

## 4-(Phenylaminomethylene)isoquinoline-1,3(2H,4H)-diones as Potent and Selective Inhibitors of the Cyclin-Dependent Kinase 4 (CDK4)

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The cyclin-dependent kinases (CDKs), as complexes with their respective partners, the cyclins, are critical regulators of cell cycle progression. Because aberrant regulations of CDK4/cyclin D1 lead to uncontrolled cell proliferation, a hallmark of cancer, small-molecule inhibitors of CDK4/cyclin D1 are attractive as prospective antitumor agents. The series of 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione derivatives reported here represents a novel class of potent inhibitors that selectively inhibit CDK4 over CDK2 and CDK1 activities. In the headpiece of the 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione, a basic amine substituent is required on the aniline ring for the CDK4 inhibitory activity. The inhibitory activity is further enhanced when an aryl or heteroaryl substituent is introduced at the C-6 position of the isoquinoline-1,3(2H,4H)-dione core. We present here SAR data and a CDK4 mimic model that explains the binding, potency, and selectivity of our CDK4 selective inhibitors.

### Introduction

Cyclin-dependent kinases (CDKs), a family of serine/threonine kinases, are critical regulators of cell cycle progression that form complexes with their activating partners, the cyclins, in order to be active.<sup>1</sup> Different CDK/cyclin complexes serve as switches that regulate each of the cell cycle transitions through four distinct phases: G1, S, G2, and M. In the G1-S transition, the retinoblastoma susceptibility protein (pRB) plays a central role. In its hypophosphorylated state, pRB blocks cell progression from G1 to S by binding with E2F transcription family members. In response to mitogenic stimulation, cells synthesized D-type cyclins that complex with CDK4 and CDK6. In turn, pRB is phosphorylated by CDK4- or CDK6-cyclin complexes in early G1 and by CDK2/cyclin E in late G1.<sup>2</sup> The hyperphosphorylation of RB causes the release of the E2F proteins, leading to transcriptional activation of a set of genes required for entry into S-phase of the cell cycle.<sup>3</sup> In normal cells, CDK4/6 activities are negatively regulated by cyclin-dependent kinase inhibitors of the INK4 family, such as p16, a tumor suppressor, and that of CDK2 is negatively regulated by CKIs of the Cip/Kip family, such as p21. The CDK4/6-cyclin D-INK4-Rb pathway is universally disrupted in human cancer.<sup>2</sup> For example, p16 is inactivated due to mutation, deletion, or genetic silencing, the catalytic subunit of CDK4 is overexpressed, and the activating partner, cyclin D1, is amplified in many human cancers. These changes lead to uncontrolled cell proliferation, a hallmark of cancer.<sup>4</sup>

Recently, our laboratory<sup>5</sup> reported that inhibition of endogenous cyclin D1 or CDK4 expression by RNA interference resulted in hypophosphorylation of RB and accumulation of cells in G1 phase. This result supports the prevailing view that

pharmacological inhibition of CDK4/cyclin D1 complexes is a useful strategy to inhibit the growth of tumors. Malumbres<sup>6</sup> provided additional evidence that knockin mice expressing a mutant form of cyclin D1 that binds to CDK4/6, but cannot activate their catalytic activity, are resistant to *c-neu/erbB-2* tumorigenesis in spite of undergoing normal epithelial cell expansion during pregnancy. Moreover, knockdown of CDK4 in mammary tumor cells prevents tumor formation.<sup>6</sup> These results strongly suggest that inhibition of CDK4 kinase activity might be beneficial for cancer therapy. In contrast, several experimental results suggest that highly selective inhibition of CDK2 may not be useful therapeutically. For example, a variety of cancer cell lines were able to proliferate after specific and acute depletion of CDK2 by siRNA or antisense oligonucleotides.<sup>7</sup> Moreover, CDK2 knockout mice are viable.<sup>8</sup>

In the past few years, several small-molecule CDK inhibitors have been advanced to clinical trials.<sup>9</sup> Among them, PD-0332991 (pyridopyrimidine)<sup>10</sup> is a selective CDK4 inhibitor, whereas flavopiridol is a pan-CDK inhibitor, R-Roscovitine (CYC202, Seliciclib)<sup>11</sup> and BMS-387032 (aminothiazole)<sup>12</sup> are inhibitors selective for CDK2 and CDK1. When we started our work, there were few examples of inhibitors selective for CDK4. Among those reported, 3-( $\alpha$ -heteroarylaminobenzylidene)-2-indolinones<sup>13</sup> were noteworthy as inhibitors selective for CDK4 over CDK1.

Our initial chemical effort involved preparation of a few preliminary analogues of ring-expanded isoquinoline-1,3-dione, a novel scaffold. To our delight, they showed selectivity for CDK4 over CDK1 and CDK2. We then began our medicinal chemistry effort to find analogues with enhanced potency which maintained a favorable selectivity profile. This paper provides details on our optimization efforts and the structure–activity relationships (SAR) relevant to the substituents at the C-4, C-6, and C-7 positions of the isoquinoline-1,3-dione core. In this paper, we also present one of our inhibitors docked in the ATP binding domain of the CDK4 mimic model to show the critical

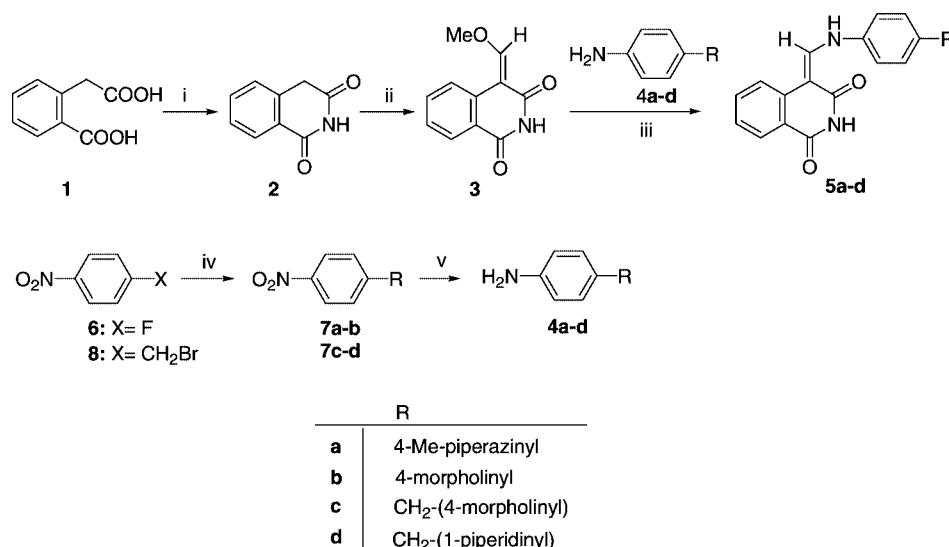
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Scheme 1<sup>a</sup>

<sup>a</sup> (i) Urea, reflux; (ii) HC(OMe)<sub>3</sub>, Ac<sub>2</sub>O, DMF, reflux; (iii) DMF, reflux; (iv) amine, EtOH; (v) Fe, NH<sub>4</sub>Cl, MeOH, H<sub>2</sub>O, reflux.

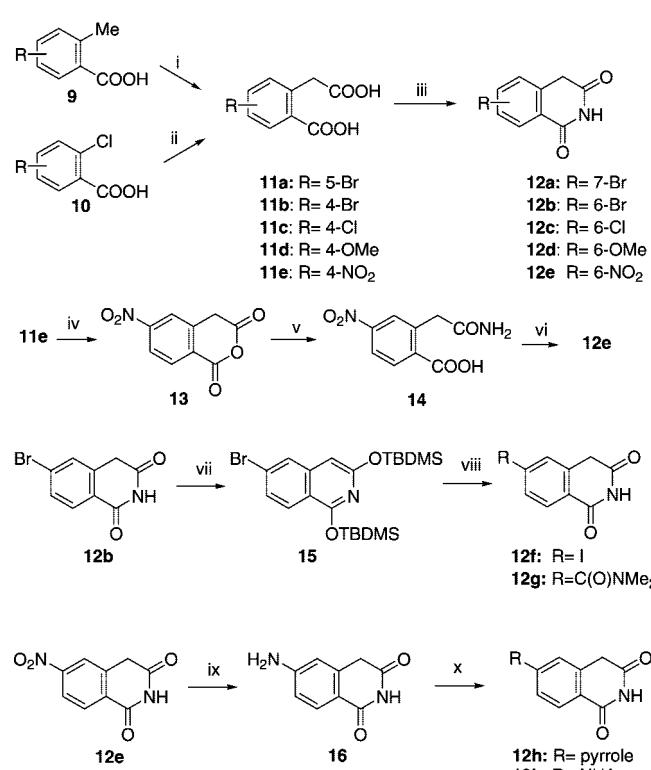
H-binding interactions that may explain the CDK4 selectivity as well as potency.

### Chemistry

As shown in Scheme 1, compounds **5a–d** were prepared starting from the commercially available homophthalic acid **1**. Urea was mixed well with **1** and heated to yield isoquinoline-1,3-dione **2**.<sup>14</sup> Condensation of **2** with methyl orthoformate in the presence of acetic anhydride gave the enol ether **3**, which was further reacted with anilines **4a–d** to afford the phenylenamines **5a–d**.<sup>15</sup> The phenylenamines could be prepared from **2** without isolation of the intermediate **3**. Substituted anilines **4a–d** were readily synthesized by treatment of the amines with *p*-fluoronitrobenzene **6** or *p*-nitrobenzyl bromide **8**, followed by reduction of the nitro group.

Substituted homophthalic acids **11a–e** were prepared by two different methods, as shown in Scheme 2. Substituted methylbenzoic acids **9** were first treated with lithium diisopropylamide, and the resulting dianions were then quenched with dimethylcarbonate<sup>16</sup> to afford **11a–d**. Alternatively, 2-chloro-4-nitrobenzoic acid was condensed with ethyl acetoacetate in the presence of sodium ethoxide and cuprous bromide, in refluxing ethanol,<sup>17</sup> to produce **11e**. The homophthalic acids **11a–e** were converted into the corresponding isoquinoline-1,3-diones **12a–e**, using the same method as for the preparation of **2**. Additionally, refluxing **11e** with acetyl chloride afforded anhydride **13**, which upon treatment with concentrated ammonium hydroxide yielded the amide **14**. Refluxing **14** in 1,2-dichlorobenzene produced the desired isoquinoline-1,3-dione **12e**.

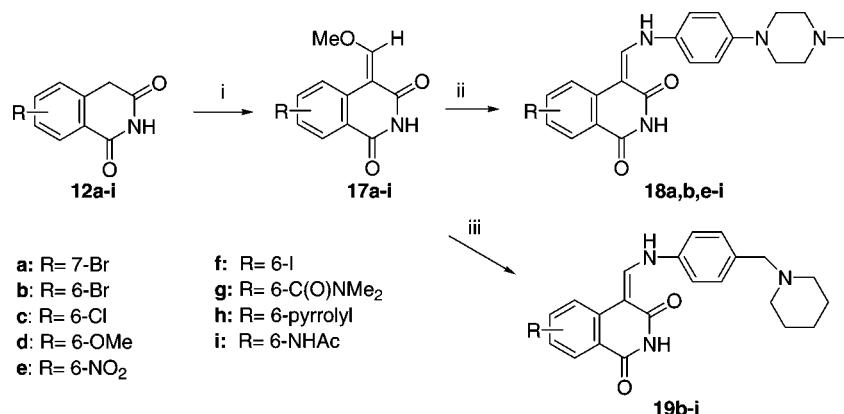
Isoquinoline-1,3-diones bearing other substituents at C-6 such as iodo (**12f**) and *N,N*-dimethylacetamido (**12g**) were prepared from the corresponding bromo derivative **12b**. Theoretically, metal–halogen exchange of **12b** would provide compounds with other functional groups. However, because of the presence of acidic methylene protons, the bromide **12b** was converted into 1,3-disiloxyisoquinoline **15** before treatment with *tert*-butyl lithium. The resulting lithium intermediate was reacted with iodine or *N,N*-dimethylcarbamoyl chloride to yield **12f** and **12g**, respectively. Other 6-substituted isoquinoline-1,3-diones **12h** and **12i** were prepared from the corresponding nitro derivative **12e**. The nitro group of **12e** was reduced to amine **16**, which was either condensed with 2,4-dimethoxytetrahydrofuran catalyzed

Scheme 2<sup>a</sup>

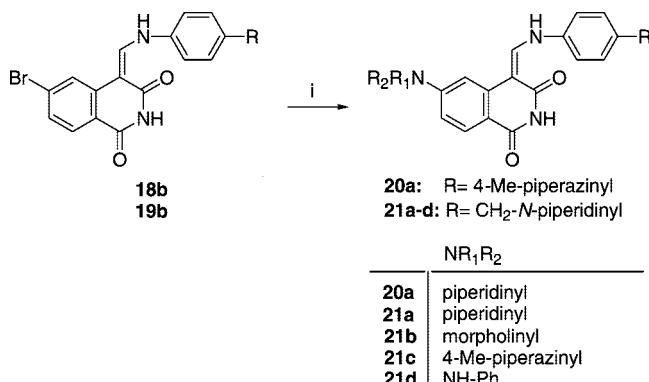
<sup>a</sup> (i) (for **11a–d**) Lithium diisopropylamide, (MeO)<sub>2</sub>CO, then H<sub>2</sub>O; (ii) (for **11e**) ethyl acetoacetate, NaOEt, CuBr, EtOH, then NaOH; (iii) urea, with or without 1,2-dichlorobenzene, reflux; (iv) MeCOCl, dioxane; (v) NH<sub>4</sub>OH; (vi) 1,2-di-Cl-benzene or carbonyldiimidazole, DMF, reflux; (vii) TBDMSCl, imidazole, DMF, room temperature; (viii) *t*-BuLi, THF, -78 °C; then for **12f**: I<sub>2</sub>, -78 °C; for **12g**: Me<sub>2</sub>NCOCl, THF, -78 °C; (ix) Pd/C, H<sub>2</sub>; (x) for **12h**: 2,4-(MeO)<sub>2</sub>-tetrahydrofuran, 4-chloropyridine HCl salt; for **12i**: Ac<sub>2</sub>O, DMF.

by 4-chloropyridine hydrochloride, to yield the pyrrole derivative **12h**, or reacted with acetic anhydride to produce the acetamide derivative **12i**.

As depicted in Scheme 3, various C-6 substituted isoquinoline-1,3-diones **12a–i** were converted into the enol ethers **17a–i** using the same conditions described in Scheme 1 for the unsubstituted isoquinoline-1,3-dione (from **2** to **3**). The enol

Scheme 3<sup>a</sup>

<sup>a</sup> (i) HC(OMe)<sub>3</sub>, Ac<sub>2</sub>O, DMF, reflux; (ii) 4-(N-Me-piperazinyl)aniline, DMF, reflux; (iii) 4-(N-piperidinylmethyl)aniline, DMF, reflux.

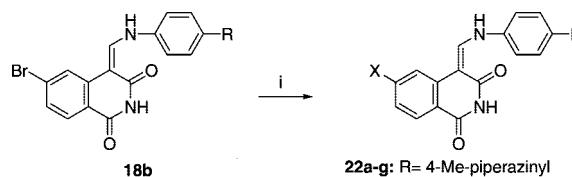
Scheme 4<sup>a</sup>

<sup>a</sup> (i) Pd<sub>2</sub>(dba)<sub>3</sub>, KOt-Bu, 1,3-bis(2,6-di-isopropylphenyl)imidazolium chloride, DMF, 100 °C, (for 20a, 21a) piperidine, (for 21b) morpholine, (for 21c) 4-Me-piperazine, (for 21d) PhNH<sub>2</sub>.

