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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.

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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.<sup>1</sup> The spliceosome regulates the processing, or splicing, of pre-mRNAs, yielding mature protein-encoding mRNAs.<sup>2,3</sup> Many human genes express more than one mRNA via alternative splicing, leading to protein diversity,<sup>4</sup> however, misregulation of alternative splicing is involved in the pathogenesis of cancer and other diseases.<sup>5,6</sup> Studies have revealed that Clk isoforms are associated with alternative splicing of PKCBII,<sup>7</sup> TF,<sup>8</sup> β-globin<sup>9</sup> and E1A pre-mRNA.<sup>10</sup> 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).<sup>11</sup> 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<sub>50</sub> values versus Clk1 and Clk4. The patent literature revealed structurally similar benzothiazole 1a from Sirtris Pharmaceuticals<sup>12</sup> and a quinoline **3** from Chronogen, Inc.<sup>13</sup> 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.<sup>14</sup> We have previously described a series of substituted 6-arylquinazolin-4-amines including NCGC00010037 (**4**, ML106) as potent inhibitors of Clk1 and Clk4.<sup>15</sup>

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<sub>50</sub> values of 12 and 27 nM for **1** and **4**, respectively). Dyrk1A<sup>16</sup> has been shown to accumulate in nuclear speckles where it interacts and activates splicing factors.<sup>17,18</sup> 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.<sup>19–25</sup> Overexpression of Dyrk1A has been associated with DS phenotypes<sup>20,26,27</sup> while loss-of-function results in potentially fatal conditions, such as decreased body and brain size.<sup>27,28</sup> 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.<sup>29</sup> 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).<sup>20</sup> The Dyrk1B homolog (also referred to as Mirk) was first isolated and described by Friedman and coworkers.<sup>30,31</sup> 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.<sup>32–36</sup> 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.<sup>32</sup> 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).<sup>22,37–39</sup> Harmine also represents the only reported inhibitor of Dyrk1B, although potency is mild and selectivity is poor.<sup>38</sup> 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.<sup>40</sup> 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<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, DME, H<sub>2</sub>O, 150 °C (μW), 1 h (typical yields: 40–50%); (ii) POCl<sub>3</sub>, *N*,*N*-dimethylaniline, toluene, reflux, 1 h; (iii) R,R'NH, *iPr*<sub>2</sub>NEt, DMF, rt to 60 °C, 2 h (typical yields: 80–95%).



**Scheme 2.** Reagents and conditions: (i) HNO<sub>3</sub>, CH<sub>3</sub>COOH, 0 °C to rt, 18 h; (ii) triflic anhydride, pyridine, DCM, 1 h, 91%; (iii) 3,4-(methylenedioxy)-phenylboronic acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, DME, H<sub>2</sub>O, 150 °C ( $\mu$ W), 1 h, 55%; (iv) Pd/C, H<sub>2</sub> (1 atm), EtOH, 18 h, 99%; (v) NH<sub>2</sub>CHO, NH<sub>3</sub>COOH, 140 °C for 3 h, then rt for 18 h, 51%; (vi) POCl<sub>3</sub>, *N*,*N*-dimethylaniline, toluene, reflux, 1 h; (vii) RNHCH<sub>3</sub>, *i*Pr<sub>2</sub>NEt, DMF, 60 °C, 18 h (typical yields: 80–95%).

IC<sub>50</sub> (nM) values of selected active quinazolines (4, 15-33)<sup>a</sup>



Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Clk4	Dyrk1A
4		Н	Н	39	62
15 <sup>b</sup>	3. 0 0	OMe	CH₃	>10,000	>10,000
16	3	Н	Н	249	1262
17	220	Н	Н	1090	3480
18	-32 OMe	Н	Н	1051	5697
19	-2-2-OMe	Н	Н	331	3152
20	Me OMe	Н	Н	3675	>10,000
21	OMe	Н	Н	>10,000	>10,000
22	CI	Н	Н	7943	9012
23	-2-CI	Н	Н	>10,000	>10,000
24	N N	Н	Н	3981	>10,000
25	2	Н	Н	7943	>10,000
26	F	Н	Н	128	164
27	2 N	Н	Н	>10,000	4517
28	Z N	Н	Н	1642	>10,000
29		Н	Н	126	180
30	COOEt	Н	Н	88	1437
31	COOH	Н	Н	199	>10,000
32	OH	Н	Н	241	4657
33	Н		Н	>10,000	>10,000

 $^a$  IC\_{50} values determined by Reaction Biology (www.reactionbiology.com). Compounds were tested in 10-dose IC\_{50} mode with 3-fold serial dilution starting at 50  $\mu$ M. Reactions were carried out at 10  $\mu$ M ATP.

