable, is less of a concern.

DYRK1A INHIBITORS FROM NATURAL SOURCES AND DERIVATIVES

Harmine **1a** (tPSA 33.62, clogP 3.13, MW 212.25, Figure 2) is a β-carboline alkaloid first isolated from the South American vine *Banisteriopsis caapi*. In 2007, studies conducted by Bain and co-workers identified harmine **1a** as a potent and relatively selective inhibitor of DYRK1A *in vitro* (IC_{50} 0.08 μM).[52] Other members of the DYRK family were also inhibited to a lesser extent (IC_{50} 0.9 μM, DYRK2; 0.8 μM, DYRK3).[52] These results were confirmed in 2009, when similar studies showed that harmine **1a** could inhibit DYRK1A *in vitro* with comparable potency against other DYRK family members (IC_{50} 0.033 μM, DYRK1A; 0.166 μM, DYRK1B; 1.9 μM, DYRK2; 80 μM, DYRK4).[53] The same report showed that **1a** was also able to inhibit DYRK1A mediated phosphorylation of its protein substrate SF3B1 in HeLa cells (IC_{50} 48 nM) as well as autophosphorylation of Tyr321 in an *in vitro* translation system (IC_{50} 1.9 μM). The higher concentration of harmine **1a** required to block autophosphorylation indicates that this is likely not the major mechanism of DYRK1A inhibition *in vivo*.[49] Furthermore, in 2011, harmine and several other analogues were assayed for their ability to inhibit DYRK1A mediated phosphorylation of AD relevant protein tau (4R2N Tau) at Ser396 *in vitro*, a site which has been shown to be phosphorylated in NFTs.[39] Harmine was found to inhibit direct phosphorylation of tau by DYRK1A at Ser396 with an IC_{50} of 700 nM. Surprisingly, harmol **1b** and 9-ethylharmine **1c** gave improved IC_{50} values of 90 and 400 nM, respectively. In addition, harmine **1a** and 9-ethyl harmine **1c** potently inhibited the expression of three forms of phosphorylated tau [pS396, pT231, and pS262/pS356 (12E8 epitope)] in H4 neuroglioma cells at concentrations that did not affect cell viability.[39] Harmine **1a** is currently considered the most potent and orally bioavailable inhibitor of DYRK1A.

However, β-carboline analogues also possess significant drawbacks to consider when exploring the potential therapeutic applications. The hallucinogenic properties of **1a** have been exploited historically,[54] and more recently shown to be the result of its affinity for the serotonin and tryptamine receptor binding sites.[55] Animal studies conducted on the β-carbolines as early as the 1930s revealed a plethora of psychoactive effects including excitation, anxiety, tremors, convulsions, ataxia, pupil dilation, and alterations in the brain's electrical activity (electroencephalographic activity or EEG activity).[56] It has been revealed that cationic β-carbolines methylated at the pyridine nitrogen can mimic the activity of the powerful neurotoxin metabolite MPP+ **1d**. MPP+ **1d** is converted to

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MPTP **1e** in the brain, affecting the extrapyramidal dopamergic system leading to permanent Parkinson's-like symptoms (Figure 2).[57] Due to their convulsive properties observed *in vivo*, it has been suggested that harmine **1a** and its analogues are likely susceptible to similar metabolic pathways. **1a** also exhibits potent inhibition of monoamine oxidase-A (MAO-A) reuptake, leading to behavioral side effects (K_i 5 nM, IC_{50} 2 nM).[58]

Epigallocatechin gallate **2** (EGCg, tPSA 197.37, clogP 1.49, MW 458.37, Figure 3) is a natural polyphenol and a major catechin component of green tea. In 2003, EGCg **2** was identified as an inhibitor of DYRK1A by Bain and co-workers, with an *in vitro* IC_{50} value of 330 nM.[59] In 2006, mutant studies revealed the mechanism of DYRK1A inhibition by **2** as ATP noncompetitive.[60] Although polyphenols are known to have rather nonselective activity against a variety of enzymes and transcription factors, EGCg **2** shows selective inhibition of DYRK1A when tested against a panel of 28 structurally and functionally related kinases. p38 regulated/activated kinase (PRAK) was identified as the next most potently inhibited target, with an IC_{50} of 1.0 μM.[59] In 2009, *in vivo* studies were published which indicated that DYRK1A overexpressing mice subjected a green tea diet, containing EGCg **2** as the major component, exhibited a significant improvement in brain structure, synaptic plasticity, and cognitive functioning involving learning and long and short-term memory, assessed through object recognition and Morris water maze learning paradigms.[61]

EGCg **2** and other flavan-3-ols are known to have a multitude of biological activities. While considered relatively safe for human consumption, EGCg is reportedly a potent antibacterial and antiviral agent leading to beneficial effects on a variety of unique and antibiotic resistant bacterial and viral infections including influenza, polio, herpes simplex, and HIV.[62,63] Antifungal activity against trichophyton, a common cause of athlete's foot and ringworm, as well as antitoxin activity against cholera and hemolysins has also been reported.[64,65] Additionally, EGCg has been shown to inhibit topoisomerases I/II, the antiapoptotic enzyme Bcl-xl, and cancer-promoting proteases leading to anticancer activity.[66−68] Furthermore, research indicates EGCg also elicits antioxidative and anti-inflammatory properties by suppressing the nitric oxide synthase pathway.[69]

Quinalizarin **3** (1,2,5,8-tetrahydroxyanthraquinone, Figure 3), identified through virtual screening, is a potent inhibitor of protein kinase CK2 (K_i 50 nM), with additional activity for DYRK1A (K_i 5.85 μM).[70] When its selectivity was tested at 1 μM concentration against a panel of 75 proteins, quinalizarin exhibited a low promiscuity score of 11.1. Thus, as a starting point for design efforts, it appears relatively unattractive.

