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Potent and selective small molecule inhibitors of specific isoforms of Cdc2-like kinases (Clk) and dual specificity tyrosine-phosphorylation-regulated kinases (Dyrk)

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ABSTRACT

Continued examination of substituted 6-arylquinazolin-4-amines as Clk4 inhibitors resulted in selective inhibitors of Clk1, Clk4, Dyrk1A and Dyrk1B. Several of the most potent inhibitors were validated as being highly selective within a comprehensive kinome scan.

The cdc2-like kinase (Clk) family contains four isoforms (Clk1-4) and is proposed to alter the function of the spliceosome by phosphorylating serine–arginine-rich (SR) proteins within the spliceosome assembly.[1] The spliceosome regulates the processing, or splicing, of pre-mRNAs, yielding mature protein-encoding mRNAs.[2,3] Many human genes express more than one mRNA via alternative splicing, leading to protein diversity,[4] however, misregulation of alternative splicing is involved in the pathogenesis of cancer and other diseases.[5,6] Studies have revealed that Clk isoforms are associated with alternative splicing of PKCβII,[7] TF,[8] β-globin[9] and E1A pre-mRNA.[10] The Clks also regulate the alternative splicing of microtubule-associated protein tau and are implicated in frontotemporal dementia and Parkinson's disease through the phosphorylation of splicing factors (SF).[11] Inhibitors of Clk isoforms may alter these events and could prove to be useful agents in disease phenotypes characterized by abnormal splicing. Hagiwara reported TG003 (**1**), a small molecule benzothiazole, as having low-nanomolar IC_{50} values versus Clk1 and Clk4. The patent literature revealed structurally similar benzothiazole **1a** from Sirtris Pharmaceuticals[12] and a quinoline **3** from Chronogen, Inc.[13] reported to have activity versus Clk1 (Fig. 1). Indole **2** was recently revealed as a potent (20 nM) ATP-competitive Clk1 inhibitor with good selectivity over Clk3 via a unique binding mode.[14] We have previously described a series of substituted 6-arylquinazolin-4-amines including NCGC00010037 (**4**, ML106) as potent inhibitors of Clk1 and Clk4.[15]

In our previous study we examined **1** and **4** within a screen of 402 kinases and found that both agents had impressive activity versus the dual-specificity tyrosine-phosphorylation regulated kinase 1A (Dyrk1A) (IC_{50} values of 12 and 27 nM for **1** and **4**, respectively). Dyrk1A[16] has been shown to accumulate in nuclear speckles where it interacts and activates splicing factors.[17,18] Although the role of Dyrk1A in the development and function of the brain is not well understood, it is proposed to play a critical role in the development of Down Syndrome (DS) due to the location of the Dyrk1A gene on Chromosome 21 in the Down Syndrome critical region.[19–25] Overexpression of Dyrk1A has been associated with DS phenotypes[20,26,27] while loss-of-function results in potentially fatal conditions, such as decreased body and brain size.[27,28] Arron and coworkers recently found that Dyrk1A regulates calcineurin/NFAT (nuclear factor of activated T cells) signaling, which

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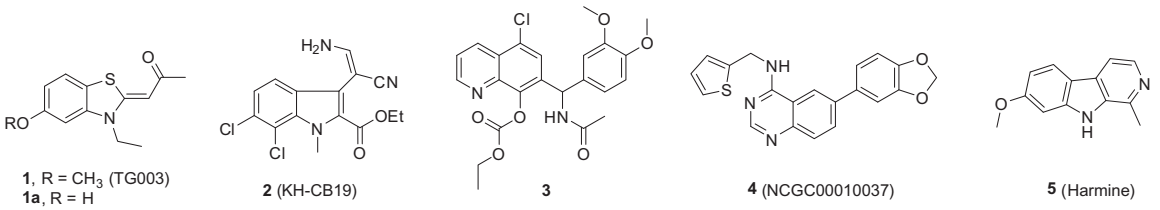


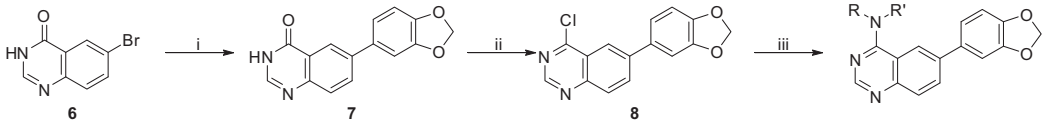
Figure 1. Structures of known Clk inhibitors including TG003 (**1**), KH-CB19 (**2**), NCGC00010037 (**4,** ML106) and Harmine (**5**).

has critical roles in human development, suggesting that increased levels of Dyrk1A can lead to many of the developmental phenotypes typically associated with DS.[29] Given these studies, selective inhibitors of Dyrk1A would be of great use to further elucidate the role this kinase plays in several disease phenotypes.