<sup>b</sup> IC<sub>50</sub> values determined using KinaseGlo (Promega) assay.

would be tolerated. To examine this we set out to synthesize a variant of our chemotype with a methoxy group in the 7-position. Nitration of methyl 3-hydroxy-4-methoxybenzoate (**9**) followed by triflation and Suzuki–Miyaura coupling afforded methyl 5-(benzo[d][1,3]dioxol-5-yl)-4-methoxy-2-nitrobenzoate (**11**) in acceptable yields (Scheme 2). Reduction of the nitro group followed by cyclization yielded the required quinazolin-4(3*H*)-one which was then chlorinated and displaced by selected amines as described.

Utilizing these synthetic procedures we were able to rapidly access numerous analogues of NCGC00010037 (**4**) to address key SAR questions. To evaluate these agents we utilized a bioluminescent luciferase-based assays capable of visualizing substrate (ATP) depletion or product (ADP) formation.<sup>28</sup> Utilizing this assay we ascertained the IC<sub>50</sub> values for our agents versus Clk4 and Dyrk1A. To further examine their selectivity, we submitted selected agents to a commercial provider of kinase assays (Reaction Biology Corp) for profiling versus Clk1-4, Dyrk1A and Dyrk1B within a <sup>33</sup>P radio-labeled kinase assay. The data from both assay formats was highly consistent, however for uniformity we will report only the values generated by Reaction Biology Corp (refer to PubChem AIDs 1969, 1970, 1983, 2705, 2710, 488872, 488883, and 488887).

Our previous structure activity evaluations highlighted the utility of a benzo[d][1,3]dioxole moiety at the 6-position of the quinazoline ring. To further our examination at this position we synthesized several derivatives maintaining a common thiophen-2-ylmethanamine substituent in the 4-position while varying the aryl substituent at the 6-position. The results are shown in Table 1 and highlight the activity of the benzo[d][1,3]dioxole and related substitutions [including 2,3-dihydrobenzo[b][1,4]dioxine (29)]. Phenyl ring substitutions were less active as where furan derivatives 16 and 17. Interestingly, furan analogues with substitutions at the 5-position were found to possess activity at Clk4 including the ethyl carboxylate (30) and carboxylic acid (31). Switching the benzo[*d*][1,3]dioxole from the 6- to the 7-position of the quinazoline ring resulted in a complete loss of activity (analogue 33) as did the incorporation of the methoxy group at the 7position (analogues 14 [IC<sub>50</sub> >10,000 for Clk4 and Dyrk1A] and 15).

We next considered alternate substitutions at the 4-position of the quinazoline ring system while maintaining the benzo[*d*][1,3]dioxole moiety at the 6-position. These agents were profiled versus all Clk isoforms and Dyrk1A and Dyrk1B (Table 2). The results confirmed that these agents possessed selectivity for Clk1 and Clk4 over Clk2 and Clk3. Five-membered heterocycles were found to have the best activity as larger ring systems began to lose activity (analogue 55, for example). The role of adding (and varying) an alkyl group to the 4-position amine was also explored (direct comparisons include analogues 4, 34 and 35; 39 and 40; 45 (NCGC00185963, ML197) and 46 (NCGC00185981, ML195); 47 and 48; 50 and 51). In general, the addition of an alkyl group led to increased activity but at least one related pair had the opposite affect (analogues 50 and 51). Several agents had pan-activity versus Clk1, Clk4, Dyrk1A and Dyrk1B including 34, 46 and 49. Other agents were found to have specified selectivity's for these targets. For instance, the profile of **38** revealed that this analogue had modest selectivity for Clk1, Clk4 and Dyrk1A while analogue 54 (NCGC00229610, ML196) possesses selectivity for Clk4 and Dyrk1A over the other isoforms. Analogue 50 possesses a modest selectivity for Clk4 over all other isoforms.