In 2010, two novel peltogynoids, Acanilol A **4a** and B **4b** (Figure 3), were isolated from *Acacia nilotica*, a plant belonging to the family of Leguminosae, subfamily Mimosoideae.[71] The extended hydrophobic system of these two flavonoids was considered similar to other potent kinase-inhibiting small molecules and *in vitro* evaluation against a small panel of kinases revealed only Alcanilol B **4b** was moderately active for DYRK1A (IC_{50} 19 μM) with no significant activity against other kinases. Moroever, the catechins and their derivatives often exhibit nonspecific activity against many protein targets, and as such, although nominal DYRK1A activity is observed, significant efforts would be required to mature these chemotypes into DYRK1A inhibitors worthy of progression along the value chain.[72] The predicted docking pose of **4b** in the active site resembled the published binding modes of other flavonoids in alternate kinases.[71]

Benzocoumarin **5a** (dNBC, Figure 3) has been recently reported as a DYRK1A inhibitor (IC_{50} 0.60 μM, [ATP] = 20 μM).[73] Authors observe that a 9-substituted nitro functionality (NBC, **5b**) is detrimental to DYRK1A activity (IC_{50} 15.0 μM, [ATP] = 20 μM), yet promotes activity for CK2 (IC_{50} **5b** 0.37 μM vs **5a** 32.34 μM), PIM kinase family members, of note PIM3 (IC_{50} **5b** 0.34 μM vs **5a** 2.05 μM), and PKB β (IC_{50} **5b** 0.78 μM vs **5a** > 30 μM). Indeed, structure based modeling efforts with CK2 suggest **5a** is likely buried more deeply than **5b** into the binding cleft of DYRK1A where **5a** interacts with both the hinge region and the phosphate binding region of the ATP binding site. Key hydrogen bonds were proposed between the hydroxyl group and the side chain primary amine of Lys188, whereas the carbonyl oxygen is hydrogen bonded to the backbone amine of Leu241 in the hinge region. The tricyclic planar system of **5a** potentially forms a series of van der Waals interactions with a variety of hydrophobic residues. The methyl group is embedded between Ile165 and Val173, in a region protected from the solvent. This docking pose suggests that insertion of a nitro group at C-9 is not tolerated due to the presence of Phe238 as the gatekeeper residue in the DYRK1A active site. This observation is consistent with the *in vitro* activity observed for NBC **5b** and dNBC **5a**.[73] The reader is referred to the original reference containing a detailed depiction of the binding modes of **5a** and **5b** for further clarification.

Indolocarbazoles are a class of natural products produced as secondary metabolites by actinomycetes, a group of bacteria commonly found in soil. Staurosporine **6** (Figure 4) was the first member isolated which bears a single sugar residue bound to both indole nitrogens at C-1 and C-5. As such, staurosporine is a well-known, highly nonselective and typically very potent kinase inhibitor often used as a screening control molecule in translational kinase campaigns. Rebeccamycin **7**, glycosylated through a single *N*-glycosidic bond to C-5, is a potent topoisomerase inhibitor. In 2009, a report describing the combinatorial biosynthesis of a collection of glycosylated

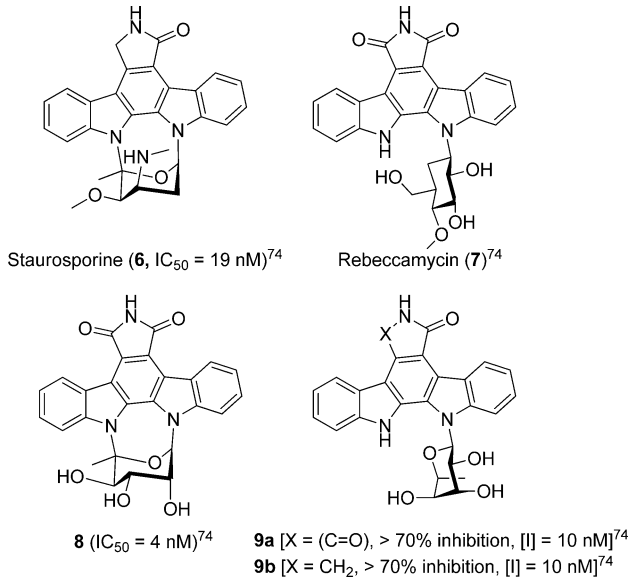

Figure 4. Chemical structures of indolocarbazoles **6**−**8** and **9a**, **b**. Inhibitory activity against DYRK1A is also indicated as percent of inhibition or IC_{50}.

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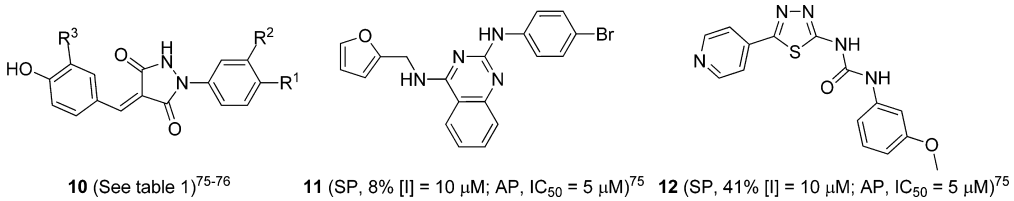


10 (See table 1)[75-76] **11** (SP, 8% [I] = 10 μM; AP, IC_{50} = 5 μM)[75] **12** (SP, 41% [I] = 10 μM; AP, IC_{50} = 5 μM)[75]

Figure 5. General structures of the most active hits of the virtual screen conducted by Song and co-workers. Activity against DYRK1A is reported in parentheses (SP, substrate phosphorylation, [ATP] = 5 μM; AP, autophosphorylation, [ATP] = 10 nM).[75,76]

indolocarbazoles and their activities against 11 kinases, revealed that staurosporine exhibited potent activity (DYRK1A IC_{50} 19 nM).[74] Being a well-mined area of study for nearly 20 years, it seems unlikely staurosporine will be used as a starting point for design efforts of DYRK1A imnhibitors; however, it does represent the ideal control molecule during screening efforts.

Analogue **8**, bearing a L-rhamnose sugar moiety substituted in a similar fashion to staurosporine **6**, also exhibited significant activity against DYRK1A (IC_{50} 4 nM). However, predictably and in similar fashion to staurosporine, it proved very nonselective, displaying strong low nanomolar inhibition of the 10 kinases evaluated. In addition, analogues **9a** and **9b** of rebeccamycin **7** have been evaluated for inhibitory activity (DYRK1A > 70% inhibition at 10 nM concentration). In summary, indolocarbazoles are documented to show promiscuous activity among a large array of kinases, although extensive structure−activity relationship (SAR) studies have been successful in advancing molecules forward into human trials for treatment of cancer, diabetes, and Parkinson's disease.[74] It seems that, considering the longevity of this class of molecules, potential problems in establishing novel composition of matter, and commonly seen behavior as pan-inhibitors, their potential therapeutic applications as DYRK1A inhibitors may be compromised.