Compounds **17a–i** were then reacted with 4-(N-methylpiperazinyl)-aniline to form **18a,b,e–i** or with 4-(piperidinylmethyl)aniline to yield **19b–i**.

As described above, the 6-bromo group of isoquinoline-1,3-dione **12b** was first converted to other functional groups (Scheme 2), followed by introduction of the aniline headgroup (Scheme 3). An alternate method is shown in Scheme 4, where the aniline headgroups were already attached to the 6-bromoisoquinoline-1,3-dione **18b** and **19b**, and the 6-bromo substituent was subsequently converted to various cyclic amines **20a**, **21a–c**, and aniline **21d** via Buchwald chemistry, using Pd<sub>2</sub>(dba)<sub>3</sub>, imidazolium ionic liquid, and potassium *tert*-butoxide in DMF. The 6-aryl (**22a**, **22e**, **23a**, **23e–k**) and 6-heteroaryl (**22b–d**, **23b–d**) were also prepared from the corresponding 6-bromo derivatives **18b** and **19g** via Suzuki cross coupling reactions, as shown in Scheme 5. The 6-alkene **22f** and 6-alkyne **22g** were prepared from **18b** via Heck coupling and Sonogashira coupling, respectively. Treatment of **19b** with zinc cyanide and Pd(PPh<sub>3</sub>)<sub>4</sub> yielded the nitrile **23l**.

Headgroups carrying bulky piperazines were prepared as shown in Scheme 6. Amination of 4-fluoronitrobenzene **6** with commercially available *N*-Boc-2,5-diaza-bicyclo[2.2.1]heptane gave **24**, which upon treatment with trifluoroacetic acid afforded **25**. Further methylation of the nitrogen of **25**, followed by reduction of the nitro group yielded the aniline **27**. Other anilines carrying bulky piperazines **30** and **31** were prepared in a similar way. Anilines **27**, **30–33** were reacted with enol ether **17f**,

Scheme 5<sup>a</sup>

X	X
22a: phenyl	23a: phenyl
22b: 2-furyl	23b: 3-furyl
22c: 3-furyl	23c: 3-pyridyl
22d: 3-thienyl	23d: 3-thienyl
22e: 4-HC(O)-phenyl	23e: 4-F-phenyl
22f: HC=CH-phenyl	23f: 4-Cl-phenyl
22g: HC≡phenyl	23g: 3-OH-phenyl
	23h: 4-OH-phenyl
	23i: 3-MeO-phenyl
	23j: 4-MeO-phenyl
	23k: 4-PhO-phenyl
	23l: CN

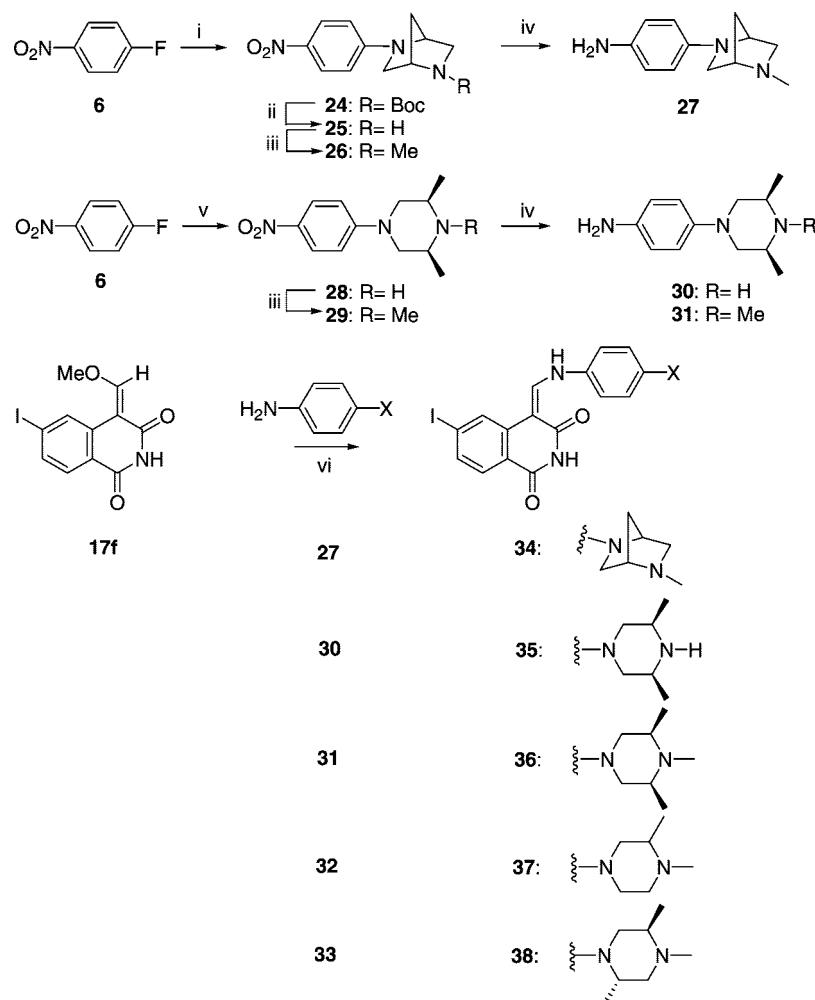
<sup>a</sup> (i) (for 22a,b) XB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, DMF, microwave, 150 °C; (for 23a–d,f,k) XB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, DMF, 120 °C; (for 22c–e, 23i,j) XB(OH)<sub>2</sub>, Pd<sub>2</sub>(dba)<sub>3</sub>, Ph-PhP(*t*-Bu)<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, DMF, 120 °C; (for 23e,g,h) XB(OH)<sub>2</sub>, Pd<sub>2</sub>(dba)<sub>3</sub>, Ph-PhP(*t*-Bu)<sub>2</sub>, Cs<sub>2</sub>CO<sub>3</sub>, DMF, 120 °C; (for 22f) styrene, *n*-Bu<sub>4</sub>NBr, (*o*-tolyl)<sub>3</sub>P, Cs<sub>2</sub>CO<sub>3</sub>, Pd(OAc)<sub>2</sub>, DMF, microwave, 200 °C, 2 min; (for 22g) phenylacetylene, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, Cul, Et<sub>3</sub>N, DMF, 70 °C; (for 23l) Zn(CN)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, DMF, 100 °C.

carrying a 6-I group to generate the desired 6-iodo-isoquinoline-1,3-dione derivatives **34–38**, respectively, as shown in Scheme 6.

Other headgroups **41a–e** (Scheme 7), where the phenyl ring of aniline **4a** was replaced with a pyridine, pyridazine, fluorophenyl, and pyrimidine, respectively, were prepared in the same manner as aniline **4a**. Whereas the pyrazine headgroup **41f** was prepared from *N*-methylpiperazine and aminobromopyrazine **42** protected as the *N,N*-diacetyl derivative. The resulting bis-amide **43** was then deprotected by acid to yield the desired headgroup **41f**. These headgroups **41a–f** were then coupled with the enol ethers **17b** and **17f** to afford the desired final compounds **44a–f**. The halogens at C-6 were further converted into 3-furyl via Suzuki coupling reactions to afford the 6-(3-furyl)isoquinoline-1,3-dione derivatives **45a–f**.

## Results and Discussion

**CDK4 Inhibitory Activity, Selectivity and Inhibition of Cell Proliferation.** Our initial efforts focused on modifying the substituent on the aniline ring of 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione. As shown in Table 1, **5a**, which has a 4-methylpiperazine group on the aniline ring, showed

Scheme 6<sup>a</sup>

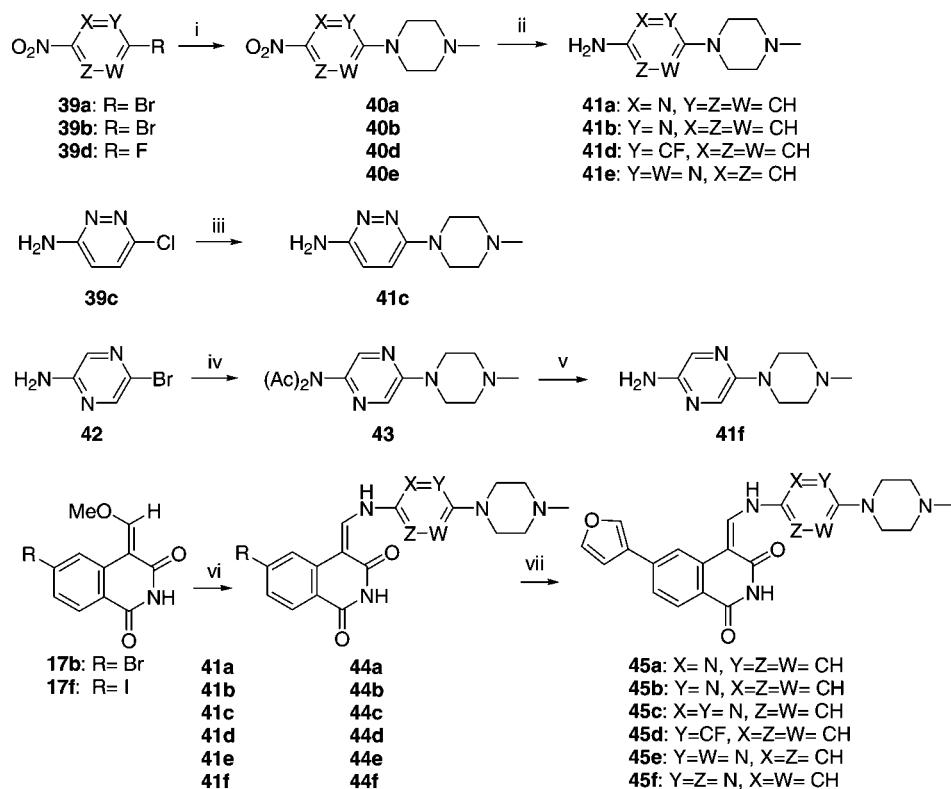
<sup>a</sup> (i) 2-Boc-(1*S*,4*S*)-2-Boc-2,5-diazabicyclo[2.2.1]heptane, i-Pr<sub>2</sub>NEt, CH<sub>3</sub>CN, 80 °C; (ii) TFA, MeOH, room temperature; (iii) HCOOH, (CH<sub>2</sub>O)<sub>n</sub>, 80 °C; (iv) Pd/C, H<sub>2</sub>; (v) DMF, 90–120 °C, 2,6-Me<sub>2</sub>-piperazine; (vi) DMF, reflux.

moderate CDK4 activity and selectivity over CDK1 and CDK2. In contrast, the morpholino derivative, **5b**, showed IC<sub>50</sub> values greater than 29  $\mu$ M for all three CDKs. Interestingly, addition of a methylene bridge between the morpholine and the phenyl ring, to provide **5c**, resulted in improved CDK4 inhibitory activity. The methylene-linked morpholine in **5c** is basic, whereas the same group in **5b** linked directly to a phenyl ring lacks basicity. This suggests that a basic amino substituent in the 4-phenylaminomethylene headpiece is needed for CDK4 activity. As expected, the methylene-linked piperidine, **5d**, is also a CDK4 inhibitor.

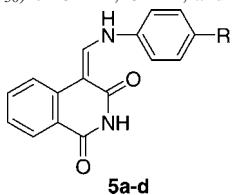
Table 2 shows the effect of varying substituents at C-6 or C-7 of **5a**, which has a 4-(methylpiperazinyl)phenylaminomethylene headpiece at C-4 of the isoquinoline-1,3(2*H*,4*H*)-dione core. The 6-Br derivative **18b** was almost a log order more potent in CDK4 inhibition than the 7-Br derivative **18a**. This result led us to focus solely on 6-substituted analogues. The larger 6-iodo analogue **18f** showed a 3-fold increase in CDK4 inhibition and more than 100-fold selectivity over CDK1 and CDK2. The 6-phenyl derivative **22a** is as potent and selective as the 6-iodo derivative **18f**. The 6-(4-formylphenyl) derivative **22e** was about 3-fold more potent than **22a**. However, addition of a double bond or a triple bond between the phenyl group and the core, yielded the respective analogues **22f** and **22g** that showed much reduced activity. Replacement of the phenyl group at C-6 of **22a** by heteroaryls, such as 2-furyl and

3-furyl, generated the corresponding compounds **22b** and **22c** that are as potent and selective as **22a**. Other 6-heteroaryl derivatives such as 6-(pyrrolyl) (**18h**) and 6-(3-thienyl) (**22d**) analogues are 3- to 4-fold more potent than the 6-phenyl analogue **22a**, with excellent selectivity (more than 357- and 500-fold for **18h** and **22d**, respectively) over CDK1 and CDK2. However, replacement of the pyrrolyl group by a piperidinyl group to provide **20a** resulted in a log order decrease in potency for CDK4 inhibition. In addition, dramatic decreases in CDK4 activity and selectivity were seen with analogues bearing electron-withdrawing substituents at C-6, such as nitro (**18e**), acetamido (**18i**), and *N,N*-dimethylcarboxamido (**18g**).

Effects of C-6 substituents on analogue **5d**, which has a 4-(piperidinylmethyl)phenylaminomethylene headpiece of the core at C-4, were also evaluated, as shown in Table 3. It is interesting to see that SAR for substitution at C-6 is quite similar for **5a** and **5d**. For example, comparable activity and selectivity were seen for the pairs of **5a** and **5d** subseries that carry bromo (**18b** vs **19b**), iodo (**18f** vs **19f**), phenyl (**22a** vs **23a**), 3-thienyl (**22d** vs **23d**), pyrrolyl (**18h** vs **19h**), piperidinyl (**20a** vs **21a**), nitro (**18e** vs **19e**), acetamido (**18i** vs **19i**), or *N,N*-dimethylcarboxamido (**18g** vs **19g**) at C-6. The only exception is the pair of 6-(3-furyl) derivatives, where **23b** was 6-fold more potent and selective than **22c**. Among the three halogen derivatives at C-6, the chloro analogue (**19c**) is the least potent, whereas the iodo compound (**19f**) is most the potent, as expected. The

Scheme 7<sup>a</sup>

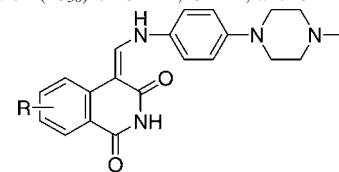
<sup>a</sup> (i) *N*-Me-piperazine,  $\text{CH}_2\text{Cl}_2$ ; (ii)  $\text{Pd/C}$ ,  $\text{H}_2$ , or  $\text{Fe}$ ,  $\text{HOAc}$ ,  $\text{MeOH}$ ; (iii) 5-Cl-pyridine HCl, *N*-Me-piperazine; (iv)  $\text{NaH}$ ,  $\text{DMF}$ ,  $\text{AcCl}$ ,  $0^\circ\text{C}$ ; then *N*-Me-piperazine,  $\text{Pd}_2(\text{dba})_3$ ,  $(t\text{-Bu})_3\text{P}$ ,  $\text{KO-}t\text{-Bu}$ ,  $\text{DMF}$ , microwave,  $120^\circ\text{C}$ ; (v)  $\text{HCl}$ ; (vi)  $\text{DMF}$ ,  $110^\circ\text{C}$ ; for  $44a\text{-}d$ :  $R = \text{Br}$ ; for  $44e\text{,}f$ :  $R = \text{I}$ ; (vii) 3-furanboronic acid,  $\text{Pd}_2(\text{dba})_3$ ,  $(t\text{-Bu})_3\text{P}$  or  $\text{Ph}_2t\text{-BuP}$ ,  $\text{Na}_2\text{CO}_3$  or  $\text{Cs}_2\text{CO}_3$ ,  $\text{DMF}$ ,  $100\text{--}120^\circ\text{C}$ .

