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Evaluation of substituted 6-arylquinazolin-4-amines as potent and selective inhibitors of cdc2-like kinases (Clk)

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ABSTRACT

A series of substituted 6-arylquinazolin-4-amines were prepared and analyzed as inhibitors of Clk4. Synthesis, structure–activity relationships and the selectivity of a potent analogue against a panel of 402 kinases are presented. Inhibition of Clk4 by these agents at varied concentrations of assay substrates (ATP and receptor peptide) highly suggests that this chemotype is an ATP competitive inhibitor. Molecular docking provides further evidence that inhibition is the result of binding at the kinase hinge region. Selected compounds represent novel tools capable of potent and selective inhibition of Clk1, Clk4, and Dyrk1A.

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The removal of intron sequences from genes occurs via the actions of the splicesome, a protein complex that removes intervening sequences at the nuclear pre-mRNA level to afford properly coded mRNA for translation.[1,2] Many genes produce multiple mRNA isoforms through the actions of alternative splicing and, importantly, numerous human diseases are caused by improper splicing.[3] Exogenous manipulation of the splicesome is theorized to be a powerful means to control gene translation and ultimately correct disease phenotypes via rectification of splicing abnormalities.

The modulation of kinases provides a powerful mechanism to control numerous aspects of cell function and offers the potential for the management of many diseases.[4] Small molecule inhibitors of kinases (both selective and promiscuous) represent important biochemical tools for basic research and several kinase inhibitors have gained FDA approval as drugs.[5,6] In 2002, Manning et al. reviewed the human kinome and found 518 putative kinase genes[7] providing a roadmap to explore the consequences of kinase modulation within cellular biology. The possible control of gene splicing through the modulation of kinase activity represents an intriguing concept.[8] There are several reports of kinases that alter the function of the splicesome. Among these is the cdc2-like kinase (Clk) family.[9] A major target of Clk kinases is the prominent family of serine- and arginine-rich (SR) splicing proteins[10,11] which are involved in the assembly of the splicesome and are implicated in both constitutive and alternative splicing control and selection of splicing sites.[12,13]

The Clk family contains four characterized isoforms (Clk1, Clk2, Clk3, and Clk4). The Clks are capable of auto-phosphorylation (at serine, threonine, and tyrosine residues) and phosphorylation of exogenous proteins (at serine and threonine residues). Members of the Clk family have been implicated in the regulation of alternative splicing of PKCβII[14], TF[15], Tau,[16] and β-globin[17] pre-mRNA. These studies suggest that small molecule modulation of the Clk family of kinases may represent an important mechanism for the control of mRNA splicing.

Hagiwara and co-workers have reported TG003 (**1**) (Fig. 1) as a small molecule with low-nanomolar IC_{50} values versus Clk1 and Clk4.[17] Additionally, Clk inhibitors are presented in patent reports from Sirtris Pharmaceuticals (no structures presented) and Chronogen Inc. (a series of substituted quinolines related to structure **2**).[18,19] The report from Hagiwara and co-workers does not define the selectivity of TG003 beyond the Clk family and four additional kinases (PKA, PKC, SRPK1, and SRPK2) and the patents do not disclose details regarding structure–activity relationships nor selectivity. Thus, a need remains for small molecule probes of this important class of enzymes. Here, we report a novel class of quinazoline small molecules as potent and selective inhibitors of Clk1 and Clk4.

We recently performed a high-throughput screen for small molecule modulators of Lamin A splicing (PubChem AID: 1487); details of this study will be reported elsewhere. Among the actives was a series of substituted quinazolines including **3** (Fig. 1). The

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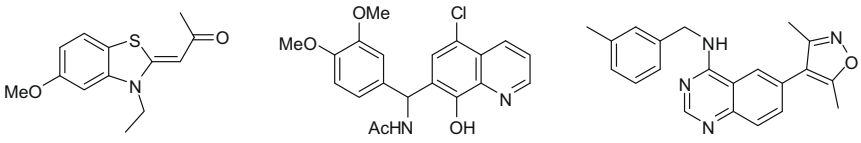


1 (TG003) **2** **3**

Figure 1. Structures of known Clk inhibitors **1** (TG003), **2** and the lead structure **3**.

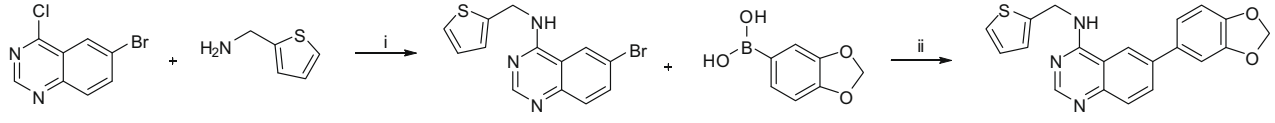
previously reported ability of quinazoline small molecules to modulate kinases through competitive inhibition at the ATP binding site suggested potential kinase stimulated influence of splicing. Given the association of the Clk family with splicing, we speculated that **3** might be a Clk inhibitor and this was confirmed via a commercial kinase profile.[20] Based on these results, we examined selected in-house compound libraries for Clk4 inhibition via two bioluminescent, luciferase-based assays capable of visualizing substrate (ATP) depletion and product (ADP) formation.[21] In primary screening, this biochemical assay revealed that **3** (Fig. 1) possessed an IC_{50} value versus Clk4 of 316 nM.