SYNTHETIC INHIBITORS

In 2006, an *in silico* pharmacophore search with a GSK-3β homology model reported several chemotypes with predicted binding affinities which subsequently were confirmed to inhibit DYRK1A autophosphorylation and substrate phosphorylation *in vitro*.[75] Interestingly, in some cases, activities across these assays were not analogous, indicating that inhibition of substrate phosphorylation and autophosphorylation of DYRK1A encompass different SAR profiles. For example, pyrazolidine-dione **10a** (Table 1) exhibited stronger inhibition of substrate phosphorylation (74% at 10 μM) than autophosphorylation (IC_{50} > 50 μM) (Table 1). In contrast, 2,4-diamino-quinazoline **11** demonstrated poor inhibition of substrate phosphorylation (8% at 10 μM) but strong inhibitory activity of autophosphorylation (IC_{50} 5 μM). Thiadiazole **12** (IC_{50} autophosphorylation 5 μM; 41% inhibition of substrate phosphorylation at 10 μM, tPSA 87.44, clogP 2.38, MW 327.36) and pyrazolidine-dione **10b** (IC_{50} autophosphorylation 2.5 μM; 82% inhibition of substrate phosphorylation at 10 μM, tPSA 78.87, clogP 2.59, MW 379.19, Table 1) were identified as the most potent hits from both *in vitro* assays and were further tested for inhibition of autophosphorylation in DYRK1A overexpressing HEK293 cells (IC_{50} 200 μM, **12** and 100 μM, **10b**), with the poor cellular activity likely attributed to poor permeability through the cell membrane. **12** and **10b** were also evaluated in a panel of 15 kinases, showing significant inhibition for only CLK3 (89% and 64%, respectively, [I] = 10 μM; [ATP] = 100 μM).[75]

Due to the superior potency of pyrazolidine-dione **10b** over **10a**, docking simulations were initiated, predicting hydrophobic interactions with several active site residues[76] and a strong interaction with the conserved hinge region, in which the protein backbone of Glu239 and Met240 forms three hydrogen bonds to the hydroxyl group, the R^3 methoxy oxygen, and the pyrrolidine-3-carbonyl oxygen, respectively. Active compounds are summarized in Table 1, where subsequent analogues **10c**

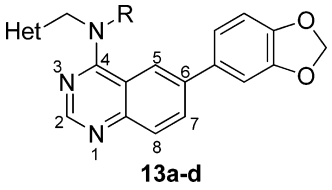
Table 1. DYRK1A Inhibitory Activities of Selected Pyrrazolidine-3,5-diones (10a−10g)

compd	R^1	R^2	R^3	IC_{50} (μM)[a]	% inhibition kinase activity (μM)[b]
10a	H	Cl	OCH_3	50	74
10b	Cl	Cl	OCH_3	2.5	82
10c	Cl	Cl	NO_2	0.6	76
10d	CN	H	OCH_3	0.6	78
10e	OCH_3	H	OCH_3	1.2	55
10f	F	H	OCH_3	2.5	44
10g	Cl	Cl	H	2.5	73

[a]Autophosphorylation assay, [ATP] = 10 nM. [b]Substrate phosphorylation assay, [I] = 10 μM, [ATP] = 5 μM ([I] = concentration of inhibitor).[76]

(tPSA 121.45, clogP 3.12, MW 394.17) and **10d** (tPSA 102.66, clogP 0.72, MW 335.31) were shown most active on both screening platforms. Moreover, the specificity of **10c** and **10d** was analyzed for eight CMGC family kinases, the only significant findings being that **10d** showed no significant inhibitory activity in the panel, whereas **10c** appreciably inhibited only the closely related kinase DYRK2 (83% at 10 μM, [ATP] = 15 μM).[76] It is important also to note a discrepancy in the activity of harmine **1a** in this report, often used as a standard in these studies, which was nearly 12-fold less potent (IC_{50} 1 μM) compared to literature values previously described.

Quinazoline based small molecules are well-known to possess inhibitory kinase activity exemplified by erlotinib (Tarceva), an inhibitor of the epidermal growth factor tyrosine kinase receptor, clinically approved for the treatment of non-small-cell lung and pancreatic cancer and an example of a Type I kinase inhibitor. Indeed, since 2009 and albeit in the oncology arena, Thomas and co-workers have been involved in the investigation of 6-arylquinazolin-4-amines as inhibitors of CLKs. During these studies, quinazoline **13a** (Figure 6) was evaluated against a panel of 400 kinases and found to have inhibitory activity for DYRK1A (K_i 27 nM) (Table 2).[77] **13a** was also somewhat nonspecific, possessing inhibitory activity for CLK1 (K_d 37 nM) and CLK4 (K_d 50 nM). Predictably, kinetic experiments suggested that **13a** acts as a CLK4 inhibitor



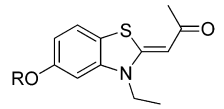
13a-d

Figure 6. General structure of quinazoline-amines **13a−d** (Table 2).[78]

through an ATP competitive mechanism. The inhibition of both DYRK1A and CLK4 from **13a** was rationalized by the high similarity existing between the two ATP binding domains.[77]

In further studies, analogues **13b−d** (Table 2) were the most potent confirmed inhibitors of DYRK1A (IC_{50} 206, 14, and 93 nM, respectively), and SAR studies clearly confirmed the importance of the benzo[*d*][1,3]dioxole moiety at the 6-position of the quinazoline scaffold.[78] Movement of the same moiety to C-7 was not tolerated for DYRK1A inhibition, and 5-membered heterocylic ring systems were optimal over 6-membered rings at the 4-position. Addition of an alkyl group (R = Me) resulted in increased activity with **13c** (tPSA 58.78, clogP 3.94, MW 376.43), and homology driven docking studies of **13c** in the target proteins CLK4 and DYRK1A suggested ATP competitive binding, in agreement with kinetic studies. In anticipation of future cell-based studies, the water solubility, stability, and membrane permeability of the most active compounds **13b−d** were also analyzed. Only **13d** exhibited exceptionally high solubility in water, and all three compounds were capable of passive membrane permeability in a Caco-2 assay, hinting at potential for sufficient BBB penetration.[78]