Table 1. Inhibition ( $\text{IC}_{50}$ ) of CDK4, CDK1, and CDK2 Activities

R	kinase assays ( $\text{IC}_{50}$ ) <sup>a</sup>		
	CDK4	CDK1	CDK2
<b>a</b> 4-Me-piperazinyl	4.1	>28	>28
<b>b</b> 4-morpholinyl	>29	>29	>29
<b>c</b> $\text{CH}_2\text{-}(4\text{-morpholinyl})$	10	>27	21
<b>d</b> $\text{CH}_2\text{-}(1\text{-piperidinyl})$	3.3	>27	19.6

<sup>a</sup> Concentration ( $\mu\text{M}$ ) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference.

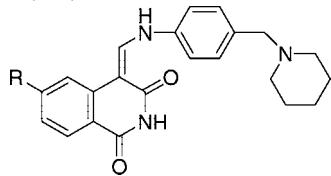
6-nitrile derivative **23i** was not only a poor inhibitor for CDK4, but also lost selectivity. Introducing a methoxy or an aniline group at C-6 on **5d** to yield **19d** and **21d** did not affect the potency. Replacement of the 6-phenyl group of **23a** with 3-pyridyl, as in derivative **23c**, resulted in enhanced potency. Further investigation was carried out by introducing substituents on the 6-phenyl ring of **23a**. The 6-(4-fluorophenyl) derivative **23e** was as potent as **23a** in inhibiting CDK4 activity. Addition of a hydroxyl substituent on the meta or para position of the 6-phenyl derivative **23a** yielded **23g** and **23h**, respectively, which interestingly showed a log order higher potency than the unsubstituted 6-phenyl derivative **23a**. This result suggests that the hydroxyl group may form a hydrogen bond interaction at the binding site of the CDK4 enzyme. Compared to the 6-(hydroxyphenyl) derivatives **23g** and **23h**, the corresponding

Table 2. Inhibition ( $\text{IC}_{50}$ ) of CDK4, CDK1, and CDK2 Activities

compd	R	kinase assays ( $\text{IC}_{50}$ ) <sup>a</sup>		
		CDK4	CDK1	CDK2
<b>5a</b>	H	4.1	>28	>28
<b>18a</b>	7-Br	12.1	>50	47
<b>18b</b>	6-Br	1.40	>50	>50
<b>18e</b>	6-NO <sub>2</sub>	11	7.2	>50
<b>18f</b>	6-I	0.48	>50	>50
<b>18g</b>	6-C(O)NMe <sub>2</sub>	41	>50	47
<b>18h</b>	6-pyrrolyl	0.14	>50	>50
<b>18i</b>	6-NHAc	11.0	13.8	>50
<b>20a</b>	6-piperidinyl	1.62	>50	>50
<b>22a</b>	6-phenyl	0.39	>50	>50
<b>22b</b>	6-(2-furyl)	0.33	>50	>50
<b>22c</b>	6-(3-furyl)	0.22	>50	>50
<b>22d</b>	6-(3-thienyl)	0.10	>50	>50
<b>22e</b>	6-[4-HC(O)-phenyl]	0.13	8.2	7.7
<b>22f</b>	6-(CH=CH-phenyl)	14.3		
<b>22g</b>	6-(C≡-phenyl)	2.30	33.2	>50

<sup>a</sup> Concentration ( $\mu\text{M}$ ) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference.

6-(methoxyphenyl) derivatives **23i** and **23j** were less potent. The *para*-methoxyphenyl derivative **23j** showed a 4-fold decrease in CDK4 potency compared to the corresponding *para*-hydroxyphenyl derivative **23h**. However, the *meta*-methoxyphenyl derivative **23j** lost its potency by 1000-fold with respect to the corresponding *meta*-hydroxyphenyl derivative **23g**. The latter

**Table 3.** Inhibition ( $IC_{50}$ ) of CDK4, CDK1, and CDK2 Activities

compd	R	kinase assays ( $IC_{50}$ ) <sup>a</sup>		
		CDK4	CDK1	CDK2
<b>5d</b>	H	3.3	>27	19.6
<b>19b</b>	Br	1.1	>50	>50
<b>19c</b>	Cl	2.5	>50	>50
<b>19d</b>	OMe	2	>50	>50
<b>19e</b>	NO <sub>2</sub>	15.1	>50	>50
<b>19f</b>	I	0.3	>50	20.4
<b>19g</b>	C(O)NMe <sub>2</sub>	21.2	>50	39.8
<b>19h</b>	pyrrolyl	0.14	>50	>50
<b>19i</b>	NHAc	3.5	>50	8.1
<b>21a</b>	piperidinyl	1.0	>50	>50
<b>21b</b>	morpholinyl	0.92	>50	>50
<b>21c</b>	4-Me-piperazinyl	34.7	>50	>50
<b>21d</b>	NH-Ph	1.8	>50	>50
<b>23a</b>	phenyl	0.32	>50	>50
<b>23b</b>	3-furyl	0.037	>50	>50
<b>23c</b>	3-pyridyl	0.05	>50	>50
<b>23d</b>	3-thienyl	0.13	>50	>50
<b>23e</b>	4-F-phenyl	0.31	>50	>50
<b>23f</b>	4-Cl-phenyl	19.4	>50	>50
<b>23g</b>	3-OH-phenyl	0.027	>50	>50
<b>23h</b>	4-OH-phenyl	0.041	>50	>50
<b>23i</b>	3-MeO-phenyl	27.8	>50	>50
<b>23j</b>	4-MeO-phenyl	0.13	>50	>50
<b>23k</b>	4-PhO-phenyl	>50	>50	>50
<b>23l</b>	CN	15.8	15.8	26.5

<sup>a</sup> Concentration ( $\mu$ M) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference.

result further supports the significance of the hydroxyl group as a participant in favorable electrostatic interaction with binding site of the CDK4 enzyme. This speculation was subsequently corroborated by a binding model for **23g** in the ATP binding site of a CDK4 mimic, which will be discussed in detail in the molecular modeling section. Although the 4-methoxy substituent is somewhat tolerated on the 6-phenyl, as shown in **23j**, the corresponding 6-(4-phenoxyphenyl) derivative **23k** lost inhibitory activity for all three CDKs, suggesting a space limitation between the inhibitor and the enzyme.

The compounds were also evaluated for their ability to inhibit the growth of selected cell lines, as shown in Table 4. Two human carcinoma cell lines were used: HCT116 (colon), which has deregulated cyclin D1 due to a mutation in the APC/ $\beta$ -catenin pathway that regulates the cyclin D promoter,<sup>18</sup> and MCF-7 (breast), which overexpresses cyclin D1 due to gene amplification.<sup>19</sup> Compared to the 6-halogen derivatives, the 6-aryl and 6-heteroaryl derivatives are among the slightly more effective inhibitors of cell growth. The magnitude of the enhancement of CDK4 inhibitory activity does not always correlate with the improvement of cellular activity. For example, compared to the 6-phenyl derivative **23a**, the 6-(hydroxyphenyl) derivatives **23g** and **23h** were a log order more potent CDK4 inhibitors, however, there was no significant improvement in cellular activity. On the other hand, the 6-(4-fluorophenyl) derivative **23e** was as potent as the 6-phenyl **23a** vs CDK4, yet the former showed 2- to 5-fold better potency than **23a** in inhibiting the proliferation of cells.

Attention was then focused on enhancing the aqueous solubility of **18f** by adding steric bulk on the piperazinyl ring

**Table 4.** Inhibition ( $IC_{50}$ ) of CDK4 Activity and Cell Proliferation

compd	R	kinase assay <sup>a</sup>		cell-based assays <sup>b</sup>	
		CDK4	HCT116	MCF-7	
<b>18b</b>	I	Br	1.4	3.1	3.0
<b>18f</b>	I	I	0.48	2.8	2.5
<b>18h</b>	I	pyrrolyl	0.14	1.5	2.3
<b>22a</b>	I	phenyl	0.39	2.8	7.3
<b>22c</b>	I	3-furyl	0.22	1.2	1.1
<b>22d</b>	I	3-thienyl	0.10	1.4	2.3
<b>19b</b>	II	Br	1.1	5	3.1
<b>19f</b>	II	I	0.3	2.7	1.0
<b>19h</b>	II	pyrrolyl	0.14	2.7	2.1
<b>21d</b>	II	NH-Ph	1.8	1.5	2.3
<b>23a</b>	II	phenyl	0.32	2.4	2.9
<b>23b</b>	II	3-furyl	0.037	1.9	1.1
<b>23d</b>	II	3-thienyl	0.05	1.0	3.4
<b>23e</b>	II	4-F-phenyl	0.31	1.0	0.55
<b>23g</b>	II	3-OH-phenyl	0.027	1.3	2.0
<b>23h</b>	II	4-OH-phenyl	0.041	1.6	3.0

<sup>a</sup> Concentration ( $\mu$ M) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference. <sup>b</sup> Dose-response curves were determined at five concentrations. The  $IC_{50}$  ( $\mu$ M) values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves.

of the headpiece to prevent stacking. Five piperazinyl derivatives with different degrees of steric bulk were prepared and evaluated, as shown in Table 5. Compared to **18f**, all five new analogues, **34–38**, showed 4- to 18-fold enhancement in aqueous solubility. However, four of these analogues, **35–38**, lost CDK4 activity by 3- to 10-fold, although they maintained good selectivity for CDK4 over CDK1 and CDK2. Only the bridged piperazinyl derivative **34** showed enzyme activity comparable to that of **18f**. It is interesting to note that, although **35** was 3-fold less potent than **18f** in inhibiting CDK4 activity, it showed a 3-fold enhancement in its ability to inhibit the proliferation of HCT116 cells.

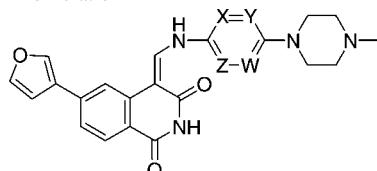
Replacements of the *p*-phenylene moiety in the *N*-methylpiperazinylphenyl headpiece of **22c** are shown in Table 6. Compared to **22c**, the fluorophenyl analogue **45d** was almost 3-fold more potent in inhibiting CDK4 activity. Replacement of the phenyl ring in the headgroup of **22c** with a pyridine, to provide **45a** and **45b**, resulted in a slight enhancement of the CDK4 inhibitory activity. Compared to **22c**, the pyrimidine derivative **45e** showed comparable CDK4 activity, but the pyrazine isomer **45f** was 6-fold less potent and the pyridazine isomer **45c** was 4-fold less potent in CDK4 inhibitory activity. These new derivatives **45a–c,e,f** bearing one or two nitrogens in the aryl ring did not display enhanced aqueous solubility at pH 7.4. Furthermore, no major improvement in their ability to inhibit cell proliferation was observed.

**Molecular Modeling.** A binding model for **23g** in the ATP binding site is presented in Figure 1. The protein coordinates used in this study are those reported in the X-ray crystal structure of the catalytic domain of CDK2, with three residues of the binding site mutated to mimic CDK4.<sup>20</sup> The CDK4 mimic structure of Ikuta et al., includes three mutations at residues F82H, L83V, and K89T. The resulting protein is a mutant of the CDK2 kinase domain that possesses the CDK2 amino acid sequence in all other regions except the ATP binding pocket,

**Table 5.** Inhibition ( $IC_{50}$ ) of CDKs Activities, Cell Proliferation, and Aqueous Solubility

compd	R	Kinase assays <sup>a</sup>			Cell-based assays <sup>b</sup>		Solubility (pH 7.4) μg/mL
		CDK4	CDK1	CDK2	HCT116	MCF-7	
18f		0.36	>50	>50	2.8	2.5	1.0
34		0.32	>50	>50	1.9	2.6	18
35		1.0	>50	>50	0.76	1.6	12
36		2.8	>50	>50	2.1	1.3	4
37		2.7	>50	>50	2	1.9	5
38		3.0	>50	>50	5.0	1.0	4

<sup>a</sup> Concentration ( $\mu$ M) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference. <sup>b</sup> Dose-response curves were determined at five concentrations. The  $IC_{50}$  ( $\mu$ M) values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves.

**Table 6.** Inhibition ( $IC_{50}$ ) of CDKs Activities and Cell Proliferation

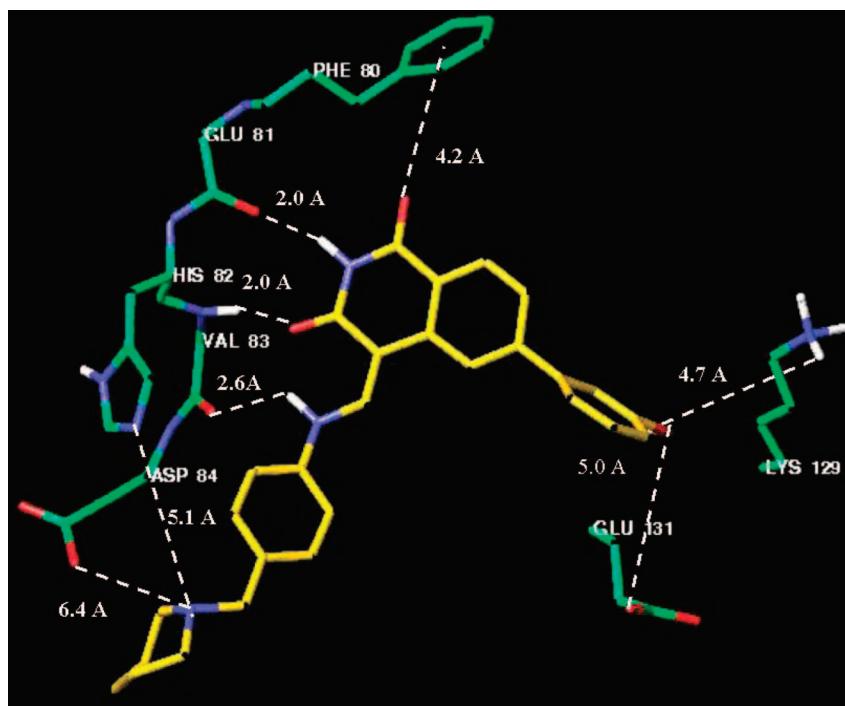
compd	X	Y	Z	W	kinase assays <sup>a</sup>			cell-based assays <sup>b</sup>	
					CDK4	CDK1	CDK2	HCT116	MCF-7
22c	CH	CH	CH	CH	0.22	>50	>50	1.2	1.1
45a	N	CH	CH	CH	0.13	>50	>50	0.76	1.6
45b	CH	N	CH	CH	0.11	>50	>50	2.1	1.7
45c	N	N	CH	CH	0.82	24.7	>50	1.9	4.5
45d	CH	CF	CH	CH	0.08	>50	>50	2.2	0.9
45e	CH	N	CH	N	0.25	31	>50	1.1	3.2
45f	CH	N	N	CH	1.25	2.2	>50	0.95	1.3

<sup>a</sup> Concentration ( $\mu$ M) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose-response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference. <sup>b</sup> Dose-response curves were determined at five concentrations. The  $IC_{50}$  ( $\mu$ M) values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves.

where the three amino acid mutations confer a sequence that more closely resembles that of CDK4. The amino acid numbering of the CDK4 mimic structure will therefore be that of CDK2, which is the parent structure. This CDK4 mimic structure was used by Ikuta et al.<sup>20</sup> to study CDK4 specific inhibitors. The inhibitor was docked using the GLIDE docking algorithm in the XP (extra precision) mode.<sup>21</sup> The resulting model successfully identifies key hydrogen bond interactions between the ligands and residues of the protein's ATP binding pocket. The specific orientation of the ligand in the binding model exploits the range of residues unique to CDK4, namely 82, 83, and 84. We do not see any interactions between the 4-(phenylaminom-

ethylene)isoquinoline-1,3(2H,4H)-dione inhibitors and Thr 89 in our binding models. A more detailed discussion on Thr 89 follows. Although the public domain CDK4 mimic structure used in this study does not include a CDK4 specific mutation at residue 84, a brief discussion of our in silico structure with residue 84 mutated to ASP follows.