Hoping to further define selective analogues we next examined several compounds with specific parings of the optimal

IC50 (nM) values of selected active quinazolines (4, 34-58)

$\mathbb{R}^{1}\mathbb{N}^{\mathbb{R}^{2}}$								
Compound	$R^1$	R <sup>2</sup>	Clk1	Clk2	Clk3	Clk4	Dyrk1A	Dyrk1B
4	Н	S Jort	59	1902	6936	39	62	697
34	CH₃	S Jore	11	173	4949	12	31	73
35	CH <sub>2</sub> CH <sub>3</sub>	S	14	447	6172	14	38	231
36	Н	S	888	>10,000	>10,000	117	1158	>10,000
37	CH <sub>3</sub>	S Jore	89	962	>10,000	29	260	1781
38	Н	S	69	2163	6555	35	35	506
39	Н	O Jore	192	301	2556	79	84	534
40	CH <sub>3</sub>	o Jore	22	692	8744	36	98	452
41	Н	C	130	801	2017	42	74	130
42	Н	O Jun	86	2725	6450	47	57	431
43	Н	O John	111	877	4650	38	126	938
44	Н	- C - Sara	39	651	>10,000	15	93	846
45	Н	N Jore	96	1327	7448	40	206	1510
46	CH <sub>3</sub>	N John S	20	186	1924	11	14	25
47	Н	S J Jul	176	414	3527	50	70	446
48	CH <sub>3</sub>	S John N	28	589	4655	20	51	180
49	CH <sub>3</sub>	N N	128	195	3019	37	26	48
50	Н		191	3594	>10,000	34	155	1125
51	CH <sub>3</sub>	HZ John	480	3331	>10,000	120	922	4036
52	Н	HN	931	2219	>10,000	161	541	2271
53	CH <sub>3</sub>	→ <sup>O</sup> → <sup>J</sup> J <sup>J<sup>2</sup></sup> N-N	98	3118	>10,000	45	120	1441
54	Н	o she	522	1055	3642	141	93	734
55	Н	0 John	598	882	5461	257	135	856
56	Н	O Jore	90	1810	3294	29	30	458
57	Н	and the second s	227	486	1320	90	76	313
58	Н	- shere	173	584	435	70	17	83

IC<sub>50</sub> values determined by Reaction Biology (www.reactionbiology.com). Compounds were tested in 10-dose IC<sub>50</sub> mode with 3-fold serial dilution starting at 50 µM. Reactions were carried out at 10 µM ATP.

amines at the 4-position and various substituted furans at the 6position. The results in Table 3 highlight the fact that, in general, these derivatives possess higher affinity for Clk4 as compared to Clk1. At least three analogues were found that were highly specific for Clk4 over all other Clk and Dyrk1 isoforms (analogues **61**, **63** and **65**). It should be noted that while these activities were consistent with in-house results (where assays were run in triplicate with good SEM values—see PubChem AIDs) the usefulness of these compounds as tools requires confirmation in cell-based assays.

IC<sub>50</sub> (nM) values of selected active quinazolines (16, 59-65)



Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Clk1	Clk2	Clk3	Clk4	Dyrk1A	Dyrk1B
16	Н	S	3-3-2- O	915	714	>10,000	249	1262	405
59	CH <sub>3</sub>	S	No.	899	1209	>10,000	269	1136	304
60	CH <sub>3</sub>	S	Unit OH	206	889	>10,000	81	557	352
61	Н	S Jore	COOEt	798	8510	>10,000	88	1437	4269
62	Н		NHEt	6164	8480	>10,000	1666	594	333
63	Н	- Vorte - Contraction - Contractio - Contraction - Contraction - Contraction - Contraction - Contrac	JAN OH	1522	1648	>10,000	136	>10,000	4420
64	Н	Vire O	COOEt	2083	6181	>10,000	229	3629	7990
65	Н	O Josef	соон	1561	1361	>10,000	104	>10,000	4927

IC<sub>50</sub> values determined by Reaction Biology (www.reactionbiology.com). Compounds were tested in 10-dose IC<sub>50</sub> mode with 3-fold serial dilution starting at 50 µM. Reactions were carried out at 10 µM ATP.