A benzothiazole denoted INDY **14a** (tPSA 40.54, cLOGP 2.70, MW 235.3, Figure 7), was reported to be a DYRK1A/CLK dual inhibitor after a high-throughput screening effort (DYRK1A, IC_{50} 0.24 μM, [ATP] = 10 μM).[79] INDY **14a** is structurally related to the known CLK inhibitor TG003 **14b** (DYRK1A IC_{50} 0.93 μM; IC_{50} CLK1 119 nM; IC_{50} CLK4 30 nM), but encouragingly is nearly 3-fold more potent for DYRK1A. Both INDY **14a** and TG003 **14b** also displayed activity against other DYRK kinase family members, exemplified by DYRK1B (IC_{50} 0.23 and 0.13 μM, respectively). Again, a significant deviation between the activity of standard compound harmine **1a** against DYRK1A (IC_{50} 0.35 μM) and reported literature values was observed. The biological activity of INDY has also been confirmed in COS-7 cells, where it partially inhibited the expression of pThr212 Tau at 3 μM and almost completely at 30 μM. In HEK293 cells, **14a** was able to block the negative regulatory activity of DYRK1A in the calcineurin/NFAT (nuclear factor of activated t-cells) pathway in a concentration-dependent manner and partially restore

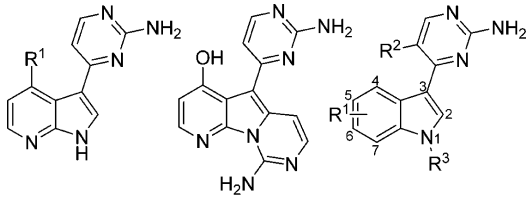


INDY (**14a**, R = H, IC_{50} = 0.24 μM)[79]
TG003 (**14b**, R = CH_3, IC_{50} = 0.93 μM)[79]
proINDY (**14c**, R = Ac, activity not reported)[79]

Figure 7. General structure of INDY **14a** and analogues **14b**, **c**. Activity against DYRK1A is reported in parentheses, [ATP] = 10 μM.[79]

NFAT dependent transcription at 10 μM. Aberrant phosphorylation leading to down-regulation of transcription factor components of the calcineurin/NFAT signaling pathway is suggested to be involved in the induction of a DS-like phenotype in that various *Nfatc* knockout mice show developmental defects and behavior similar to those observed in DS.[42,80] Furthermore, a novel vertebrate *in vivo* assay was performed in which DYRK1A or xDYRK1A (92.4% sequence identity to human DYRK1A) overexpressing *X. laevis* tadpole embryos were treated with a pro-drug of INDY (proINDY, **14c**) acetylated at the hydroxyl oxygen. Interestingly, proINDY **14c** showed similar cellular activity to INDY **14a** and was effective in relieving noticeable deformities to the eyes and head of tadpole embryos, and resulted in normal development in 86% of xDYRK1A overexpressing tadpoles (injected with 250 pg of xDYRK1A mRNA, [proINDY] = 2.5 μM) in the sample population.[79]

Meriolins, 3-(pyrimidin-4-yl)-7-azaindoles **15**, are hybrid structures between variolins **16** and meridianins, 3-(2-aiminopyrimidin-4-yl)indoles **17** (Figure 8). Meriolins **15**



Meriolins (**15**) Variolin B (**16**) Meridianins (**17**)

Figure 8. General structure of compounds **15−17** (Tables 3 and 4).[81−83]

display strong inhibition of CDKs, with weaker activity against GSK3 and DYRK1A and aroused early interest in the oncology field due to their strong cellular antiproliferative and proapoptotic effects.

Subsequently, in 2008, the *in vitro* activity of several Meriolin derivatives **15** (Table 3) against CMGC kinase family members including CDK1/2/5/9, GSK3, and DYRK1A was reported.[81]

Table 2. IC_{50} and K_d Values of Quinazoline Analogues 13a−d against DYRK1A and Other Related Kinases[a]

Cmpd	Het	R	DYRK1A	DYRK1B	CLK1	CLK2	CLK3	CLK4
13a		H	(27)		(37)			(50)
13b		H	206 (82)	1510	96 (50)	1327 (380)	7448	40 (43)
13c		Me	14 (13)	25 (300)	20 (18)	186 (59)	1924	11 (5)
13d		H	93 (27)	734	522 (72)	1055 (320)	3642	141 (30)

[a]IC_{50} and K_d are both expressed in nM. K_d shown in parentheses; IC_{50} determined using [ATP] = 10 μM.[78]

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Table 3. IC_{50} Values for Meriolin Derivatives 15 and Variolin B 16 against 7 Kinases[a]

compd	R^1	DYRK1A	CDK1	CDK2	CDK5	CDK9	GSK3	CK1
15a	OH	35	57	18	50	18	400	50
15b	OMe	29	170	11	170	6	230	200
15c	OEt	32	10	7	5	7	30	100
15d	OPr	37	7	3	3	5.6	25	200
15e	OiPr	40	8	5.1	3	5.6	21	140
16		80	60	80	90	26	70	5

[a]IC_{50} expressed in nM. ATP] = 15 uM.[81−83]

Table 4. Inhibitory IC_{50} Values of Synthetic Meridianin Derivatives 17a−k against a Panel of Selected Kinases[a]

compd	R^1	R^2	R^3	DYRK1A	CDK5	CK1	GSK3	Erk2	CLK1	tPSA	clogP	MW
17a	H	3-OCH_3−Ph	H	0.6	>10	0.49	3.0	3.10	NR	72.00	3.29	316.36
17b	H	3-NH_2−Ph	H	0.64	>10	3.3	6.0	6.9	NR	88.79	2.26	301.35
17c	H	4-(C(O)NH_2)-Ph	H	0.58	>10	3.4	2.0	L1.9	NR	105.86	1.92	329.36
17d	H	4-COOH-Ph	CH_3	0.9	>10	>10	>10	>10	NR	91.28	3.41	344.37
17e	H	I	CH_3	0.4	2.6	1.1	4.2	4.0	NR	53.98	2.95	350.16
17f	7-Br	H	H	0.068	3.1	4.2	>10	NR	0.065	62.77	2.86	289.13
17g	6-Br	I	H	0.034	0.68	0.7	1.1	NR	0.032	62.77	3.53	415.03
17h	7-Br	I	H	0.039	2.1	1.6	4	NR	0.042	62.77	3.53	415.03
17i	7-NO_2	H	H	0.085	6.3	10	>10	NR	0.07	114.58	1.94	255.23
17j	6-NO_2	I	H	0.095	1.6	1	2.6	NR	0.067	114.58	2.61	381.13
17k	H	I	H	0.066	1.4	0.9	3.7	NR	0.03	62.77	2.63	336.13

[a]Expressed in μM. [ATP] = 15 μM. NR, not reported.[83]

Variolin B **16** was used as a standard in this report (DYRK1A IC_{50} 80 nM), and several new meriolins exhibited submicromolar activity for DYRK1A, but invariably were more active against other kinases of therapeutic note. Compounds with the strongest activity **15a−e** (DYRK1A IC_{50} < 100 nM, [ATP] = 15 μM) are depicted in Table 3, and significant antiproliferative effects were observed in various cancer cell lines, likely due to inhibition of multiple cell cycle kinases targets.[81] No reports have been found detailing the activity of these molecules in DYRK1A cellular phosphorylation assays, which may represent an opportunity for further study.