In the most favored bound conformation, the NH of the isoquinoline-1,3-dione core is found 2.0 Å from the backbone carbonyl of Glu 81. The binding model places a carbonyl oxygen of the isoquinoline-1,3-dione core at 2.0 Å from the backbone NH of Val 83, a position in the binding cavity where the L83V mutation differentiates CDK4 from CDK2. Additionally, the



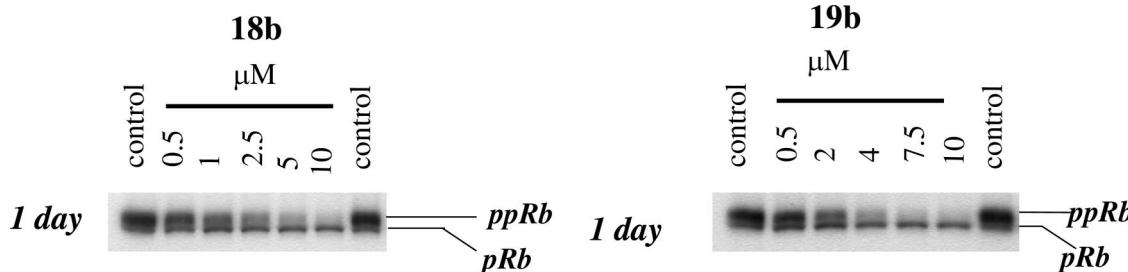
**Figure 1.** Proposed Binding Model for **23g** in the ATP binding site of the CDK4 mimic model.

amino NH of the enamine headpiece is found within 2.6 Å from the backbone carbonyl oxygen of Val 83. As the sequence of amino acids confers specific spatial features to the protein cavity, we postulate that the close proximity of these interactions lends support to the selectivity of the isoquinoline-1,3-dione core for the CDK4 binding pocket. A second carbonyl oxygen of the isoquinoline-1,3-dione core is found within 4.2–4.8 Å of the center of the phenyl ring of Phe 80. It is postulated that interactions between at least two of the aromatic hydrogens of the phenyl ring and the carbonyl oxygen of the isoquinoline-1,3-dione core play a significant role in supporting inhibitor binding. Two more residues were mutated in our computational studies, and this is evident in the structures of the protein complexed with **23g** (Figure 1). These were carried out to evaluate resulting models and possible protein–ligand interactions. The CDK4 mimic structure of Ikuta et al. contains a His 84, which is unique for CDK2. However, we mutated this residue to the Asp that is found at this position in CDK4. Similarly, residue 131 in the CDK4 mimic structure is Gln, which is a unique residue for CDK2, was mutated in our models to Glu, which is found in CDK4. In our models, Glu 131 is found to be approximately 5.0 Å from the hydroxy oxygen at the meta position of the 6-phenyl substituent in **23g** and the ring nitrogen of the piperidine headpiece of **23g** is found ~5 Å from the His 82 side chain and approximately 6.4 Å from the Asp 84 side chain. Both of these groups are found in solvent exposed regions, involving very flexible side chains. Short dynamics runs were carried out and they indicate a closer approach of these interacting pairs, however, the distances do not vary dramatically in our simulation runs. It is expected that the flexibility and the solvent exposed nature of these interactions will result in closer distances of approach between the respective interacting partners, and possibly water-mediated interactions.

Other researchers such as McInnes,<sup>22a</sup> Honma,<sup>22b</sup> and Aubry<sup>22c</sup> carried out computational studies using homology models of CDK4, produced using the known crystal structures of CDK2 and CDK6 as templates. As such, the amino acid

numbering of these homology models differs from those of the CDK4 mimic models used by our group, Ikuta<sup>20</sup> and Pratt,<sup>24</sup> by a value of 13 for the residues of the ATP binding site. Docking studies using CDK4 homology models identified interactions of inhibitors with the backbone NH and carbonyl group of Val 96 (Val 83 in our model) and the carbonyl of Glu 94 (Glu 81 in our model) along with the side chain of His 95 (His 82 in our model). As well, Aubry et al.<sup>22c</sup> observed  $\pi$ – $\pi$  interactions with the side chain of Phe 93 (Phe 80 in our model). A key finding of the study by McInnes et al.<sup>22a</sup> is the presence of a positively charged moiety proximal to the Asp and Glu residues that correspond to residues numbered 86 and 131 in the CDK2 structure used in our study. Although the 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione analogues of our study are not found to interact with Asp 86, our binding models place a piperidine moiety proximal to the His 82 and Asp 84 side chains and a hydroxy moiety proximal to the Glu 131 side chain; all these are residues that are unique to CDK4 and found in regions of the binding pocket where protein flexibility and the presence of solvent molecules are expected. While the Gln 131 of the CDK4 mimic structure was mutated to Glu 131 and short molecular dynamics simulation runs were carried out, we believe this is insufficient to realize any significant rearrangement resulting from protein–ligand binding. Park et al.<sup>23</sup> have shown that protein flexibility is crucial to inhibitor binding and that CDK4 undergoes significant conformational changes resulting from solvent and inhibitor interactions. We postulate that detailed molecular dynamics studies will identify the key role that Glu 131 plays in stabilizing protein–4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione binding.

The K89T mutation, which places a threonine at the entrance to the ATP binding cleft of CDK4, is singled out as a significant source of CDK2/CDK4 selectivity by several investigators.<sup>18,24</sup> Our binding studies, however, do not indicate any involvement of the threonine residue in ligand binding. Pratt et al.<sup>24</sup> have pointed to the K89T mutation as being the primary source of CDK4 selectivity for the bis-anilino-pyrimidine analogues. We



**Figure 2.** Effect of **18b** and **19b** on pRB phosphorylation in MCF-7 cells. MCF-7 cells were incubated in the presence of **18b** or **19b** at the indicated concentrations for 24 h at 37 °C. Protein extracts were analyzed by SDS-PAGE, followed by immunoblotting using Rb antibody.

point to intrinsic differences in the shapes of the 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione analogues, which result in optimal orientations in the CDK4 binding cavity that do not involve the threonine residue in question. Consequently, the 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione inhibitors of our study represent a class of molecules that form interactions with several CDK4 specific amino acids that are described in earlier literature, as well as with the side chains of His 82 and Asp 84, both of which are CDK4 specific amino acid residues proximal to the hinge region of the CDK4 catalytic domain.

We believe that the protein–ligand interactions detailed in our binding models characterize the selectivity and potency of the 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione analogues of this study with corroboration of several trends in the SAR. First, the interactions of the inhibitor core with the backbone of residues 81–83, and the presence of a basic nitrogen proximal to the side chains of His 82 and Asp 84, as shown in Figure 1, are fundamental to inhibitor potency. The R groups, listed in Table 3, that facilitate such an orientation (i.e., compounds **19f**, **23a**, **23c**, **23d**, **23g**, **23h**) result in increased potency. However, bulky R groups (i.e., compounds **23f**, **23k**) and R groups with undesirable moieties (i.e., compounds **19g**, **21c**) where steric hindrance or electrostatic incompatibility with residues such as Glu 131 and Lys 129, preclude the optimal alignment of the basic piperidinyl nitrogen. A similar trend is seen in Table 2, where R groups that facilitate the basic nitrogen of the piperizinyl moiety to orient proximally to His 82 and Asp 84 (i.e., compounds **18f**, **22a**, **22d**) render enhanced potency with excellent selectivity. In contrast, the best binding models obtained for compound **5b** of Table 1 place a morpholinyl oxygen proximal to the side chains of His 82 and Asp 84, where we see a significant drop in potency.

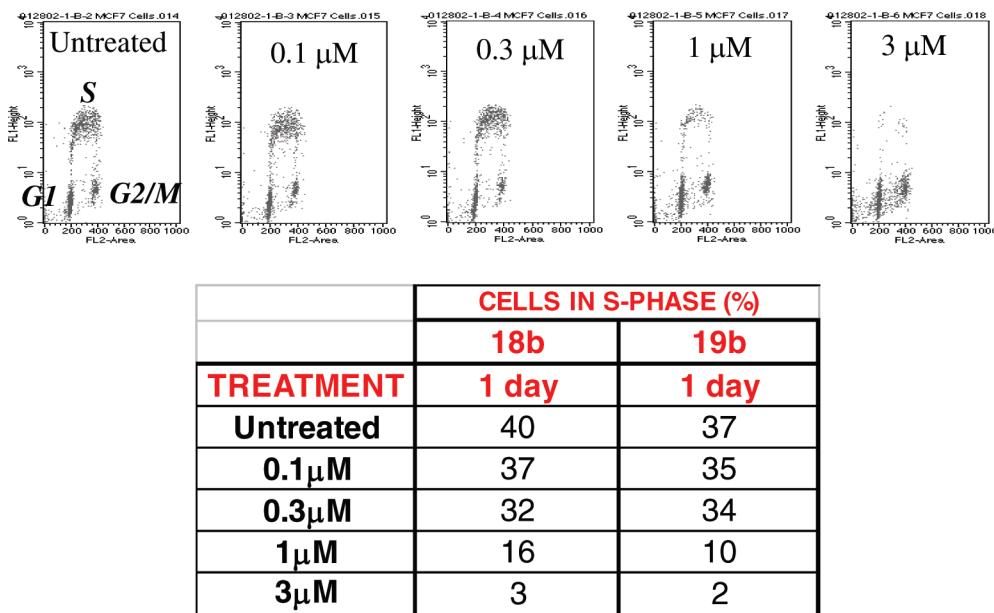
**Inhibition of pRB Phosphorylation and Cell Cycle Analysis.** The biological activity of **18b** and **19b** was evaluated in MCF-7 cells, which overexpress cyclin D1 due to gene amplification. We first examined the phosphorylation state of pRB, the physiological substrate for CDK4/cyclin D complexes, after overnight incubation with **18b** or **19b**. As shown in Figure 2, treatment of MCF-7 cells with either compound, resulted in a reduction of phosphorylation of pRB, as indicated by the decrease of the band corresponding to ppRB in polyacrylamide-SDS gels. Inhibition was clearly detected at the lowest concentration tested (0.5 μM) and increased in a concentration-dependent manner. This effect is observed at a concentration which is consistent with the effect of these compounds in the cellular proliferation assays ( $IC_{50}$  3 μM). Inhibition of pRB phosphorylation was also confirmed with a specific antibody against the phosphorylated Ser 807/811 in pRB (data not shown). Consistent with the effect of these compounds on inhibition of pRB phosphorylation, overnight treatment of MCF-7 cells with these inhibitors (**18b** and **19b**) caused a G1-

phase cell cycle arrest, as indicated by the decrease in the S-phase fraction in flow cytometry (Figure 3). Compared with untreated cells, the percentage of cells in S-phase decreased with increasing concentration of the inhibitors. Almost complete disappearance of S-phase cells (2–3% of total cells) was observed with 3 μM of each compound, corroborating the data from the pRB phosphorylation studies and the cell proliferation assays. No significant sub-G1 fraction was observed in these experiments, indicating that the primary effect of these compounds is cell cycle arrest rather than apoptosis. We next examined the effects of inhibiting CDK4 on other cell cycle regulatory proteins. Overnight treatment of MCF-7 cells with **19b** results in substantial decrease in cyclin D1 levels (85% at 7.5–10 μM). Smaller decreases were observed for CDK4 (60% at 7.5–10 μM), CDK2 (55% at 7.5 μM), cyclin B (60% at 4 μM), and CDK1 (40% at 7.5 μM), as shown in Figure 4. There appeared to be a slight overall increase (5–20%) in cyclin E levels at all concentrations >2 μM. This could result from compensatory effects of cyclin D1 loss. Alternatively, it may reflect arrest of the cells in late G1, when cyclin E levels begin to rise. Surprisingly, although the flow cytometric analyses indicate a cell cycle arrest at G1, a decrease in the cell cycle kinase inhibitor p27 was also observed (45% decrease at 7.5 μM). Taken together, the biological activities of these compounds are consistent with the expected effects of CDK4 inhibition.

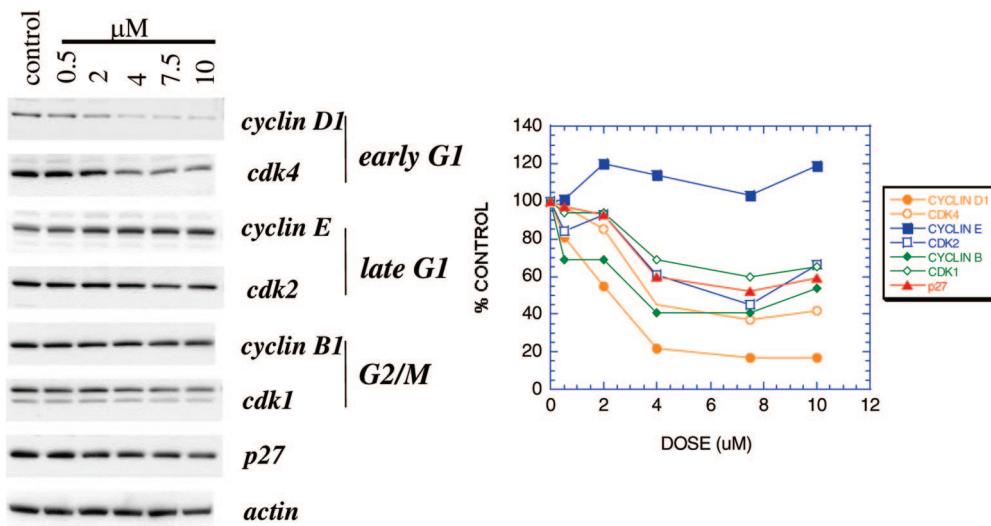
## Conclusions

In summary, we reported the discovery of 4-(phenylaminomethylene)isoquinoline-1,3(2H,4H)-dione derivatives as a novel class of potent inhibitors that selectively inhibit CDK4 over CDK2 and CDK1. We have shown that attaching a basic functional group onto the 4-(phenylaminomethylene) headpiece is essential for CDK4 inhibition. Systematic addition of a variety of substituents on the C-6 and C-7 positions of the isoquinoline-1,3(2H,4H)-dione led us to identify **23g**, carrying a 3-hydroxyphenyl substituent at C-6, that is 122-fold more potent than the corresponding C-6 unsubstituted compound **5d**. We also presented a proposed binding model for **23g** at the ATP binding site of a CDK4 mimic structure that contains three mutations at residues F82H, L83V, and K89T with two additional in silico mutations at H84D and Q131E. On the basis of this binding model, several hydrogen bond interactions were postulated between the NH and carbonyl oxygen of the core, the NH of the enamine headpiece, the basic amine on the aniline ring of the 4-(phenylaminomethylene) headpiece and the amino acid residues that are unique to the CDK4 enzyme. These interactions could explain the high selectivity of this series of compounds for CDK4 over CDK2 and CDK1. In cells, selected compounds **18b** and **19b** from this series have demonstrated biological activity: they inhibit phosphorylation of pRB, the physiological substrate for CDK4/cyclin D1 and cause cell cycle arrest.

### Treatment with 19b



**Figure 3.** Effect of **18b** and **19b** on cell cycle progression in MCF-7 cells. MCF-7 cells were treated with the indicated concentrations of **18b** or **19b** for 24 h at 37 °C. Untreated and treated cells were pulse-labeled for 30 min with BrDU. Cells were stained with BrDU antibodies/FITC conjugates, counterstained with propidium iodide and analyzed by flow cytometry. The percentage of cells in S-phase of the cell cycle is shown as determined from the histogram of propidium iodide-stained cells.