While understanding the activity for these agents versus the related Clk and Dyrk1 isoforms is a key element to defining novel probes of these targets, we also endeavored to understand their selectivity in the context of the entire kinome. As previously discussed, guinazoline-based kinase inhibitors are well known to possess a wide range of activity and it remained critical to show that these agents were not promiscuous across numerous targets. Our previous report included a profile of analogue 4 across a commercial kinase panel (DiscoveRx). We also included TG003 (1) in this profile and the results demonstrated that both agents were remarkably selective (**4** was shown to possess activity  $[K_d]$  values less than 300 nM] versus Clk1, Clk4, Dyrk1A and EGFR while 1 possessed activity versus Clk1, Clk2, Clk4, Csnk1d, Csnk1e, Dyrk1A, Dyrk1B, Pim1, Pim3, and Ysk4).<sup>15</sup> Often, when new analogues are examined (even highly structurally related compounds) new activities are formed. To assure that these analogues still possessed the high selectivity for the Clk and Dyrk1 class of kinases we selected four representative inhibitors (45, 46, 54 and 63) to examine in the same kinome-wide assay (possessing 442 kinases at the time of the profile). The kinome scan records activity as a percentage of kinase bound to an immobilized ligand in the presence and absence of each compound. In accordance with our previous work, activities beyond a selected threshold were submitted for  $K_d$  determination. The resulting  $K_d$  values further validated the selectivity of **45** and 54 for the Clk and Dyrk classes of kinases (Fig. 2). Compound 46, although slightly less selective, is highly active against the desired targets as well as undesired kinases, Mek5 ( $K_d$  = 47 nM), a potential prostate cancer target,<sup>41</sup> and the kinase encoded by PIK3C2G (PI3K family)( $K_d$  = 40 nM), which is involved in the pathophysiology of diabetes.<sup>42</sup> The results for **63** suggested that this agent is somewhat promiscuous across several kinases and not acceptable as a probe of Clk and Dyrk1 activity (and highlights the utility of these profiles).

Our previous report included a docking study of 4 within a homology model of Clk4. This model highlighted a potential Hbond between an amide NH within the ATP binding pocket and the guinazoline core. In this study, we hoped to utilize these models to better understand the interaction mode and selectivity profiles of the lead compounds within the Clk and Dyrk subfamilies. In order to generate useful models (particular of the Dyrk1 family) we performed multiple protein sequence alignments to derive homology models for Clk4 and Dyrk1B for which there are no published X-ray structures.<sup>15</sup> The homology model of Clk4 was developed by utilizing the X-ray structure of Clk1 as the template (86% sequence identity at the catalytic domain), while the Dyrk1B homology model was built based upon the highly homologous Dyrk1A (77% sequence identity) using MOE molecular modeling software (Fig. 3A).<sup>43</sup> Several of our lead compounds were then docked into the ATP binding domains of these Clk and Dyrk1 models to achieve an optimal binding pose using FRED (OpenEye Scientific Software suite) (Fig. 3B).<sup>44</sup> The resulting docking poses were considered in the context of the experimentally determined  $IC_{50}$  and  $K_d$  values. In agreement with our previous docking results, the quinazoline core adopted a common pose within the ATP binding pocket forming previously validated hydrogen bonds with the hinge region (Fig. 3B highlights the docking of 46 with Clk4). As previously discussed, when an alkyl group was added to the 4-position amine (either a methyl or ethyl) activity generally improved. Our model rationalizes this result due to a small hydrophobic pocket (as indicated by a white line) in which the alkyl group is oriented which would likely increase specified van der Waals interactions and lock the inhibitor in a preferred conformation (Fig. 3C). Interestingly, the SAR surrounding the amine side-chain suggests that several variations are well tolerated. This model suggests that the primary role of this moiety is space-filling rather than interacting with specific protein residues via H-bonding or electrostatic interactions.



**Figure 2.** Dendrogram representation of the human kinome demonstrating kinase selectivity of reported inhibitors over a panel of 442 kinases. Activity for **45**: Clk1 = 50 nM, Clk2 = 380 nM, Clk4 = 43 nM, Dyrk1A = 82 nM, PIP5K2C protein = 280 nM. Activity for **46**: Clk1 = 18 nM, Clk2 = 59 nM, Clk4 = 5 nM, Dyrk1A = 13 nM, Dyrk1B = 300 nM, Dyrk2 = 480 nM, Erk8 = 430 nM, Mek5 = 47 nM, PIK3C2B protein = 340 nM, PIK3C2G protein = 40 nM, PIK3CG protein = 370 nM, PIP5K2C protein = 360 nM, Ysk4 = 190 nM. Activity for **54**: Clk1 = 72 nM, Clk2 = 320 nM, Clk4 = 30 nM, Dyrk1A = 27 nM, PIK3C2B protein = 410 nM, PIK4CB protein = 430 nM, PIP5K2C protein = 310 nM. Data from DiscoveRx (http://kinomescan.com).