The following year, studies of *in vitro* kinase inhibition and anticancer activity of structurally related synthetic meridianin derivatives **17** were published by Moreau and co-workers. Meridianins **17** are marine alkaloids, and several analogues **17a−e** were shown to have potent and somewhat selective activity for DYRK1A over four other CMGC kinase family members (CDK5, CK1, GSK3β, and Erk2) (Table 4).[82] In general, analogues bearing a nonmethylated indole nitrogen **17a−c** were less selective for DYRK1A. Of interest, meridianin analogue **17d** elicited an IC_{50} of 0.9 μM against DYRK1A while possessing nominal IC_{50} values (>10 μM) for the other four structurally related kinases. Although antiproliferative effects in PA1 cells were also investigated, analogues with significant activity against DYRK1A interestingly displayed insignificant antiproliferative cellular activity.[82] A subsequent article published in 2011[83] describes the synthesis and SAR of novel Meridianin analogues (Table 4), reported with submicromolar IC_{50}'s against DYRK1A. In general, analogues bearing a 7-bromo substituent exhibited the most potent and selective activity for DYRK1A. Indeed, compounds **17f−h** showed >45 fold selectivity for DYRK1A and CLK1 over other kinases tested. Additionally, compounds with a nitro- or amino-substituent at the 6- or 7-position of the indole ring **17i**, **j** also led to stronger DYRK1A/CLK1 activity, compared to those with similar groups at the 4- or 5-positions.[83] The authors were unable to separate DYRK1A from CLK1 activity, and in all cases high potency against DYRK1A was coupled with similar activity against CLK1. As such, this may represent a future avenue for elucidation of more specific probelike DYRK1A small molecules given the strength of structure-based design in the kinase field.

In 2011, Mérour and co-workers published the biological evaluation of thirteen 3-(6-hydroxyindol-2-yl)-5-(phenyl)-pyridine and pyrazine analogues **18** against DYRK1A, CDK5, and GSK3β (Figure 9, Table 5).[84] Although efforts were focused on the design of CDK inhibitors, compounds with significant affinity for DYRK1A were identified.

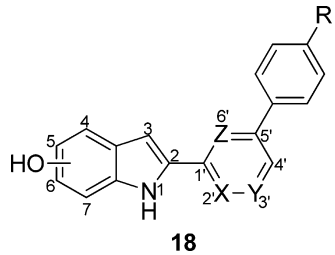

Figure 9. General structure of 3-(6-hydroxyindol-2-yl)-5-(phenyl)-pyridine and pyrazine analogues **18a−g** (Table 5).[84]

In general, compounds bearing a hydroxyl substituent on C-6 rather than C-5 of the indole ring were more potent (DYRK1A **18a** IC_{50} 0.34 μM and **18b** IC_{50} 0.54 μM). Additionally, analogues containing a pyridine rather than pyrazole ring were stronger inhibitors (DYRK1A **18c** IC_{50} 0.06 μM and **18b** IC_{50} 0.54 μM). Similarly, analogues bearing a pyridine nitrogen in the *meta*-position to the phenyl and indole substituents (Y, C-3′) rather than *ortho*- to the indole ring (X, C-2′) also show better activity (DYRK1A **18c** IC_{50} 0.06 μM and **18d** IC_{50} 0.3 μM). Furthermore, all analogues containing a phenol ring at C-5′ were more active against DYRK1A than those bearing cyano-

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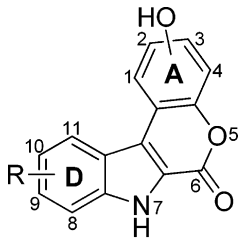
Table 5. IC_{50} Values for Selected 3-(Hydroxyindol-2-yl)-5-(phenyl) Pyradines and Pyrazines 18[a]

compd	OH	R	X	Y	Z	DYRK1A	CDK5	GSK3β
18a	C-5	OH	CH_2	N	N	0.34	1.5	4.7
18b	C-6	OH	CH_2	N	N	0.54	1.5	5.3
18c	C-6	OH	CH_2	N	CH_2	0.06	0.16	1.1
18d	C-6	OH	N	CH_2	CH_2	0.3	0.83	ND
18e	C-6	CN	CH_2	N	CH_2	1.6	0.9	4
18f	C-6	SO_2Me	CH_2	N	CH_2	0.35	0.38	1.1
18g	C-5	OH	N	CH_2	CH_2	0.23	0.36	ND

[a]IC_{50} expressed in μM. [ATP] = 15 μM.[84]

phenyl or methylsulfonyl-phenyl substituents (**18c** vs **18e** and **18f**),[84] and clear opportunities exist to exploit **18a** (tPSA 77.21, clogP 3.20, MW 303.31) and **18c** (tPSA 64.85, clogP 3.62, MW 302.33) for the design of the new potentially selective DYRK1A inhibitors.

In 2012, the same group reported the development of lamellarin D analogues, chromeno[3,4b]indoles **19** (Figure 10),



19

Figure 10. General structure of Chromenoindoles **19** (Table 6).[85]

Table 6. Inhibitory Activities of Selected Chromenoindoles 19 against Three Kinases[a]

compd	R	OH	DYRK1A	CDK5	GSK3
19a	H	C2	0.074	2.7	0.38
19b	H	C3	0.5	4.7	0.44
19c	C10-OH	C2	0.067	0.72	0.31
19d	C10-OH	C3	0.3	4.2	0.42
19e	C10-CF_3	C2	≥10	≥10	≥10
19f	C11-OMe	C2	0.35	≥10	3.3
19g	C9-OH	C2	4.3	>10	>10
19h	C11-(4-phenol)	C2	≥10	≥10	>10

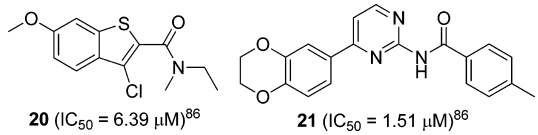
[a]IC_{50} expressed as μM. [ATP] = 15 μM. [86]

as a novel class of DYRK1A inhibitors (Figure 10, Table 6).[85] Lamellarins are isolated from marine organisms, and lamellarin D has been shown to exhibit potent inhibition of topoisomerase I as well as nonselective inhibition of various kinases.