**Figure 4.** Effect of **19b** on cell cycle regulatory proteins. MCF-7 cells were incubated in the absence or presence of **19b** at the indicated doses for 24 h at 37 °C. Protein extracts were analyzed by SDS-PAGE, followed by immunoblotting using the indicated antibodies. Equivalent protein loading was confirmed using actin antibodies.

Consistent with these effects, the compounds inhibit the proliferation of cells in vitro. Further refinement of these series of compounds is required to demonstrate antitumor activity in animal tumor models.

### Experimental Section

**Chemistry.**  $^1\text{H}$  NMR spectra were determined with a Bruker DRX400 spectrometer at 400 MHz or a NT-300 WB spectrometer at 300 MHz. Chemical shifts ( $\delta$ ) are expressed in parts per million relative to the internal standard tetramethylsilane. Electrospray mass spectra were recorded in positive mode on a Micromass Platform spectrometer. Electron impact (EI) and high-resolution mass spectra (HRMS) were obtained on a Finnigan MAT-90 spectrometer. Some high-resolution electrospray mass spectra with higher precision were obtained on a Brucker 9.4T FTMS spectrometer. Chromatographic

purifications were by flash chromatography using Baker 40  $\mu$  silica gel. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. Semipreparative reverse-phase high-pressure liquid chromatography (RP-HPLC) was performed using a Gilson Preparatory HPLC. The sample was dissolved in DMSO, applied on a Phenomenex C18 Luna column (21.2 mm  $\times$  60 mm, 5  $\mu$ ), and eluted at 22.5 mL/min with a 19 min of gradient: 5% B; 2.5 min: 5% B; 18 min: 95% B; 19 min: 95% B, where solvent A = water (0.02% TFA buffer) and solvent B = acetonitrile (0.02% TFA buffer). Analytical high-pressure liquid chromatography (HPLC) and LC-MS analyses were conducted using the following two instruments and conditions. LCMS: analytical HPLC was conducted on an HP 1100 liquid chromatography system over a 2.1 mm  $\times$  30 mm xterra C18 Luna column (5  $\mu$ ) at 50 °C using multiple wavelength UV detection (typically 215,

254 nm) and MS detection (API-ES scanning mode positive/negative 100–1000, fragmentor: positive 140 mV, negative 170 mV). A gradient elution of increasing concentrations of acetonitrile in water containing 0.02% formic acid (10–90% over 3.5 min, and was held at 90% for additional 1.5 min) and a flow rate of 1 mL/min were employed. LCMS2: Analytical HPLC was conducted on a Agilent LC-1100-MSD liquid chromatography system over a 50 mm × 2.1 mm Aquasil C18 column (Thermo Electron Corporation, 5  $\mu$ ) using multiple wavelength UV detection (typically 215, 254 nm) and MS detection (single quadrupole mass filter scanning from 100–1000 Da). A gradient elution of increasing concentrations of acetonitrile in water containing 0.1% formic acid (0–100% over 2.5 min, and was held at 100% for additional 1.5 min) and a flow rate of 1 mL/min were employed.

**Molecular Modeling.** The inhibitor structures were minimized using the MMFF94 force field of SYBYL.<sup>25</sup> A public domain structure of CDK4<sup>20</sup> was used as the 3-D representation for molecular docking studies. This is a CDK4 mimic structure with the mutations in the ATP binding cleft at residues F82H, L83V, and K89T. The inhibitor structures were docked using the GLIDE<sup>21</sup> docking algorithm in the XP (extra precision) mode. Details of the algorithm are found in GLIDE documentation. Briefly, GLIDE's proprietary conformational expansion and exhaustive search of the binding site produces a multitude of ligand poses that undergo an initial refinement, energy minimization on a precomputed grid, and a final scoring and ranking. GLIDE uses proprietary scoring functions that are variations of the ChemScore<sup>26</sup> empirical scoring function and the OPLS-AA<sup>27</sup> force field to compute van der Waals and electrostatic grids for the receptor. The final ligand binding poses are ranked according to a computed E model score that encompasses the grid score, the proprietary Glide Score, and the internal energy strain. The HIS to ASP mutation at position 84 and the GLN to GLU mutation at position 131 were carried out using the GLIDE software package. The molecular dynamics simulations mentioned above were carried out using the SYBYL software package and the MMFF94 force field.

**Preparation of the Substituted Cores. 4-Bromo-2-(carboxymethyl)benzoic acid (11b).** In a 500 mL 3-neck round-bottom flask, an amount of diisopropylamine (28.0 mL, 200 mmol) in 65 mL of tetrahydrofuran was cooled to –78 °C and slowly added 80.0 mL (200 mmol) of *n*-butyllithium (2.5 M in hexane) with vigorous stirring. Allow to warming up to 0 °C and keeping at this temperature for 5 min, the reaction was cooled back to –78 °C. To this mixture was slowly added a solution of 10.8 g (50.0 mmol) of 4-bromo-2-methylbenzoic acid (**9b**) and 8.42 mL (100 mmol) of dimethylcarbonate in 65 mL of tetrahydrofuran keeping the internal temperature of the reaction mixture below –50 °C. After addition, the dry ice bath was removed and the reaction mixture was stirred at room temperature for 4 h. Precipitate was observed as the internal temperature raising to room temperature. The reaction was quenched with 80 mL of water and stirred overnight to give a homogeneous solution. The organic layer was separated, and the aqueous solution was acidified with concentrated HCl to pH ~ 2 and extracted with 3 × 100 mL of ethyl acetate. The combined organic solution was washed twice with water. After drying over MgSO<sub>4</sub>, the organic solution was filtered and concentrated to give a white solid. Recrystallization from EtOAc (hot)/hexane yielded 8.86 g (68.2%) of white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 12.62 (bs, 1H); 7.82 (d, *J* = 6.3 Hz, 1H), 6.18 (m, 2H), 3.95 (s, 2H). MS (ESI) *m/z* 257.1 and 259.1 (M – H)<sup>–1</sup>. Anal. (C<sub>9</sub>H<sub>7</sub>BrO<sub>4</sub>•0.2 EtOAc) C, H.

**6-Bromoisoquinoline-1,3(2H,4H)-dione (12b).** A suspension of **11b** (343 g, 1.324 mol) and urea (171.5 g, 2.858 mol) in 1,2-dichlorobenzene (3 L) was stirred and heated. The starting material went into solution at 115 °C and urea melted at 120 °C. The product started to precipitate as a yellow solid at 135 °C. The reaction mixture was heated at 150 °C for 2.5 h. After it was cooled to room temperature, the yellow solid was collected by filtration, washed with ethyl acetate, water, and methanol and air-dried to give 271.6 g (85.5%) of **12b** as a solid. (ESI) *m/z* 237.9 (M – H)<sup>–1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 4.04 (s, 2H), 7.65

(d, *J* = 8.1 Hz, 1H), 7.66 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 1H) 11.37 (s, 1H). Anal. (C<sub>9</sub>H<sub>6</sub>BrNO<sub>2</sub>) C, H, N.

**(4E)-6-Bromo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (17b).** A solution of **12b** (4.15 g, 7.29 mmol), 3.78 mL (34.55 mmol) of trimethyl orthoformate, and 30 mL of acetic anhydride in 10 mL of *N,N'*-dimethylformamide was heated at 120 °C under N<sub>2</sub> for 2 h. After cooling, ethyl ether was added, and the precipitate was collected and washed successively with MeOH, Et<sub>2</sub>O, and hexane. After drying in oven (60 °C) overnight, 3.4 g (70% yield) of **17b** was obtained as a solid. MS (ESI) *m/z* 281.9 (M + H)<sup>–1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 4.26 (s, 3H), 7.62 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 8.08 (s, 1H), 8.38 (d, *J* = 2.2 Hz, 1H), 11.40 (s, 1H). Anal. (C<sub>11</sub>H<sub>8</sub>BrNO<sub>3</sub>) C, H, N.

**6-Bromo-1,3-bis[*tert*-butyl(dimethylsilyl)oxy]isoquinoline (15).** A solution of **12b** (1.0 g, 4.17 mmol), 1.875 g (12.51 mmol) of *tert*-butyldimethylsilyl chloride, and 1.13 g (16.68 mmol) of imidazole in *N,N'*-dimethylformamide was stirred at room temperature overnight. After evaporating to dryness, the brown oil was extracted with 4 × 100 mL of 25% diethyl ether/hexane. The organic solution was washed with 3 × 100 mL of water, dried over MgSO<sub>4</sub>, filtered, and concentrated to give a brown oil. This brown oil was dissolved in 50 mL of 20% CH<sub>2</sub>Cl<sub>2</sub>/hexane and passed through a pad of magnesol, followed by rinsing with 500 mL of the same solvent mixture. The organic solution was concentrated to give 1.184 g (60.6%) of **15** as a colorless solid. MS (ESI) *m/z* 468.2 and 470.2 (M + H)<sup>–1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 0.31 (s, 6H), 0.42 (s, 6H), 1.00 (s, 9H), 1.07 (s, 9H), 6.46 (s, 1H), 7.33 (dd, *J* = 8.81, 1.76 Hz, 1H), 7.71 (d, *J* = 1.76 Hz, 1H), 7.94 (d, *J* = 8.81 Hz, 1H). Anal. (C<sub>21</sub>H<sub>34</sub>BrNO<sub>2</sub>Si<sub>2</sub>) C, H, N.

**6-Iodoisoquinoline-1,3(2H,4H)-dione (12f).** An amount of **15** (15.0 g, 32.0 mmol) in 100 mL of anhydrous tetrahydrofuran was cooled to –78 °C and then 47 mL (80.0 mmol) of *tert*-butyllithium (1.7 M in pentane) was added slowly with stirring. After stirring at this temperature for 2 h, 12.0 g (48 mmol) of fresh iodine crystal was quickly added into the mixture and stirred at this temperature for additional 5 h. The dry ice bath was removed, and the reaction mixture was allowed to warm up to room temperature and stirred over a weekend. Evaporating the brown solution yielded a brown oil. The reaction mixture was acidified with 48 mL of 2 M HCl solution and stirred at room temperature for 1 h. The mixture was filtered to give a light-tan solid. The solid was dissolved in hot DMSO, and then 20% MeOH/H<sub>2</sub>O solution was added to give a precipitate. The precipitate was collected and washed successively with water, methanol, ether, and hexane to afford 5.1 g (55.6%) of **12f** as an off-white solid. MS (ESI) *m/z* 286.08 (M – H)<sup>–1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 4.01 (s, 2H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.84 (s, 1H), 11.35 (s, 1H).

**6-(*N,N*-Dimethylcarbamyl)isoquinoline-1,3(2H,4H)-dione (12g).** An amount of **15** (0.5 g, 1.07 mmol) in 10 mL of anhydrous tetrahydrofuran was cooled to –78 °C and then 1.42 mL (2.41 mmol) of *tert*-butyllithium (1.7 M in pentane) was added slowly with stirring. After stirring at this temperature for 30 min, 0.118 mL (1.28 mmol) of *N,N*-dimethylcarbamyl chloride was added and stirred at this temperature for 45 min. After it was warmed up to room temperature, 1.6 mL (3.2 mmol) of 2 M aqueous HCl solution was added and stirred for 3 h. The solvents were removed, and the resulting gum was purified by column chromatography eluted with 5% methanol in chloroform. The desired fractions were pooled and dried to give 42 mg (57%) of **12g** as a yellow solid. MS (ESI) *m/z* 233.2 (M + H)<sup>–1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.87 (s, 3H), 2.98 (s, 3H), 4.06 (s, 2H), 7.40 (s, 1H), 7.45 (d, *J* = 6.9 Hz, 1H), 8.05 (d, *J* = 6.9 Hz, 1H), 8.05 (s, 1H), 11.38 (s, 1H).

**2-Carboxy-5-nitrobenzeneacetamide (14).** A stirred mixture of 2-carboxy-5-nitrobenzeneacetic acid, **11e** (2.25 g, 10 mmol) (prepared from 2-chloro-4-nitrobenzoic acid, **10** according to literature procedure<sup>28</sup>), 2.5 mL (35 mmol) of acetyl chloride, and 8 mL of acetone was refluxed for 60 min. The resulting solution was evaporated to dryness. The resulting tan solid was shown to be the corresponding cyclic anhydride, **13**, by <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (singlet of two protons at  $\delta$  4.40). The anhydride **13** was mixed at 0 °C with 16 mL of conc NH<sub>4</sub>OH and 16 mL of H<sub>2</sub>O. The

resulting mixture was warmed to 25 °C, stirred 15 min, and evaporated to dryness at <30 °C. The residue was stirred in 25 mL of H<sub>2</sub>O, acidified at 10 °C with 4 mL of 4 N HCl, and stirred 10 min. The resulting tan solid was filtered, washed with H<sub>2</sub>O, and dried to give 2.01 g (90%) of **14** as a solid: mp 185–190 °C (dec). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ ppm: 13.50 (s, 1H), 8.20 (d, *J* = 2.4, 1H), 8.16 (dd, *J* = 2.4, 8.4, 1H), 8.02 (d, *J* = 8.4, 1H), 7.47 (s, 1H), 6.96 (s, 1H), 3.96 (s, 2H). MS (ES<sup>−</sup>) *m/z* 223.1 (M − H)<sup>−1</sup>. Anal. (C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

**6-Nitroisoquinoline-1,3(2H,4H)-dione (12e).** A stirred suspension of **14** (11.1 g, 49.3 mmol) in 99 mL of 1,2-dichlorobenzene was refluxed for 3 h. The residue obtained after evaporation of the solvent under vacuum was washed with ether and dried to give 7.34 g (72%) of **12e** as a tan solid: mp 255–260 °C (dec). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.6 (s, 1H), 8.2–8.3 (m, 3H), 4.17 (s, 2H). MS (ES<sup>−</sup>) *m/z* 205.2 (M − H)<sup>−1</sup>.

**(4E)-4-(Methoxymethylene)-6-nitroisoquinoline-1,3(2H,4H)-dione (17e).** To a stirred mixture of **12e** (0.41 g, 2.0 mmol), 3.2 mL (34 mmol) of acetic anhydride, and 0.80 mL of DMF was added trimethyl orthoformate (0.44 mL, 4.0 mmol). The mixture was heated to 125 °C and maintained for 30 min, cooled, diluted with ether, and stirred for 10 min. The resulting brown solid was filtered, washed with ether, and dried to give 372 mg (74%) of **17e** as a solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.55 (s, 1H), 8.99 (d, *J* = 2.0, 1H), 8.30 (d, *J* = 8.6, 1H), 8.19 (dd, *J* = 2.0, 8.6, 1H), 4.33 (s, 3H).

**6-Aminoisoquinoline-1,3(2H,4H)-dione (16).** A solution of **12e** (6.19 g, 30 mmol) in 15 mL of MeOH and 150 mL of DMF was hydrogenated at 1 atm of H<sub>2</sub> at 25 °C in the presence of 1.5 g of 10%Pd/C for 7 h. The catalyst was removed by filtration through a pad of Celite. The filtrate was evaporated to give 5.4 g (100%) of **16** as a tan solid: mp 200–220 °C (dec). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 10.75 (s, 1H), 7.66 (d, *J* = 8.8, 1H), 6.55 (dd, *J* = 2.1, 8.8, 1H), 6.36 (d, *J* = 2.1, 1H), 6.09 (s, 2H), 3.82 (s, 2H). MS (ES<sup>+</sup>) *m/z* 177.2 (M + H)<sup>+</sup>. Anal. (C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**6-Pyrrol-1-yl-isoquinoline-1,3(2H,4H)-dione (12h).** A solution of **16** (1 g, 5.676 mmol), 2,4-dimethoxytetrahydofuran (0.736 mL, 5.676 mmol), 4-chloropyridine hydrochloride (0.417 g, 2.78 mmol) in 20 mL of dioxane was heated at 70 °C for 2 h. After filtration, the filtrate was evaporated to dryness. The residue was washed with ether, filtered, and washed with water, ether, and hexane to give 0.86 g (67%) of **12h** as a light-brown solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 4.06 (s, 2H), 6.33 (m, 2H), 7.51 (m, 2H), 7.66 (d, s, 1H), 7.71 (dd, *J* = 8.3, 2.1 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 1H), 11.28 (s, 1H). MS (ES<sup>+</sup>) *m/z* 227.1 (M + H)<sup>+</sup>. Anal. (C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>·0.4 H<sub>2</sub>O) C, H, N.