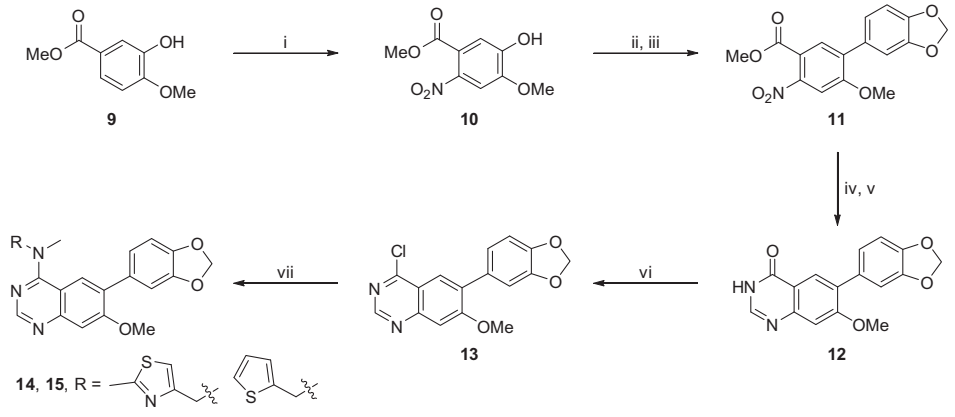
The Dyrk family contains several additional isoforms (Dyrk1B, Dyrk2, Dyrk3, and Dyrk4).[20] The Dyrk1B homolog (also referred to as Mirk) was first isolated and described by Friedman and coworkers.[30,31] Several reports suggest that Dyrk1B plays a significant role in cancer biology and muscle differentiation. Extensive studies on the Dyrk1B gene found that overexpression is associated with pancreatic and other cancers potentially due to its downstream effect on oncogenic K-ras and the hedgehog pathway.[32–36] It has been hypothesized that inhibition of Dyrk1B would diminish the ability of cancer cells to mitigate the effects of reactive oxygen species resulting in cell damage and induction of apoptosis.[32] Accordingly, selective inhibitors of Dyrk1B could be used to understand the role of this kinase in cancer progression. A prior art search of Dyrk1A and Dyrk1B inhibitors revealed the naturally occurring chemotherapeutic harmine (**5**) as a nonspecific inhibitor with nanomolar potency (Fig. 1).[22,37–39] Harmine also represents the only reported inhibitor of Dyrk1B, although potency is mild and selectivity is poor.[38] Given the potential associated with these targets we aimed to further define small molecules with alternative selectivity profiles. Here, we report the results of continued evaluations of substituted 6-arylquinazolin-4-amines as selective and highly potent small molecule inhibitors of Clk1, Clk4, Dyrk1A and Dyrk1B.

Previously, we utilized a synthetic strategy that relied upon an amine displacement step starting with 6-bromo-4-chloroquinazoline followed by an end-stage Suzuki–Miyaura coupling with various aryl boronic acids. In this study, we utilized a Suzuki–Miyaura coupling to introduce a variety of aryl boronic acids (e.g., 3,4-(methylenedioxy)-phenylboronic acid) to the commercially available 6-bromoquinazolin-4-one (**6**) scaffold (Scheme 1). The resulting substituted quinazolinone **7** was chlorinated using phosphorous oxychloride to yield chloro-pyrimidine **8**, which upon reaction with a variety of commercially available amines under mild heating gave the desired analogues.

Substituted quinazolines are a common pharmacophore for ATP-competitive kinase inhibitors and our previous work confirmed that these agents are inhibiting Clk isoforms through an ATP-competitive mechanism. Agents such as Tarceva (a clinically used cancer therapeutic that inhibits epidermal growth factor receptor 1) utilize substitutions on both the 6- and 7-position of the core quinazoline structure to boost target affinity and to endow the molecule with improved aqueous solubility.[40] We had previously shown that an aryl substitution on the 6-position was a critical element of the pharmacophore of these compounds; however, we were also interested to see if substitution at the 7-position



Scheme 1. Reagents and conditions: (i) aryl-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 150 °C (μW), 1 h (typical yields: 40–50%); (ii) $POCl_3$, *N*,*N*-dimethylaniline, toluene, reflux, 1 h; (iii) R,R′NH, *i*Pr_2NEt, DMF, rt to 60 °C, 2 h (typical yields: 80–95%).



Scheme 2. Reagents and conditions: (i) HNO_3, CH_3COOH, 0 °C to rt, 18 h; (ii) triflic anhydride, pyridine, DCM, 1 h, 91%; (iii) 3,4-(methylenedioxy)-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 150 °C (μW), 1 h, 55%; (iv) Pd/C, H_2 (1 atm), EtOH, 18 h, 99%; (v) NH_2CHO, NH_3COOH, 140 °C for 3 h, then rt for 18 h, 51%; (vi) $POCl_3$, *N*,*N*-dimethylaniline, toluene, reflux, 1 h; (vii) $RNHCH_3$, *i*Pr_2NEt, DMF, 60 °C, 18 h (typical yields: 80–95%).