The SAR of **19** was analyzed and clearly defined for the A and D rings, where the presence of the hydroxyl group on the A ring was essential for kinase activity and hydroxyl substitution at C-2 afforded more potent and selective DYRK1A inhibition than at C-3 (**19a** vs **19b**; **19c** vs **19d**). Additionally, substitution on the D ring with bulky, hydrophobic, and/or electron withdrawing groups reduced potency, whereas small electron donating groups, such as a hydroxyl and methoxyl group, often increased the activity (**19c** vs **19e**). Furthermore, substitution at C-10 was better tolerated than at C-9 or C-11 (**19c** vs **19f** and **19g**). Summarizing, analogues **19a** (tPSA 58.56, clogP 3.06, MW 251.24) and **19c** (tPSA 78.79, clogP 2.39, MW 267.24) exhibited the most potent DYRK1A inhibition of this series, with **19a** being slightly more selective over CDK5 and GSK3 than **19c**.[85]

Encouragingly, although the chromenoindoles are highly planar in nature coupled with significant conjugation in the 4-ring system, the authors indicate that these compounds do not display significant DNA binding activity and docking studies indicate a binding mode for **19c** similar to that of harmine **1a**, in which the lactone carbonyl oxygen interacts with the hinge region residue Leu241 and the D-ring C-10 hydroxyl group engages the conserved Lys188 residue.[85]

In January 2012, virtual screening of a DYRK1A homology model, constructed from CLK1 (PDB ID: 1Z57), another dual specificity protein kinase,[86] identified six novel potential inhibitors of our kinase of interest. Two of them, **20** and **21** (Figure 11), possessed moderate inhibitory activity (DYRK1A



20 (IC_{50} = 6.39 μM)[86] **21** (IC_{50} = 1.51 μM)[86]

Figure 11. Structures of the two most potent DYRK1A inhibitors **20** and **21** discovered by virtual screening ([ATP] = 1 μM).[86]

20 IC_{50} 6.39 μM and **21** IC_{50} 1.51 μM). Despite the structural differences between **20** and **21**, subsequent docking studies using a reported DYRK1A crystal structure predicted both compounds to be ATP competitive, fitting nicely into the ATP binding pocket. The intracellular activity of **20** was also investigated using a luciferase reporter assay measuring phosphorylation of GLI1, a downstream component of the Hedgehog signaling pathway and protein substrate of DYRK1A. Interestingly, **20** (25−75 μM) caused a concentration dependent inhibition of luciferase output. Its inhibitory activity was stronger than the activity of EGCg **2** used as positive control in the experiment. Moreover, it was also found that **20** blocks DYRK1A-mediated reduction of NFAT transcriptional activity which may be involved in promoting developmental and behavioral DS-like phenotypes.[42,80,86]

In 2009, Thiéry and co-workers described the biological activity of various bis-indole indigoid analogues against five CMGC kinase family members reportedly involved in the promotion of Alzheimer's dementia (CDK1/5,[87] GSK3,[88] CK1,[89] DYRK1A).[90] Although some indigoids **22a−d** (Table 7, Figure 12) exhibited moderate *in vitro* inhibition of DYRK1A, low selectivity and far better activity was observed versus other kinases implicated in Alzheimer's progression. However, it should be noted that, in the field of kinase inhibition, gaining selectivity among kinase targets with high

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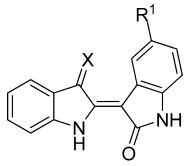
Table 7. IC_{50} Values for the Inhibitory Activity of Indigoid Analogues 22a−d against Five Selected CMGC Kinases[a]

compd	R^1	X	DYRK1A	CDK1	CDK5	GSK3	CK1	tPSA	clogP	MW
22a	NH_2	O	2.5	1.5	0.59	0.08	0.65	84.22	1.94	277.28
22b	NHAc	O	2.5	0.05	0.018	0.0075	8.0	87.30	2.19	319.31
22c	NO_2	NOH	1.3	0.019	0.006	0.0021	>10	125.53	2.70	322.27
22d	NH_2	NOH	4.2	0.1	0.15	0.36	0.13	99.74	1.17	292.29

[a] IC_{50} values expressed in μM. [ATP] = 15 μM.[90]

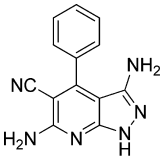


22a-d

Figure 12. General structure of bis-indole indigoids **22a−d** (Table 7).[90]

structural similarity in the ATP binding pocket can be extremely challenging, and while indigoids **22a−d** do show significant activity for closely related kinases, these compounds should be considered relatively selective DYRK1A inhibitors. Additionally, the same rationale should be given to select meriolins **15**, meridianins **17**, pyridine and pyrazines **18**, and chromenoindoles **19**. Furthermore, in many cases, the overall biological effects of marketed medications cannot be attributed to one biological target, and activity against more than one target *in vivo* can prove to be more beneficial in eliciting the desired phenotype than a "cleaner" acting drug, especially when these targets are implicated in related pathologies, as are DYRK1A, CDK1/5, GSK3, and CK1.