**Figure 3.** (A) Ribbon representation of the catalytic clefts in the Clk1 crystal structure (green; PDB 1257), Clk4 homology model (cyan), Dyrk1A crystal structure (orange, PDB 2VX3) and Dyrk1B homology model (purple). The ligand shown is the co-crystal ligand in Dyrk1A. (B) Docking model of **46** in the Clk4 catalytic cleft. The binding pocket is depicted by molecular surface and the hydrogen bonds are labeled as yellow dotted lines. (C) The close view of the small hydrophobic pocket (as indicated by a white line) in which the methyl group is sitting in the docking model of **46** within the Clk4 catalytic cleft. (D) Docking model of **63** in the Clk4 catalytic cleft superimposed with Clk1, Dyrk1a and Dyrk1b. The hydrogen bond from the hydroxyl group to Asp248 is labeled as a yellow dotted line. Clk4 kinase domain is a homology model derived from the X-ray structure of Clk1 (PDB code: 1257). This figure was prepared with the program VIDA (OpenEye Scientific Software).

A primary hope was that these models might yield insight into defining more selective inhibitors of the Clk and Dyrk1 targets. One insight surrounds Clk4, which is nearly identical to Clk1 at the catalytic domain, but shows a key divergence in residue Asp248. Models docking **63** at Clk4 and Clk1 suggest that this molecule's hydroxyl group is within an appropriate proximity to Asp248  $(\sim 3.2 \text{ Å})$  to form a hydrogen bond (Fig. 3D). This potential interaction would help rationalize the potency for **63** versus Clk4 (136 nM) as compared to Clk1 (1522 nM). In the Dyrk1A crystal structure and the Dyrk1B homology model, the Asp residue at the same position shifted more than 6 Å away from the hydroxyl group in analogue **63** due to a backbone conformational change.

3	1	5	8
-	•	~	~

Analogue	Kinetic solubility <sup>a</sup> ( $\mu M$ )	$P_{\rm app} (A-B)^{\rm b} (10^{-6}  {\rm cm/s})$	$P_{\rm app} (B-A)^{\rm b} (10^{-6}  {\rm cm/s})$	48 h aqueous stability <sup>c</sup> (DPBS buffer, pH 7.4)
45	4.3	25.5	12.7	>95
46	6.6	18.9	8.9	>95
54	143.3	35.4	23.1	>95

Aqueous solubility and stability and Caco-2 permeability data for selected agents

<sup>a</sup> Solubility measurements done at Analiza, Inc. (http://analiza.com).

<sup>b</sup> Caco-2 permeability was measured at Cyprotex (www.cyprotex.com/adme).

<sup>c</sup> Aqueous stability measured over 48 h in pH 7.4 DPBS buffer. Value reflects the % remaining.

This distance is too far for a viable hydrogen bond and offers a basis for the potency decrease versus Dyrk1A and Dyrk1B (>10,000 and 4420 nM, respectively). This interaction also highlights a potential route to furthering the SAR of this chemotype.

As it was our goal to define probe compounds for these targets that would have use within cell-based studies it was important for us to examine the water solubility, stability and membrane permeability of selected analogues. To accomplish this, we submitted selected analogues (**45**, **46** and **54**) to commercial providers of kinetic aqueous solubility data (Analiza, Inc.) and Caco-2 permeability data (Cyprotex PLC). We further examined the stability of these agents in aqueous buffered environment for 48 h (results are based upon retention of UV/Vis signal and mass assessment within a standard HPLC gradient). The outcome of these studies is shown in Table 4. Compounds **45** and **46** were found to possess only modest aqueous solubility while **54** was highly soluble. The Caco-2 profile suggested that each agent was capable of passive membrane permeability and had favorable efflux ratios. Finally, each agent was highly stable in an aqueous environment for up to 48 h.