**N-(1,3-Dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl)acetamide (12i).** A solution of 500 mg (2.8 mmol) of **16** in 2 mL of *N,N*-dimethylacetamide at 0 °C was dropwise added to 1.1 mL (14.2 mmol) of acetyl chloride and 1.1 mL (14.2 mmol). After 0.5 h, it was warm up to room temperature. After the solvents were removed, the solid was isolated and washed with ether to give 450 mg (73%) of **12i** as a green solid. MS (ESI) *m/z* 217.1 (M-1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 3.65 (s, 3H), 3.81 (s, 3H), 4.66 (d, *J* = 6.30 Hz, 2H), 7.15–7.43 (m, 3H), 7.70 (d, *J* = 8.56 Hz, 1H), 7.87 (d, *J* = 8.31 Hz, 1H), 8.10 (d, *J* = 1.51 Hz, 1H), 8.71 (d, *J* = 13.35 Hz, 1H), 9.37 (s, 1H), 10.53–10.76 (m, 1H), 11.10 (s, 1H).

**Preparation of the Headgroups. 4-(N-Nitro-benzyl)-morpholine (7c).** An amount of 10 g (46.30 mmol) of 1-bromomethyl-4-nitro-benzene (**8**) was stirred in dichloromethane (125 mL), followed by addition of triethylamine (12.90 mL, 92.6 mmol) and morpholine (4.03 g, 46.30 mmol). The reaction mixture was refluxed for 1 h, subsequently washed three times with aqueous sodium bicarbonate, dried over sodium sulfate, followed by evaporation to dryness, to give 7.5 g (73%) of **7c** as white crystals.

**4-Mopholin-4-yl-methyl-phenylamine (4c).** Seven g (31.52 mmol) of **7c**, ammonium chloride (15.14 g, 283.68 mmol), and iron (10.56 g, 189.12 mmol) were added to 266 mL of methanol/water (4.75:1) and refluxed until there was no appearance of starting materials. After filtering through celite, the solvent was evaporated. The residue was dissolved in water, basified with potassium

carbonate, and extracted three times with ethyl acetate. The organic solution was dried with magnesium sulfate and evaporated to afford 6 g (99%) of **4c** as an orange solid.

**3,5-Dimethyl-1-(4-nitrophenyl)piperazine (28).** An amount 4-nitrophenylfluoride, **6** (2.0 mL, 18.85 mmol), in 20 mL of acetonitrile was added to 2,6-dimethylpiperazine (2.58 g, 22.62 mmol). The reaction mixture was reflux under N<sub>2</sub> overnight. Mass spectroscopy suggested the completion of reaction. Solvent was subsequently evaporated under vacuum. The collected yellow solid was dissolved in chloroform and washed twice with 200 mL of saturated NaHCO<sub>3</sub> solution and once with 100 mL of brine. The organic portion was dried over MgSO<sub>4</sub>, filtered, evaporated to give yellow solid, which was recrystallized from EtOAc/hexane to give 3.98 g (89.8%) of **28** as bright-yellow crystals: mp 124–125 °C. MS (ESI) *m/z* 236.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 1.03 (d, *J* = 6.04 Hz, 6H) 2.30 (s, 1H) 2.34–2.45 (m, 2H) 2.75 (d, *J* = 3.02 Hz, 2H) 3.89 (dd, *J* = 12.34, 2.01 Hz, 2H) 7.02 (d, *J* = 9.57 Hz, 2H) 8.03 (d, *J* = 9.32 Hz, 2H). Anal. (C<sub>12</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**[4-(3,5-Dimethylpiperazin-1-yl)phenyl]amine (30).** An amount of **28** (1.86 g, 7.90 mmol) in EtOH was hydrogenated with Pd/C catalyst for 4 h. The solid was removed by filtration, and solvent was evaporated under vacuum to give a pinkish residue. Recrystallization of this residue from MeOH/ether gives 1.30 g (80.2%) of **30** as pinkish crystals: mp 124–125 °C. MS (ESI) *m/z* 206.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 1.12 (d, *J* = 6.55 Hz, 6H) 2.21 (t, *J* = 10.83 Hz, 3H) 2.86–3.20 (m, 3H) 3.32 (dd, *J* = 11.83, 2.27 Hz, 3H) 6.56–6.69 (m, 2H) 6.75–6.91 (m, 2H). Anal. (C<sub>12</sub>H<sub>19</sub>N<sub>3</sub>) C, H, N.

**1-Methyl-4-(5-nitro-pyridin-2-yl)-piperazine (40b).** An amount of 2 g (9.85 mmol) of 2-bromo-5-nitro-pyridine (**39b**) was stirred in dichloromethane (50 mL), followed by addition of 1-methylpiperazine (10.9 mL, 98.5 mmol). The reaction mixture was refluxed for 1 h. After cooling, the mixture was washed with sodium bicarbonate, followed by additional washing with brine, dried over sodium sulfate, filtered, and evaporated to afford 2 g (91%) of **40b** as yellow crystals: mp 75–76 °C. MS (ESI) *m/z* 223.1 (M + 1)<sup>+</sup>.

**6-(4-Methylpiperazin-1-yl)pyridin-3-amine (41b).** An amount of 1 g (4.48 mmol) of **40b** was dissolved in methanol (50 mL), followed by catalytic amount of 10% Pd/C. The mixture was hydrogenated at 35–40 psi for 3 h. After filtration through a pad of Celite, the solution was evaporated to give 900 mg (100%) of **41b** as a purple solid: mp 97–98 °C. MS (ESI) *m/z* 193.1 (M + 1)<sup>+</sup>.

**[5-(4-Methylpiperazin-1-yl)pyridin-2-yl]amine (41a).** Using the procedure described for the preparation of **41b**, 2.6 g (90%) of **41a** was obtained as a purple solid from 3.4 g (15.3 mmol) of 1-methyl-4-(2-nitro-pyridin-2-yl)-piperazine (**40a**). MS (ESI) *m/z* 193.1 (M + 1)<sup>+</sup>.

**[6-(4-Methylpiperazin-1-yl)pyridazin-3-yl]amine (41c).** An amount of 1.0 g (7.72 mmol) of 3-amino-6-chloropyridazine (**39c**), 5-chloro pyridine hydrogen chloride (4.46 g, 38.6 mmol), and *N*-Me-piperazine (5.1 mL, 72 mmol) were placed in a preheated oil bath at 165–170 °C for 4 h. After cooling, the mixture was basified with saturated potassium carbonate, extracted with ethyl acetate, and dried over magnesium sulfate. The oily residue was purified by column chromatography to give 800 mg (53%) of **41c** as a brown solid. MS (ESI) *m/z* 194.3 (M + 1)<sup>+</sup>.

**1-(2-Fluoro-4-nitrophenyl)-4-methylpiperazine (40d).** Using the procedure described for the preparation of **40b**, 4.89 g (45%) of **40d** as a light-brown solid was obtained from 5 mL (45.16 mmol) of 3,4-difluoronitrobenzene (**39d**) and 5 mL (45.16 mmol) of *N*-methyl-piperazine in 20 mL of dichloromethane and 3 equivalent of triethylamine: mp 69–70 °C. MS (ESI) *m/z* 240.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 2.37 (s, 3H) 2.48–2.81 (m, 4H) 3.20–3.44 (m, 4H) 6.92 (t, *J* = 8.81 Hz, 1H) 7.90 (dd, *J* = 13.22, 2.64 Hz, 1H) 7.98 (dd, *J* = 9.06, 1.01 Hz, 1H).

**[3-Fluoro-4-(4-methylpiperazin-1-yl)phenyl]amine (41d).** Using the procedure described for the preparation of **41b**, 3.8 g (90.5%) of **41d** was obtained as a colorless solid from 4.8 g (20.06 mmol) of **40d**: mp 89–90 °C. MS (ESI) *m/z* 210.1 (M + H)<sup>+</sup>. <sup>1</sup>H

NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.35 (s, 3H) 2.59 (s, 4H) 3.01 (s, 4H) 3.54 (s, 2H) 6.19–6.53 (m, 2H) 6.81 (t,  $J$  = 9.06 Hz, 1H).

**[2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl]amine (41e).** By the procedure described above for **41b**, 2-(4-methyl-piperazin-1-yl)-5-nitro-pyrimidine<sup>29</sup> (**40e**) (0.446 g, 2 mmol) was reduced in the presence of iron (0.432 g, 7.7 mmol), acetic acid (0.884 mL), and methanol (8 mL) to give 0.3 g (78%) of **41e**. MS (ESI)  $m/z$  194.1 (M + 1)<sup>+</sup>.

**[5-(4-Methylpiperazin-1-yl)pyrazin-2-yl]amine (41f).** An amount of 3.0 g (17.24 mmol) of 2-amino-5-bromopyrazine (**42**) was dissolved in *N,N*-dimethylformamide (20 mL) and cooled to 0 °C. Sodium hydride (1.05 g, 43.09 mmol) was added and stirred at 0 °C for 10 min. Acetyl chloride (6.2 mL, 86.2 mmol) was added and stirred at room temperature overnight. The mixture was quenched with water and extracted with ether. The organic layer was dried over magnesium sulfate, dried, and purified by column chromatography to give 1.35 g (31%) of *N*-acetyl-*N*-(5-bromo-pyrazin-2-yl)-acetamide as a yellow oil. MS (ESI)  $m/z$  259.0 (M + 1)<sup>+</sup>. A mixture of *N*-acetyl-*N*-(5-bromo-pyrazin-2-yl)-acetamide (1.33 g, 5.14 mmol), *N*-methylpiperazine (2.9 mL, 25.7 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (470 mg, 0.1 mmol), (t-Bu)<sub>3</sub>P (1 mL, 10.28 mmol), KO-t-Bu (1.15 g, 10.28 mmol) in 10 mL of DMF was heated at 120 °C in a microwave for 20 min. After it was filtered, it was dried and washed with ether to give 500 mg (35%) of *N*-acetyl-*N*-(4-methyl-3,4,5,6-tetrahydro-2*H*-[1,2']bipyrazinyl-5'-yl)-acetamide (**43**). Then **43** (500 mg, 1.8 mmol) was stirred with 6 mL of conc hydrochloric acid at room temperature overnight. It was basified with potassium carbonate and extracted with methylene chloride. It was dried to give 200 mg (57%) of **41f** as a dark oil. MS (ESI)  $m/z$  195.1 (M + 1)<sup>+</sup>.

**Preparation of the Final Compounds. (4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (5a).** A mixture of 4-methoxymethylene-4*H*-isoquinoline-1,3-dione (**3**) (101.5 mg, 0.5 mmol) and 4-(4-methyl-piperazin-1-yl)-phenylamine (**4a**) (95.6 mg, 0.5 mmol) in 1 mL of dimethylformamide was heated at 110 °C for 1 h. After cooling in the refrigerator, the precipitate was collected, and washed with ether to give 163 mg (90%) of **5a** as a yellow solid: mp 245–246 °C. MS (ESI)  $m/z$  363.19 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.22 (s, 3H), 2.39–2.49 (m, 4H), 3.02–3.18 (m, 4H), 6.99 (d,  $J$  = 9.1 Hz, 2H), 7.24 (m, 1H), 7.43 (d,  $J$  = 9.1 Hz, 2H), 7.55–7.66 (m, 1H), 8.02 (dd,  $J$  = 8.1, 1.26 Hz, 1H), 8.13 (d,  $J$  = 8.31 Hz, 1H), 8.81 (d,  $J$  = 12.8 Hz, 1H), 11.25 (s, 1H), 12.47 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

**(4Z)-4-{[4-(4-Morpholin-4-ylphenyl)amino]methylene}isoquinoline-1,3(2H,4H)-dione (5b).** By the procedure described above for **5a**, **3** (101.5 mg, 0.5 mmol) and 4-morpholin-4-yl-phenylamine (**4b**) (89.12 mg, 0.5 mmol) were reacted to give 139 mg (80%) of **5b** as a greenish-yellow solid: mp 257–258 °C. MS (ESI)  $m/z$  350.17 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.97–3.21 (m, 4H), 3.66–3.86 (m, 4H), 7.01 (d,  $J$  = 9.0 Hz, 2H), 7.25 (m, 1H), 7.46 (d,  $J$  = 9.0 Hz, 2H), 7.49–7.79 (m, 1H), 8.02 (dd,  $J$  = 7.9, 1.1 Hz, 1H), 8.13 (d,  $J$  = 8.3 Hz, 1H), 8.82 (d,  $J$  = 12.8 Hz, 1H) 11.24 (s, 1H), 12.46 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>) C, H, N.

**(4Z)-4-{[4-(Morpholin-4-ylmethyl)phenyl]amino}methylene-isoquinoline-1,3(2H,4H)-dione (5c).** By the procedure described above for **5a**, **3** (300 mg, 1.47 mmol) and **4c** (283.5 mg, 1.47 mmol) were reacted to give 283.5 mg (1.47 mmol) of **5c** as a yellow solid: mp 221–222 °C. MS (ESI)  $m/z$  463.1 (M – 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.35 (m, 4H), 3.36 (s, 2H), 3.57 (m, 4H), 7.28 (m, 1H), 7.35 (d,  $J$  = 8.3 Hz, 2H), 7.52 (d,  $J$  = 8.3 Hz, 2H), 7.62 (m, 1H), 8.06 (d,  $J$  = 7.1 Hz, 1H), 8.16 (d,  $J$  = 8.3 Hz, 1H), 8.89 (d,  $J$  = 12.5 Hz, 1H), 11.32 (s, 1H), 12.41 (d,  $J$  = 12.5 Hz, 1H). Anal. (C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>•0.3H<sub>2</sub>O) C, H, N.

**(4Z)-4-{[4-(Piperidin-1-ylmethyl)phenyl]amino}methylene-isoquinoline-1,3(2H,4H)-dione (5d).** By the procedure described above for **5a**, **3** (101.5 mg, 0.5 mmol) and 4-piperidin-1-ylmethyl-phenylamine (**4d**) (95.14 mg, 0.5 mmol) were reacted to give 92 mg (51%) of **5d** as a yellow solid: mp 185–186 °C. HRMS (ESI)  $m/z$  calcd for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub> 362.18546, found 362.18631 (M + H)<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.45–1.54 (m, 4H), 2.32 (s, 4H), 3.41 (s, 2H), 7.21–7.39 (m, 3H), 7.50 (d,  $J$  = 8.3 Hz, 2H), 7.55–7.72 (m, 1H), 8.03 (dd,  $J$  = 7.93, 1.4 Hz, 1H), 8.16 (d,  $J$  = 8.3 Hz, 1H), 8.88 (d,  $J$  = 12.6 Hz, 1H), 11.32 (s, 1H), 12.41 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>•0.6H<sub>2</sub>O) C, H, N.