In work by Chioua, a series of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridines were synthesized and screened against DYRK1A, CDK5, and GSK3.[91] Compound **23** (Figure 13) exhibited modest activity against DYRK1A (IC_{50} 11 μM, [ATP] = 15 μM) and had the most affinity for CDK5 (IC_{50} 0.41 μM) and GSK3 (IC_{50} 1.5 μM).[91]



23 (IC_{50} = 11 μM)[92]

Figure 13. Structure of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine **23** ([ATP] = 15 μM).[91]

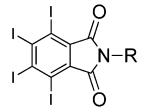
PROMISCUOUS KINASE INHIBITORS

Several DYRK1A inhibitors were originally developed or hypothesized to potentially target other kinases. Aminopurine analogue Purlavanol A **24a** (Figure 14) was one of the 14 compounds profiled by Bain and co-workers against a panel of 28 kinases, shown to be ATP competitive (DYRK1A IC_{50} 300 nM, [ATP] = 0.1 mM).[59] Significant activity was also observed for CDK2 (IC_{50} 100 nM) and other kinases in the panel.[56] Roscovitine **24b** (Figure 14) exhibited more moderate activity (DYRK1A IC_{50} 3.1 μM, [ATP] 0.1 mM) and in analogous fashion possessed CDK2 activity (IC_{50} 250 nM).[59] **24b** also inhibited the activity of ERK8 and other CDKs, such as CDK1, CDK5, and CDK7.

In additional profiling of small molecules in a kinase panel, Bain and co-workers discovered that the GSK3 inhibitor SB-216763 **25** and the PKB inhibitor A-443654 **26** (Figure 14) possessed low inhibitory activity and specificity for DYRK1A.[52] As such, no IC_{50} values were determined, and due to the poor selectivity observed high caution is recommended for their use as potential molecular probes.

Pagano and co-workers also investigated the specificity of TBB **27a** (4,5,6,7-tetrabromo-1*H*-benzotriazole), TBI **27b** (4,5,6,7-tetrabromo-1*H*-benzimidazole), and DMAT **27c** (2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole) all originally designed as creatine kinase (CK) inhibitors (Figure 15).

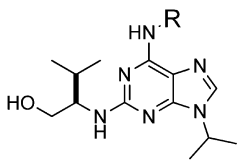
TBB (**27a**, X = N, IC_{50} = 4.36 μM)[92]
TBI (**27b**, X = CH, IC_{50} = 2.1 μM)[92]
DMAT (**27c**, X = C(NMe_2), IC_{50} = 0.4 μM)[92]



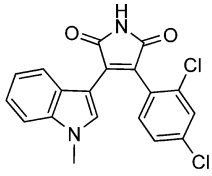
TID (**28**, R varies, % inhibition 60-80%, [I] =1 0 μM)[93]

Figure 15. General structures of the tetrahalo-bicycles evaluated against DYRK1A (**27a−c**, [ATP] = 50 μM; **28**, [I] = 10 μM, [ATP] = 50 μM]).[92,93]

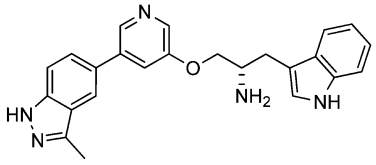
Evaluation against a panel of 76 kinases revealed that **27a−c** were not as highly specific for CK2 as previously claimed.[92] Indeed, they were also shown to inhibit DYRK1A (IC_{50} 4.36, 2.1, and 0.41 μM, respectively). Moreover, all the three



Purlavanol A (**24a**, R = 3-Cl-Ph, IC_{50} = 300 nM)[59]
Roscovitine (**24b**, R = Bn, IC_{50} = 3.1 μM)[59]



SB-21673
(**25**, 92% inhibition, [I] = 2 μM)[52]



A-443654
(**26**, 90% inhibition, [I] = 10 nM)[52]

Figure 14. DYRK1A inhibitors originally developed as inhibitors of other kinases. (**24a,b**, [ATP] = 0.1 mM; **25**, [I] = 2 μM, [ATP] = 50 μM; **26**, [I] = 10nM, [ATP] = 50 μM).[52,59]

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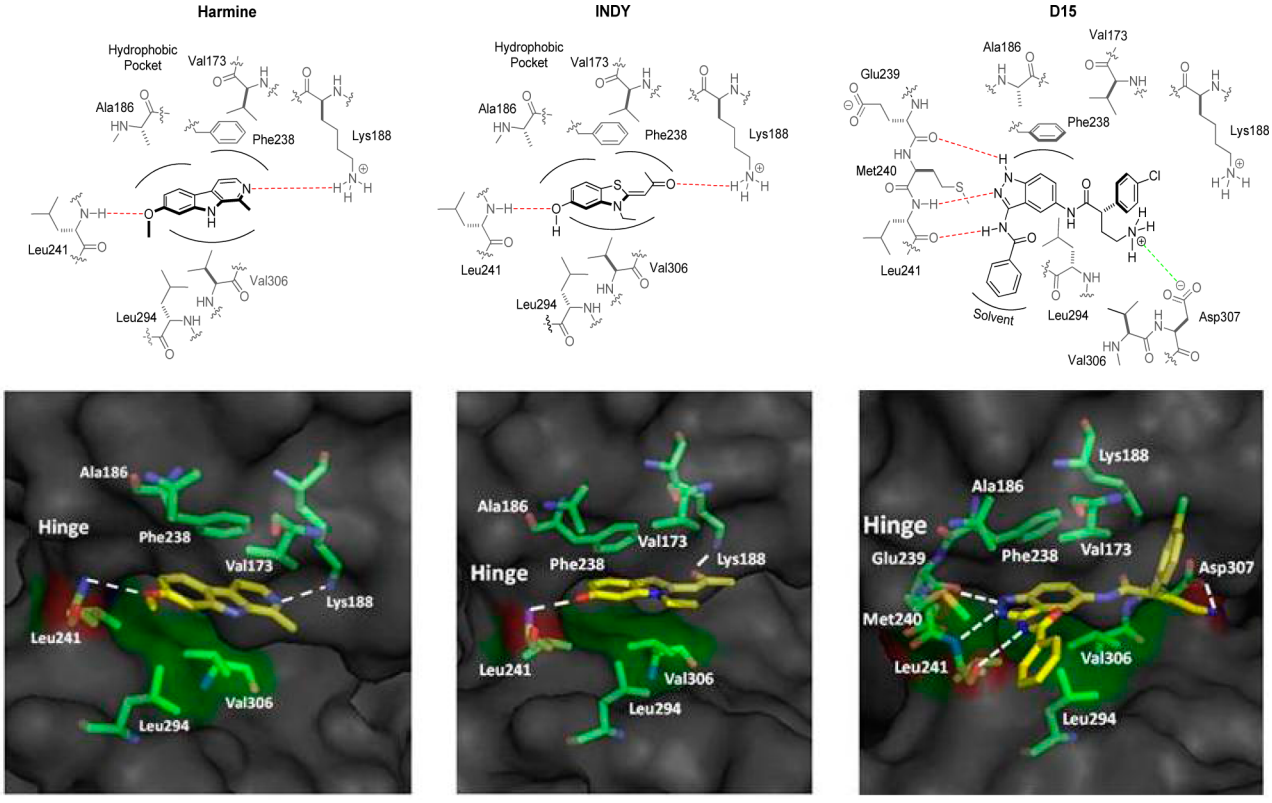