In conclusion, the expansion of our previous efforts surrounding specific 6-arylquinazolin-4-amines as potent Clk and Dyrk1 inhibitors has yielded several agents with impressive potency and selectivity. Selected inhibitors possess activity versus Clk1, Clk4 and Dyrk1A below 100 nM. A broad kinome scan has confirmed that these compounds are highly selective for these targets and molecular docking studies highly suggest that they bind at the kinase hinge region. This work also defined the first reported inhibitor of Dyrk1B. These agents provide useful tool compounds to probe the role of these targets in pre-mRNA splicing and, in the case of Dyrk1B, specified roles in cancer.

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# Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.114.

### **References and notes**

- 1. Prasad, J.; Manley, J. L. Mol. Cell. Biol. 2003, 23, 4139.
- 2. Black, D. L. Annu. Rev. Biochem. 2003, 72, 291.
- 3. Wang, Z.; Burge, C. B. RNA 2008, 14, 802.
- 4. Faustino, N. A.; Cooper, T. A. Genes Dev. 2003, 17, 419.
- 5. He, C.; Zhou, F.; Zuo, Z.; Cheng, H.; Zhou, R. PLoS ONE 2009, 4, e4732.

- 6. Matlin, A. J.; Clark, F.; Smith, C. W. J. Nature 2005, 6, 386.
- Jiang, K.; Patel, N. A.; Watson, J. E.; Apostolatos, H.; Kleiman, E.; Hanson, O.; Hagiwara, M.; Cooper, D. R. *Endocrinology* 2009, 150, 2087.
- Schwertz, H.; Tolley, N. D.; Foulks, J. M.; Denis, M. M.; Risenmay, B. W.; Buerke, M.; Tilley, R. E.; Rondina, M. T.; Harris, E. M.; Kraiss, L. W.; Mackman, N.; Zimmerman, G. A.; Weyrich, A. S. J. Exp. Med. 2006, 203, 2433.
- Muraki, M.; Ohkawara, B.; Hosoya, T.; Onogi, H.; Koizumi, J.; Koizumi, T.; Sumi, K.; Yomoda, J.-i.; Murray, M. V.; Kimura, H.; Furuichi, K.; Shibuya, H.; Krainer, A. R.; Suzuki, M.; Hagiwara, M. J. Biol. Chem. 2004, 279, 24246.
- Yomoda, J.-i.; Muraki, M.; Kataoka, N.; Hosoya, T.; Suzuki, M.; Hagiwara, M.; Kimura, H. Genes Cells 2008, 13, 233.
- Hartmann, A. M.; Rujescu, D.; Giannakouros, T.; Nikolakaki, E.; Goedert, M.; Mandelkow, E.-M.; Gao, Q. S.; Andreadis, A.; Stamm, S. *Mol. Cell. Neurosci.* 2001, 18, 80.
- Perni, R. B.; Bemis, J.; Nunes, J. J.; Szczepankiewicz, B. G. Inhibitors of the CDC2like Kinases and Methods of Use Therof. WO 2009/085226 A2, 9 July 2009.
- Hekimi, S.; McBride, K.; Hihi, A. K.; Kianjcka, I.; Wang, Y.; Hayes, S. L.; Guimond, M.-P.; Sevigny, G.; Dumas, D.; Smith, J. New Quinoline Derivatives. 60/832937, 25 July 2006.
- Fedorov, O.; Huber, K.; Eisenreich, A.; Filippakopoulos, P.; King, O.; Bullock, A. N.; Szklarczyk, D.; Jensen, L. J.; Fabbro, D.; Trappe, J.; Rauch, U.; Bracher, F.; Knapp, S. Chem. Biol. 2011, 18, 67.
- Mott, B. T.; Tanega, C.; Shen, M.; Maloney, D. J.; Shinn, P.; Leister, W.; Marugan, J. J.; Inglese, J.; Austin, C. P.; Mistelli, T.; Auld, D. S.; Thomas, C. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6700.