**(4Z)-7-Bromo-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (18a).** By the procedure described above for **5a**, 300 mg (1.06 mmol) of (4E)-7-bromo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**17a**) and (211.10 mg, 1.06 mmol) of 4-(4-methylpiperazin-1-yl)-phenylamine (**4a**) were reacted to give 380 mg (81%) of **18a** as a green-yellow solid: MS (ESI)  $m/z$  441.33 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.39–2.48 (m, 4H), 3.07–3.19 (m, 4H), 6.99 (d,  $J$  = 8.9 Hz, 2H), 7.44 (d,  $J$  = 8.9 Hz, 2H), 7.73 (dd,  $J$  = 8.4, 2.2 Hz, 1H), 8.12 (dd,  $J$  = 8.4, 2.2 Hz, 2H), 8.84 (d,  $J$  = 12.8 Hz, 1H), 11.40 (s, 1H), 12.50 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>21</sub>H<sub>21</sub>BrN<sub>4</sub>O<sub>2</sub>•0.5H<sub>2</sub>O) C, H, N.

**(4Z)-6-Bromo-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (18b).** A mixture of **17b** (3.49 g, 12.4 mmol) and **4a** (2.36 g, 12.4 mmol) in 12 mL of DMF was heated in a preheated oil bath at 110 °C for 25 min. After the solvent was evaporated, the solid was collected and washed with cold DMF, ether, and hexane to give 3.0 g (55%) of **18b** as an amber solid: mp 220–223 °C. MS (ES+)  $m/z$  441.0, 443.0 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.44–2.49 (m, 4H), 3.07–3.21 (m, 4H), 7.00 (d,  $J$  = 9.1 Hz, 2H), 7.37 (dd,  $J$  = 8.4, 1.6 Hz, 1H), 7.48 (d,  $J$  = 9.1 Hz, 2H), 7.90 (d,  $J$  = 8.4 Hz, 1H), 8.43 (d,  $J$  = 1.6 Hz, 1H), 8.88 (d,  $J$  = 12.8 Hz, 1H), 11.34 (s, 1H), 12.59 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>21</sub>H<sub>21</sub>BrN<sub>4</sub>O<sub>2</sub>•0.4H<sub>2</sub>O) C, H, N: calcd, 12.49; found, 12.06.

**(4Z)-6-Nitro-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (18e).** By the procedure described above for **18b**, 0.116 g (0.469 mmol) of **17e** and 0.09 g (0.469 mmol) of **4a** were reacted to give 0.13 g (68%) of **18e** as an amber solid, mp 250–260 °C (dec). MS (ES+)  $m/z$  408.2 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.27 (s, 3H), 2.54 (m, 4H), 3.01–3.26 (m, 4H), 7.02 (d,  $J$  = 9.1 Hz, 2H), 7.51 (d,  $J$  = 9.1 Hz, 2H), 7.92 (dd,  $J$  = 8.6, 2.0 Hz, 1H), 8.22 (d,  $J$  = 8.6 Hz, 1H), 8.94 (d,  $J$  = 2.0 Hz, 1H), 9.02 (d,  $J$  = 12.8 Hz, 1H), 11.58 (s, 1H), 12.67 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>21</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>•0.5H<sub>2</sub>O) C, H, N: calcd, 16.82; found, 16.06.

**(4Z)-6-Iodo-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (18f).** To a solution of 0.15 g (0.61 mmol) of (4E)-6-iodo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**17f**) in 2 mL of *N,N*'-dimethylformamide was added **4a** (0.096 g, 0.67 mmol). The reaction mixture was heated at 120 °C for 2 h. The reaction mixture was concentrated under high-pressure vacuum, then treated with MeOH to give a tan precipitate. It was filtered and washed successively with MeOH, Et<sub>2</sub>O, and hexane to afford 0.202 g (91%) of **18f** as a brown solid: mp 220–221 °C. MS (ESI)  $m/z$  489.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.42–2.48 (m, 4H), 3.09–3.20 (m, 4H), 7.00 (d,  $J$  = 8.8 Hz, 2H), 7.47 (d,  $J$  = 8.8 Hz, 2H), 7.56 (d,  $J$  = 8.5 Hz, 1H), 7.72 (d,  $J$  = 8.5 Hz, 1H), 8.56 (s, 1H), 8.86 (d,  $J$  = 12.8 Hz, 1H), 11.31 (s, 1H), 12.59 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>21</sub>H<sub>21</sub>IN<sub>4</sub>O<sub>2</sub>•0.33H<sub>2</sub>O) C, H, N.

**(4Z)-*N*-Dimethyl-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)-1,3-dioxo-1,2,3,4-tetrahydroisoquinoline-6-carboxamide (18g).** A slurry of 81.7 mg (0.298 mmol) of **17g** and 56.9 mg (0.298 mmol) of **4a** in 1.5 mL of DMF was heated at 100 °C under N<sub>2</sub> for 0.5 h. After the reaction was chilled in ice, the solid product was collected, washed with DMF and Et<sub>2</sub>O and dried to give 111 mg (86%) of **18g** as yellow crystals: mp >300 °C. HRMS (ESI)  $m/z$  calcd for C<sub>24</sub>H<sub>27</sub>N<sub>5</sub>O<sub>3</sub> 432.20411, found 432.20337 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.22 (s, 3H), 2.46 (m, 4H), 2.90 (s, 3H), 3.04 (s, 3H), 3.14 (m, 4H), 6.98 (d,  $J$  = 8.9 Hz, 2H), 7.16 (d,  $J$  = 8.1 Hz, 1H), 7.46 (d,  $J$  = 8.9, 2H), 8.03 (d,  $J$  = 8.1 Hz, 1H), 8.14 (s, 1H), 8.88 (d,  $J$  = 12.8 Hz, 1H), 11.25 (s, 1H), 12.55 (d,  $J$  = 12.8 Hz, 1H); Anal. (C<sub>24</sub>H<sub>27</sub>N<sub>5</sub>O<sub>3</sub>) C, H, N.

**(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-(1H-pyrrol-1-yl)isoquinoline-1,3(2H,4H)-dione (18h).** A mixture of **17h** (100 mg, 0.3727 mmol) and **4a** (71.3 mg, 0.3727 mmol) in 1 mL of *N,N*-dimethylformamide was heated at 100 °C for 0.5 h. After the solvent was evaporated, ether was added to the residue. The precipitate was collected and washed with ether to give 122 mg (77%) of **18h** as a light-brown solid: mp 239–240 °C. HRMS (ESI) *m/z* calcd for  $C_{25}H_{25}N_5O_2$  428.20811, found 428.20865 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.40–2.48 (m, 4H), 3.02–3.23 (m, 4H), 6.18–6.45 (m, 2H), 7.02 (d,  $J$  = 9.1 Hz, 2H), 7.33–7.58 (m, 3H), 7.59–7.76 (m, 2H), 8.06 (d,  $J$  = 8.6 Hz, 1H), 8.11 (d,  $J$  = 1.8 Hz, 1H), 8.95 (d,  $J$  = 12.8 Hz, 1H), 11.22 (s, 1H), 12.59 (d,  $J$  = 12.8 Hz, 1H). Anal. ( $C_{25}H_{25}N_5O_2 \cdot 0.33H_2O$ ) C, H, N.

**N-[(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-1,3-dioxo-1,2,3,4-tetrahydroisoquinolin-6-yl]acetamide (18i).** By the procedure described above for **5a**, 410 mg (1.57 mmol) of (4E)-6-acetamido-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**17i**) and 300 mg (1.57 mmol) of **4a** were reacted to give 450 mg (68%) of **18i** as a yellow solid: mp 292–293 °C. MS (ESI) *m/z* 420.2 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.10 (s, 3H), 2.24 (s, 3H), 2.45–2.49 (m, 4H), 2.96–3.22 (m, 4H), 7.02 (d,  $J$  = 9.2 Hz, 2H), 7.34 (d,  $J$  = 9.2 Hz, 2H), 7.52 (dd,  $J$  = 8.8, 0.8 Hz, 1H), 7.94 (d,  $J$  = 8.8 Hz, 1H), 8.01 (d,  $J$  = 0.8 Hz, 1H), 8.42 (d,  $J$  = 12.8 Hz, 1H), 10.21 (s, 1H), 11.13 (s, 1H), 12.26 (d,  $J$  = 12.8 Hz, 1H). Anal. ( $C_{23}H_{25}N_5O_3 \cdot 0.2H_2O \cdot 0.5TFA$ ) C, H, N.

**(4Z)-6-Bromo-4-({[4-piperidin-1-ylmethyl]phenyl]amino}methyleneisoquinoline-1,3(2H,4H)-dione (19b).** (way-186084). By the procedure described above for **5a**, 141 mg (0.50 mmol) of **17b** and 100 mg (0.525 mmol) of **4d** were reacted to give 138 mg (63%) of **19b** as an off-white solid, mp 222–225 °C. MS (ESI) *m/z* 440.1, 442.1 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.42–1.62 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 7.33 (d,  $J$  = 8.3 Hz, 2H), 7.41 (d,  $J$  = 8.0 Hz, 1H), 7.55 (d,  $J$  = 8.6 Hz, 2H), 7.92 (d,  $J$  = 8.3 Hz, 1H), 8.46 (s, 1H), 8.94 (d,  $J$  = 12.7 Hz, 1H), 11.41 (s, 1H), 12.53 (d,  $J$  = 12.7 Hz, 1H). Anal. ( $C_{22}H_{22}BrN_3O_2 \cdot 0.3H_2O$ ) C, H, N.

**(4Z)-6-Chloro-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methyleneisoquinoline-1,3(2H,4H)-dione (19c).** By the procedure described above for **5a**, 0.15 g (0.63 mmol) of (4E)-6-chloro-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**17c**) and 0.145 g (0.756 mmol) of **4d** were reacted to give 0.165 g (66%) of **19c** as a yellow solid: mp 225–226 °C. MS (ESI) *m/z* 396.1 ( $M + H$ )<sup>+</sup>. HRMS (ESI) *m/e* calcd for  $C_{22}H_{22}ClN_3O_2$  396.14733, found 396.14670 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.49 (m, 4H), 2.33 (s, 4H), 3.44 (m, 2H), 7.28 (dd,  $J$  = 8.5, 1.7 Hz, 1H), 7.34 (d,  $J$  = 8.1 Hz, 2H), 7.55 (d,  $J$  = 8.1 Hz, 2H), 8.00 (d,  $J$  = 8.5 Hz, 1H), 8.32 (d,  $J$  = 1.7 Hz, 1H), 8.94 (d,  $J$  = 12.6 Hz, 1H), 11.40 (s, 1H), 12.52 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{22}H_{22}ClN_3O_2 \cdot 0.16DMF \cdot 0.25H_2O$ ) C, H, N.

**6-Methoxy-4-[(4-piperidin-1-ylmethyl-phenylamino)methylene]-4H-isoquinoline-1,3-dione (19d).** By the procedure described above for **5a**, 117 mg (0.50 mmol) of (4E)-6-methoxy-4-methoxymethylene-4H-isoquinoline-1,3-dione (**17d**) and **4d** (95 mg, 0.50 mmol) in *N,N*-dimethylformamide (1 mL) were reacted to give 152 mg (78%) of **19d** as a yellow solid: mp 272–5 °C (dec). MS (ESI) *m/z* 392.4 ( $M + H$ ). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.34–1.43 (m, 2H), 1.44–1.63 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 3.92 (s, 3H), 6.87 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.33 (d,  $J$  = 8.3 Hz, 2H), 7.50 (d,  $J$  = 8.3 Hz, 2H), 7.54 (d,  $J$  = 2.1 Hz, 1H), 7.95 (d,  $J$  = 8.7 Hz, 1H), 8.85 (d,  $J$  = 12.6 Hz, 1H), 11.13 (s, 1H), 12.49 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{23}H_{25}N_3O_3$ ) C, H, N.

**(4Z)-6-Nitro-4-[(4-piperidin-1-ylmethyl)phenyl]amino)methyleneisoquinoline-1,3(2H,4H)-dione (19e).** By the procedure described above for **5a**, 115 mg (0.46 mmol) of (4E)-4-(methoxy)methylene-6-nitroisoquinoline-1,3(2H,4H)-dione (**17e**) was reacted with 93 mg (0.49 mmol) of **4d** to give 137 mg (73%) of **19e** as a brown solid: mp 225–235 °C (dec). MS (ESI) *m/z* 405.2 ( $M - H$ )<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.50 (m, 4H), 2.35 (m, 4H), 3.46 (s, 2H), 7.36 (d,  $J$  = 8.3 Hz, 2H),

7.59 (d,  $J$  = 8.3 Hz, 2H), 7.96 (dd,  $J$  = 8.8, 2.0 Hz, 1H), 8.23 (d,  $J$  = 8.8 Hz, 1H), 8.98 (d,  $J$  = 2.0 Hz, 1H), 9.10 (d,  $J$  = 12.6 Hz, 1H), 11.65 (s, 1H), 12.62 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{22}H_{22}N_4O_4$ ) C, H, N.

**(4Z)-6-Iodo-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (19f).** By the procedure described above for **5a**, 0.2 g (0.60 mmol) of (4E)-6-iodo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**17f**) was reacted with **4d** (0.127 mL, 0.67 mmol) to give 0.143 g (48.3%) of **19f** as a tan solid: mp 202–203 °C. MS (ESI) *m/z* 488.1 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.43–1.65 (m, 4H), 2.32 (s, 4H), 3.42 (m, 2H), 7.33 (d,  $J$  = 8.31 Hz, 2H), 7.54 (d,  $J$  = 8.5 Hz, 2H), 7.60 (dd,  $J$  = 8.1, 1.4 Hz, 1H), 7.73 (d,  $J$  = 8.3 Hz, 1H), 8.59 (s, 1H), 8.92 (d,  $J$  = 12.6 Hz, 1H), 11.38 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{22}H_{22}I_2N_3O_2$ ) C, H, N.

**(4Z)-N,N-Dimethyl-1,3-dioxo-4-({[4-(piperidinylmethyl)phenyl]amino}methylene)-1,2,3,4-tetrahydroisoquinoline-6-carboxamide (19g).** By the procedure described above for **5a**, 70 mg (0.255 mmol) of **17g** and 48.5 mg (0.255 mmol) of **4d** were reacted to give 82.4 mg (74%) of **19g** as a bright-yellow crystals: mp 266–267 °C (dec). HRMS (ESI) *m/e* calcd for  $C_{25}H_{28}N_4O_3$  431.20886, found 431.20820 ( $M - H$ )<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.38 (m, 2H), 1.48 (m, 4H), 2.32 (m, 4H), 2.90 (s, 3H), 3.04 (s, 3H), 3.42 (s, 2H), 7.20 (d,  $J$  = 6 Hz, 1H), 7.32 (d,  $J$  = 9 Hz, 2H), 7.53 (d,  $J$  = 9 Hz, 2H), 8.06 (d,  $J$  = 6 Hz, 1H), 8.17 (s, 1H), 8.95 (d,  $J$  = 9 Hz, 1H), 11.40 (s, 1H), 12.45 (d,  $J$  = 9 Hz, 1H). Anal. ( $C_{25}H_{28}N_4O_3 \cdot 0.25H_2O$ ) C, H, N.

**(4Z)-4-({[4 Piperidin-1-ylmethyl]phenyl]amino)methylene}-6-(1H-pyrrol-1-yl)isoquinoline-1,3(2H,4H)-dione (19h).** By the procedure described above for **5a**, 100 mg (0.38 mmol) of (4E)-4-(methoxymethylene)-6-(1H-pyrrol-1-yl)isoquinoline-1,3(2H,4H)-dione (**17h**) and **4d** (70.70 mg, 0.38 mmol) were reacted to give 50 mg (31%) of **19h** as a yellow solid: mp 207–208 °C. MS (ESI) *m/z* 426.21 ( $M + H$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.50 (m, 4H), 2.34 (m, 4H), 3.45 (s, 2H), 6.35 (m, 2H), 7.36 (d,  $J$  = 8.4 Hz, 2H), 7.46–7.57 (m, 3H), 7.66 (m, 2H), 8.07 (d,  $J$  = 8.8 Hz, 1H), 8.13 (d,  $J$  = 2.0 Hz, 1H), 9.01 (d,  $J$  = 12.59 Hz, 1H), 11.30 (s, 1H), 12.6 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{26}H_{26}N_4O_2 \cdot 0.1H_2O \cdot 0.1TFA$ ) C, H, N.