Figure 16. Crystal structure binding modes of DYRK1A inhibitors harmine **1a** (PDB ID: 3ANR), INDY **14a** (PDB ID: 3ANQ), and D15 **29** (PDB ID: 2WO6).

compounds elicited inhibition of DYRK2 (IC_{50} 0.99, 0.34, and 0.35 μM, respectively) and DYRK3 (IC_{50} 5.3, 3.7, and 1.7 μM, respectively), in addition to PIM kinase (PIM1−3) and HIP kinase (HIPK2−3) in the nanomolar to low micromolar IC_{50} range.[92]

Structurally related to **27a−c** are the 4,5,6,7-tetrahalogeno-1*H*-isoindole-1,3(2H)-diones **28** (60−80% inhibition at 10 μM, Figure 15). Six analogues were synthesized and evaluated as inhibitors of the human protein kinase CK2 by Yarmoluk and co-workers, and all compounds showed some inhibition of DYRK1A. However, their activity did not warrant IC_{50} determinations.[93]

X-RAY CRYSTAL STRUCTURES

To date, three ligand-DYRK1A X-ray co-crystal structures have been reported that include harmine **1a** (tPSA 33.62, clogP 3.13, MW 212.25), INDY **14a** (tPSA 40.54, clogP 2.70, MW 235.3), and D15 **29** (free base tPSA 108.61, clogP 3.93, MW 447.92). Analysis of their binding modes (Figure 16) and identification of key interactions within the protein binding site provides excellent insight for the future rational design of novel potent and selective DYRK1A inhibitors. Harmine **1a** (PDB ID: 3ANR) was found to bind in the adenine binding pocket with the pyridyl ring apparently involved in a quadrupole $\pi-\pi$ interaction with the gatekeeper residue Phe238. Two hydrogen bonds also appear to play key roles: the first between the pyridine nitrogen and the conserved residue Lys188, and the second between the methoxy group and the backbone amide nitrogen of the hinge residue Leu241. INDY **14a** (PDB ID: 3ANQ) binds the active site of DYRK1A in a similar fashion where the hydroxyl oxygen seems to be hydrogen bonding to the hinge backbone amide NH of Leu241, whereas the carbonyl oxygen is anchored through engagement with the conserved lysine. The crystal structure of D15 **29** (PDB ID: 2WO6) indicates the indazole moiety is a key warhead interacting via a series of three hydrogen bonds to the backbone hinge residues Leu241 and Glu239. Away from the warhead, a primary amine forms a salt bridge with the carboxylate side chain of Asp307. Surprisingly, despite being deposited in the PDB database, to date no study describing the activity of D15 **29** versus DYRK1A can be found in the primary literature.

CONCLUSIONS

Inhibition of DYRK1A represents a relatively new field of investigation that is rapidly gaining attention as emerging studies validating DYRK1A as a target for a disease modifying treatment of certain neurodegenerative pathologies, particularly Alzheimer's disease, and Down syndrome continue to grow in number. Indeed, interest is further heightened as investigators search for alternate therapeutic targets to BACE-1 (beta-site APP cleaving enzyme-1, also known as β-secretase) and γ-secretase, which to date have been unsuccessful in human trials.[94] On this theme, natural products with high potency and selectivity for DYRK1A, in particular harmine **1a** and EGCg **2**, may still prove to be fruitful avenues for further study. Indeed, harmine **1a** remains the most potent and orally bioavailable inhibitor discovered to date, but a plethora of pharmacological side effects currently limit its therapeutic application. Despite the nonselective nature of most polyphenolic compounds, the potency of **2** and its beneficial effects on cognitive functioning observed on *in vivo* studies make EGCg **2** also deserving of further investigation. In addition to the discovery of potential therapeutics from natural sources and small molecule drug discovery campaigns directed toward the discovery of potent

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and selective DYRK1A, inhibitors are rapidly increasing in number yet the majority still reside in the early stages of lead identification. INDY **14a** is one of the most potent inhibitors so far identified, but some promiscuity toward other kinases has been detected. Nonetheless, determination of the cellular activity of **14a** in conjunction with the promising activity of its acetylated analogue **14c** in a vertebrate model of neurodegenerative diseases further confirms its potential therapeutic application. Among other active scaffolds identified to date, the pyrazolidine-diones **10**, amino-quinazolines **13**, meridianins **17**, pyridine and pyrazines **18**, and chromenoidoles **19** all showed potent and somewhat selective activity when analyzed against limited panels of kinases. Most encouragingly, the recent disclosure of the crystal structures of three inhibitors (**1a**, **14a**, and **29**) bound to the active site of the DYRK1A has added considerable insight into the development of more selective DYRK1A inhibitors providing new impetus for translational efforts.

In summary, a detailed evaluation of the *in vitro*, cellular and *in vivo* data of the most promising DYRK1A inhibitors developed to date suggests that their potential therapeutic use may still be partially limited by broad specificity and/or undesirable side-effects. Progressing an active compound from Tier 1 *in vitro* screening to clinical evaluation in man requires significant work; however, as demonstrated by the druggability of this highly therapeutically significant target family, such bench to bedside progression seems eminently feasible with sufficient dedicated resources. Indeed, as a target family already commanding significant investment in the private sector, it seems inevitable that small molecules devoid of toxicological liabilities with desired potency, selectivity, and *in vivo* efficacy will ultimately emerge for evaluation of this biologically exquisite target in a clinical setting.

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Author Contributions

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Author Contributions

Breland Smith and Dr. Federico Medda were responsible for writing the entirety of the review and searching databases to identify current small molecule DYRK1A inhibitors with associated activity and selectivity profiles. Breland Smith was also responsible for construction of the small molecule drawings. Dr. Vijay Gokhale was responsible for additional literature searching, editing, investigating binding modes, and discussion thereafter. Breland Smith spearheaded the rational discussion for DYRK1A as a molecular target for Alzheimer's, and Dr. Travis Dunckley made significant improvements within this section as the lead biologist on the project. Finally, Dr. Hulme spent time on final edits and additional theory.

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Notes

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