- Becker, W.; Weber, Y.; Wetzel, K.; Eirmbter, K.; Tejedor, F. J.; Joost, H.-G. J. Biol. Chem. 1998, 273, 25893.
- 17. Alvarez, M.; Estivill, X.; Luna, S. d. l. J. Cell Sci. 2003, 116, 3099.
- de Graaf, K.; Czajkowska, H.; Rottmann, S.; Packman, L.; Lilischkis, R.; Luscher, B.; Becker, W. BMC Biochemistry 2006, 7, 7.
- 19. Park, J.; Song, W.-J.; Chung, K. C. Cell. Mol. Life Sci. 2009, 66, 3235.
- 20. Yoshida, K. Biochem. Pharmacol. 2008, 76, 1389.
- Kim, N. D.; Yoon, J.; Kim, J. H.; Lee, J. T.; Chon, Y. S.; Hwang, M.-K.; Hac, I.; Songc, W.-J. Bioorg. Med. Chem. Lett. 2006, 16, 3772.
- 22. Seifert, A.; Allan, L. A.; Clarke, P. R. FEBS J. 2008, 275, 6268.
- 23. Guimera, J.; Casas, C.; Estivill, X.; Pritchard, M. Genomics 1999, 57, 407.
- 24. Tejedor, F. J.; Hammerle, B. FEBS J. 2011, 278, 223.
- 25. Wegiel, J.; Gong, C.-X.; Hwang, Y.-W. FEBS J. 2011, 278, 236.
- Toiber, D.; Azkona, G.; Ben-Ari, S.; Toran, N.; Soreq, H.; Dierssen, M. Neurobiol. Dis. 2010, 40, 348.
- 27. Guo, X.; Williams, J. G.; Schug, T. T.; Li, X. J. Biol. Chem. 2010, 285, 13223.
- 28. Yabut, O.; Domogauer, J.; D'Arcangelo, G. J. Neurosci. 2010, 30, 4004.
- Arron, J. R.; Winslow, M. M.; Polleri, A.; Chang, C.-P.; Wu, H.; Gao, X.; Neilson, J. R.; Chen, L.; Heit, J. J.; Kim, S. K.; Yamasaki, N.; Miyakawa, T.; Francke, U.; Graef, I. A.; Crabtree, G. R. *Nature* **2006**, *441*, 595.
- 30. Mercer, S. E.; Friedman, E. Cell Biochem. Biophys. 2006, 45, 303.
- 31. Lee, K.; Deng, X.; Friedman, E. Cancer Res. 2000, 60, 3631.
- 32. Friedman, E. Cancers 2010, 2, 1492.
- 33. Jin, K.; Park, S.; Ewton, D. Z.; Friedman, E. Cancer Res. 2007, 67, 7247.
- 34. Friedman, E. J. Cell Biol. 2007, 102, 274.
- Jin, K.; Ewton, D. Z.; Park, S.; Hu, J.; Friedman, E. J. Biol. Chem. 2009, 284, 22916.
  Gao, J.; Zheng, Z.; Rawal, B.; Schell, M. J.; Bepler, G.; Haura, E. B. Cancer Biol.
- *Ther.* **2009**, 8, 1671. 37. Bain, J.; Plater, L.; Elliott, M.; Shpiro, N.; Hastie, C. J.; McLauchlan, H.; Klevernic,
- I.; Arthur, J. S. C.; Alessi, D. R.; Cohen, P. *Biochem, J.* **2007**, 408, 297.
- Gockler, N.; Jofre, G.; Papadopoulos, C.; Soppa, U.; Tejedor, F. J.; Becker, W. FEBS J. 2009, 276, 6324.
- 39. Becker, W.; Sippl, W. FEBS J. 2011, 278, 246.
- 40. <www.tarceva.com>.
- Mehta, P. B.; Jenkins, B. L.; McCarthy, L.; Thilak, L.; Robson, C. N.; Neal, D. E.; Leung, H. Y. Oncogene 2003, 22, 1381.
- Makoto, D.; Hidenori, S.; Toshihide, O.; Sayumi, T.; Takafumi, S.; Shigeru, K.; Yumi, J.; Kiriko, W.; Wataru, K.; Shinji, S.; Hiroshi, Y.; Mitsuru, E.; Masaaki, M.; Isao, K.; Sumio, K.; Kato, T. Biochem. Biophys. Res. Commun. 2008, 365, 466.
- MOE Molecular Operating EnVironment, 2008.10; Chemical Computing Group Inc.: Montreal, Canada, 2008.
- FRED, OpenEye Scientific Software, Inc.: Santa Fe, NM, 2010. <<a href="http://www.eyesopen.com/">http://www.eyesopen.com/</a>>.