Based upon this result, we examined the SAR for this chemotype versus Clk4. Key to this effort was the synthetic preparation of these agents. In our hands, the most straightforward synthesis began with 6-bromo-4-chloroquinazoline (Scheme 1). Addition of primary and secondary amines (including aliphatic, benzylic, heteroaromatic and substituted analines) was accomplished in DMF with Hunig's base at room temperature. The resulting 6-bromo-N-substituted-quinazolin-4-amines were then subjected to standard Suzuki–Miyaura couplings with various aryl boronic acids using tetrakis(triphenylphosphine)palladium(0) and sodium carbonate in DMF and heating using microwave irradiation. This general procedure[22] resulted in the production of numerous N-substituted-6-arylquinazolin-4-amines including **4** which were purified via reverse-phase HPLC methods prior to testing in biological assays.

The primary phenotypic screen and the follow-up biochemical Clk4 assay were performed on the NIH Molecular Libraries Small Molecule Repository and several internal NCGC compound libraries. Each assay was performed using the quantitative high-throughput screening method whereby each molecular entity is screened in a dose–response format.[23] This screening format provides an unprecedented level of detail with regards to nascent SAR from the primary screening data. The resulting analysis of both of the aforementioned screens provided several clues as to which substitution patterns were likely to result in active compounds. Substitutions at the 4 position of the quinazoline core scaffold were limited to substituted benzylic systems (for instance *m*-tolylmethanamine, pyridin-3-ylmethanamine, and (3-fluorophenyl)methanamine)) and various heterocyclic methylamines (for instance 1-(furan-3-yl)-*N*-methylmethanamine, (4-methylthiophen-2-yl)methanamine, and thiophen-2-ylmethanamine). Substitutions at the 6 position of the quinazoline core scaffold were limited to various aryl groups (for instance 3-methoxybenzene, benzo[*d*][1,3]dioxole, 2,3-dihydrobenzo[*b*][1,4]dioxine, furan, and 3,5-dimethylisoxazole). As SAR is often not additive, we chose to incorporate all of these substitutions in matrix format in hopes of identifying more potent Clk4 inhibitors and revealing as much SAR as possible. The results of this library are listed in Figure 2. The original lead **3** was found to possess an IC_{50} value of 282 nM (very similar to the IC_{50} value determined in the primary biochemical screen). Additionally, two novel small molecules were found with potencies of 63 nM including 6-(benzo[*d*][1,3]dioxol-5-yl)-*N*-(thiophen-2-ylmethyl)quinazolin-4-amine (**4**). The SAR revealed that the benzo[*d*][1,3]dioxole was consistently favored in the 6 position of the quinazoline ring. The SAR of the amine substitutions was more complex. The thiophen-2-ylmethanamine substitution was typically favored, but several structural combinations favored other moieties at this position.

Prior to additional investigations into the SAR and optimization of this chemotype, it was important for us to gain an appreciation of the selectivity of these compounds towards the Clk family of kinases. Quinazoline based small molecules have been reported as potent inhibitors of numerous kinase families. Any small molecule Clk inhibitor that is additionally capable of promiscuous inhibition across the kinome will be of limited value as a tool compound for evaluating Clk biochemistry. To assess the selectivity of this general chemotype we choose to submit a representative reagent (analogue **4**) across a commercial panel of kinases. In 2008, Ambit Biosciences reported a quantitative analysis of 38 known kinase inhibitors across a panel of 317 kinases.[24] This commercially available panel contained 402 kinases at the time of submission and is based upon a competition binding assay of kinases fused to a proprietary tag. The data is first recorded as a % of kinase bound to an immobilized ligand in the presence and absence of the test reagent as compared to DMSO. Activities beyond a selected threshold are submitted for K_d determination. In addition to profiling **4** versus this panel, we submitted the reported small molecule Clk1/4 inhibitor TG003 (**1**) to generate a comparison between both agents. The results (tabulated in a dendrogram representation[25]) are shown in Figure 3 and demonstrate that both agents are remarkably selective. TG003 (**1**) was determined to have K_d's of 19 nM, 95 nM, and 30 nM versus Clk1, Clk2, and Clk4, respectively. The K_d for TG003 (**1**) versus Clk3 was 3 μM. It was also found that TG003 had activity versus CSNK1D (150 nM), CSNK1E (300 nM), Dyrk1A (12 nM), Dyrk1B (130 nM), PIM1 (130 nM), PIM3 (280 nM), and Ysk4 (290 nM). The novel quinazoline **4** was found to have K_d's of 37 nM, 50 nM and 27 nM versus Clk1, Clk4, and Dyrk1A, respectively. The only other locus of relevant activity (below 500 nM) was found for binding to the endothelial growth factor receptor (EGFR) (230 nM).

Based upon the potency and selectivity for **4** we next aimed to understand the binding mechanism of this chemotype at Clk4. We explored the binding modality by examining the inhibitory capacity of **4** in settings that varied both compound and substrate concentrations. The results are shown in Figure 4. The dose–response curve of **4** in the presence of three different ATP



Scheme 1. Reagents and conditions: (i) DIPEA, DMF, rt, 2 h (typical yields: 80–95%); (ii) $Pd(PPh_3)_4$, Na_2CO_3, DMF, 150 °C (wave), 1 h (typical yields: 50–80%).