**N-[(4Z)-1,3-Dioxo-4-({[4-piperidin-1-ylmethyl]phenyl]amino)methylene]-1,2,3,4-tetrahydroisoquinolin-6-yl]acetamide (19i).** By the procedure described above for **5a**, 94 mg (0.36 mmol) of *N*-[(4E)-1,3-dioxo-4-(methoxy)methylene-1,2,3,4-tetrahydroisoquinolin-6-yl]acetamide **17i** and **4d** (76 mg, 0.40 mmol) were reacted to give 89 mg (59%) of **19i** as a brown solid, mp >320 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.41 (m, 2H), 1.54 (m, 4H), 2.10 (s, 3H), 2.13 (m, 4H), 3.56 (m, 2H), 7.42 (m, 2H), 7.57 (m, 2H), 7.98 (m, 1H), 8.10 (m, 1H), 8.60 (m, 1H), 9.00 (d,  $J$  = 12.7, 1H), 10.24 (m, 1H), 11.31 (m, 1H), 12.38 (m, 1H). MS (ESI) *m/z* 419.3 ( $M + H$ )<sup>+</sup>. Anal. ( $C_{24}H_{26}N_4O_3 \cdot 2.6H_2O$ ) C, H, N: calcd, 6.76; found, 4.47.

**(4Z)-6-Piperidin-1-yl-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (21a).** A mixture of 300 mg (0.68 mmol) of (4Z)-6-bromo-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (**19b**), tris(dibenzylideneacetone)-dipalladium(0) (118.30 mg, 0.129 mmol), 1,3-bis(2,6-di-isopropylphenyl)imidazolium chloride (Ipr<sup>+</sup>·HCl) 78.04 (0.184 mmol), potassium *t*-butoxide, (194.41 mg, 1.36 mmol), and piperidine (200 mg, 2.04 mmol) was placed in a three-neck flask. Under N<sub>2</sub>, *N,N*-dimethylformamide (8 mL) was added, and the mixture was then stirred in a preheated oil bath 100 °C for 45 min. After cooling, the mixture was treated with CH<sub>2</sub>Cl<sub>2</sub> and filtered through celite. After evaporating all the solvents, the residue was dissolved in methylene chloride, washed three times with sodium bicarbonate solution, dried over magnesium sulfate, and evaporated. The yellow oily residue was purified by preparative thin layer chromatography (5:95 = methanol:methylene chloride), to give **21a** as a yellow solid, 80 mg (25%): mp 211–212 °C. MS (ESI) *m/z* 445.3 ( $M + 1$ ). HRMS (ESI) *m/e* calcd for  $C_{27}H_{32}N_4O_2$  445.25981, found 445.25909 ( $M + H$ )<sup>+</sup>. <sup>1</sup>H NMR (400 MHz,

DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.38 (m, 2H), 1.48 (m, 4H), 1.61 (m, 6H) 2.32 (m, 4H), 3.42 (m, 6H), 6.87 (dd, *J* = 9.2, 1.6 Hz, H), 7.26 (d, *J* = 1.6 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 9.2 Hz, 1H), 8.78 (d, *J* = 12.4 Hz, 1H), 10.89 (s, 1H), 12.48 (d, *J* = 12.4 Hz, 1H). Anal. (C<sub>27</sub>H<sub>32</sub>N<sub>4</sub>O<sub>2</sub>•0.4H<sub>2</sub>O) C, H, N: calcd, 12.14; found, 11.23.

**(4Z)-6-Piperidin-1-yl-4-({[4-(methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (20a).** By the procedure described for the preparation of **21a**, **18b** (300 mg, 0.68 mmol) and piperidine (200 mg, 2.04 mmol) were reacted to give 70 mg (23%) of yellow solid: mp 223–224 °C. MS (ESI) *m/z* 445.56 (M + 1). HRMS (ESI) *m/e* calcd for C<sub>26</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> 446.25506, found 446.25472 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.61 (m, 4H), 1.99 (s, 3H), 2.22 (m, 2H), 2.44–2.47 (m, 4H), 3.12–3.15 (m, 4H), 3.42 (m, 4H), 6.85 (dd, *J* = 8.8, 2.0 Hz, 1H) 7.00 (d, *J* = 9.2 Hz, 2H) 7.23 (d, *J* = 2.0 Hz, 1H), 7.39 (d, *J* = 9.2 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 1H), 8.72 (d, *J* = 12.8 Hz, 1H), 10.81 (s, 1H), 12.51 (d, *J* = 12.8 Hz, 1H). Anal. (C<sub>26</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub>•0.5H<sub>2</sub>O) C, H, N: calcd, 15.35; found, 13.56.

**(4Z)-6-Morpholin-4-yl-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (21b).** By the procedure described above for **21a**, **19b** (300 mg, 0.68 mmol) and morpholine (178 mg, 2.04 mmol) were reacted to give 95 mg (31% yield) of **21b** as a yellow solid: mp 216–217 °C. MS (ESI) *m/z* 446.55 (M + 1). HRMS (ESI) *m/e* calcd for C<sub>26</sub>H<sub>30</sub>N<sub>4</sub>O<sub>3</sub> 447.23907, found 447.23969 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.38 (m, 2H), 1.48 (m, 4H), 2.32 (m, 4H), 3.38–3.4 (m, 4H), 3.42 (s, 2H), 3.72–3.85 (m, 4H), 6.91 (dd, *J* = 9.2, 1.8 Hz, 1H), 7.31 (d, *J* = 1.8 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 9.2 Hz, 1H) 8.80 (d, *J* = 12.4 Hz, 1H) 10.96 (s, 1H) 12.48 (d, *J* = 12.4 Hz, 1H). Anal. (C<sub>26</sub>H<sub>30</sub>N<sub>4</sub>O<sub>3</sub>•H<sub>2</sub>O) C, H: calcd, 12.06; found, 11.44.

**(4Z)-6-[(4-Methyl-piperizin-1-yl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (21c).** By the procedure described above for **21a**, **19b** (300 mg, 0.68 mmol) and 4-methyl-piperazine (204.41 mg, 2.04 mmol) were reacted to give 90 mg (29% yield) of **21c** as a yellow solid: mp 192–193 °C. MS (ESI) *m/z* 459.59 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.48 (m, 4H), 2.24 (s, 3H), 2.32 (m, 4H), 2.47 (m, 4H), 3.41 (m, 6H), 6.91 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.29 (d, *J* = 2.0 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 9.2 Hz, 1H), 8.80 (d, *J* = 12.4 Hz, 1H), 10.93 (s, 1H), 12.49 (d, *J* = 12.4 Hz, 1H). Anal. (C<sub>27</sub>H<sub>33</sub>N<sub>5</sub>O<sub>2</sub>•0.4H<sub>2</sub>O) C, H, N.

**(4Z)-6-Anilino-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (21d).** By the procedure described above for **21a**, **19b** (300 mg, 0.68 mmol) and aniline (125 mg, 1.36 mmol) were reacted to give 120 mg (39% yield) of **21d** as a yellow solid: mp 234–235 °C. MS (ESI) *m/z* 453.2 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.46–1.51 (m, 4H), 2.31 (m, 4H), 3.40 (s, 2H), 6.89–7.01 (m, 2H), 7.24 (dd, *J* = 8.4, 1.6 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.37 (m, 2H), 7.4 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 8.55 (d, *J* = 12.8 Hz, 1H), 8.66 (m, 1H), 10.98 (s, 1H), 12.29 (d, *J* = 12.8 Hz, 1H). Anal. (C<sub>28</sub>H<sub>28</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

**(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-phenylisoquinoline-1,3(2H,4H)-dione (22a).** To a suspension of **18b** (40 mg, 0.1 mmol) in *N,N*-dimethylformamide (1 mL) was added phenyl boronic acid (12.2 mg, 0.1 mmol), followed by 60  $\mu$ L of 2 M aqueous cesium carbonate and tetrakis(triphenylphosphine)palladium(0) (6 mg, 0.005 mmol). The reaction mixture was subjected to microwave heating at 150 °C for 100 s. The reaction mixture was then diluted to 2 mL with *N,N*-dimethylformamide and purified by C18 reverse phase HPLC. The pure fractions were combined and concentrated to yield 43 mg (98%) of **22a** as a solid: LC/MS1 Rt 1.918 min, *m/z* 439.1 (M + 1); LC/MS2 Rt 1.99 min, HRMS 439.2132 [M + H]<sup>+</sup> obs, 439.2129 [M + H]<sup>+</sup> calcd. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.87 (s, 3H), 2.97–3.19 (m, 4H), 3.52–3.84 (m, 4H), 7.10 (d, *J* = 8.5 Hz, 2H), 7.29–7.37 (m, 1H), 7.45–7.56 (m, 4H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* =

7.8 Hz, 2H), 8.10 (d, *J* = 8.3 Hz, 1H), 8.30 (s, 1H), 8.98 (d, *J* = 12.7 Hz, 1H), 11.27 (s, 1H), 12.54 (d, *J* = 12.7 Hz, 1H).

**(4Z)-6-(2-Furyl)-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (22b).** By the procedure described above for **22a**, **18b** (40 mg, 0.1 mmol) and 2-furanboronic acid (11.2 mg, 0.1 mmol) were reacted to give 13 mg (30%) of **22b**: LC/MS1 Rt 1.874 min, *m/z* 429.1 (M + 1); LC/MS2 Rt 1.89 min, *m/z* 429 (M + 1); HRMS 429.1925 [M + H]<sup>+</sup> obs, 429.1921 [M + H]<sup>+</sup> calcd. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.88 (s, 3H), 2.97–3.19 (m, 4H), 3.52–3.84 (m, 4H), 7.08 (d, *J* = 8.6 Hz, 2H), 7.34 (dd, *J* = 7.8, 7.1 Hz, 1H), 7.47 (d, *J* = 7.1 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 7.80 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 8.31 (s, 1H), 8.98 (d, *J* = 12.7 Hz, 1H), 11.27 (s, 1H), 12.54 (d, *J* = 12.7 Hz, 1H).

**(4Z)-6-(3-Furyl)-4-({[4-(4-methylpiperazin-1-yl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (22c).** A mixture containing 0.15 g (0.34 mmol) of **18b**, 0.076 g (0.64 mmol) of 3-furanboronic acid, 0.02 g (0.017 mmol) of tris(dibenzylideneacetone)-dipalladium(0), 0.02 g (0.064 mmol) of 2-(di-*t*-butylphosphino)-biphenyl, and 0.072 g (0.64 mmol) of sodium carbonate in 2 mL of DMF and 0.4 mL of water was heated at 100 °C for 1.5 h. It was filtered through a pad of celite and dried up. The residue was purified by column chromatography over silica gel using 5% MeOH/CHCl<sub>3</sub> as eluent to yield 0.096 mg (66.2%) of **22c** as a yellow solid: mp 190–191 °C.; MS (ESI) *m/z* 429.2 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.45–2.49 (m, 4H), 2.96–3.23 (m, 4H), 7.02 (d, *J* = 9.1 Hz, 2H), 7.25 (d, *J* = 1.01 Hz, 1H), 7.46 (d, *J* = 9.1 Hz, 2H), 7.51 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.82 (t, *J* = 1.64 Hz, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 8.19 (s, 1H), 8.43 (s, 1H), 8.91 (d, *J* = 12.6 Hz, 1H), 11.21 (s, 1H), 12.55 (d, *J* = 12.6 Hz, 1H). Anal. (C<sub>25</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>•0.2H<sub>2</sub>O) C, H, N.

**(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-thien-3-ylisoquinoline-1,3(2H,4H)-dione (22d).** By the procedure described above for **22c**, **18b** (1.0 g, 2.26 mmol) and 3-thiopheneboronic acid (0.43 g, 3.4 mmol) were reacted to yield 0.38 g (38%) of **22d** as a yellow solid: mp 224–226 °C. MS (ESI) *m/z* 445.2 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.41–2.48 (m, 4H), 3.02–3.20 (m, 4H), 7.01 (d, *J* = 9.06 Hz, 2H), 7.46 (d, *J* = 8.81 Hz, 2H), 7.61 (dd, *J* = 8.31, 1.51 Hz, 1H), 7.71 (dd, *J* = 5.04, 2.9 Hz, 1H), 7.84 (dd, *J* = 5.0, 1.3 Hz, 1H), 8.04 (d, *J* = 8.31 Hz, 1H), 8.19 (dd, *J* = 2.9, 1.3 Hz, 1H), 8.31 (s, 1H), 8.95 (d, *J* = 12.8 Hz, 1H), 11.22 (s, 1H), 12.56 (d, *J* = 12.8 Hz, 1H). Anal. (C<sub>25</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>•0.9H<sub>2</sub>O) C, H, N.

**4-[(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-thien-3-ylisoquinoline-1,3(2H,4H)-dione (22e).** By the procedure described above for **22c**, **18b** (0.5 g, 1.13 mmol) and 4-formylphenylboronic acid (0.25 g, 1.7 mmol) were reacted to give 0.054 g (10.2%) of **22e** as a yellow solid: mp 152–153 °C, MS (ESI) *m/z* 467.2 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.23 (s, 3H), 2.47 (s, 4H), 2.97–3.20 (m, 4H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.32–7.52 (m, 3H), 7.60 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.98–8.19 (m, 4H), 8.39 (s, 1H), 8.99 (d, *J* = 12.8 Hz, 1H), 10.10 (s, 1H), 11.31 (s, 1H), 12.59 (d, *J* = 12.8 Hz, 1H). Anal. (C<sub>28</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>•0.67H<sub>2</sub>O) C, H, N.

**4Z-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-(2-phenylvinyl)isoquinoline-1,3(2H,4H)-dione (22f).** To a mixture of cesium carbonate (39 mg, 0.12 mmol), tetrabutylammonium bromide (32.2 mg, 0.1 mmol), tri-*o*-tolylphosphine (30.4 mg, 0.1 mmol) and palladium acetate (9 mg, 0.04 mmol) in *N,N*-dimethylformamide (1.2 mL) were added to **18b** (47 mg, 0.1 mmol) and styrene (15.6 mg, 0.15 mmol). The reaction mixture was subjected to microwave heating at 200 °C for 120 s. The reaction mixture was then diluted to 2 mL with *N,N*-dimethylformamide and purified by C18 reverse phase HPLC. The pure fractions were combined and concentrated to yield 6 mg (13%) of **22f** as a solid. LC/MS1 Rt 2.134 min, *m/z* 465 (M + 1); LC/MS2 Rt 2.84 min, *m/z* 465 (M + 1); HRMS 465.2288 [M + H]<sup>+</sup> obs 465.2285 [M + H]<sup>+</sup> calcd. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.89 (s, 3H), 2.97–3.19 (m, 4H), 3.53–3.90 (m, 4H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.33–7.46 (m, 4H), 7.53–7.59 (m, 4H), 7.66 (d, *J* = 8.4 Hz, 2H), 8.01 (d, *J* =

= 8.2 Hz, 1H), 8.25 (s, 1H), 8.90 (d,  $J$  = 12.7 Hz, 1H), 11.27 (s, 1H), 12.54 (d,  $J$  = 12.7 Hz, 1H).

**(4Z)-4-({[4-(4-Methylpiperazin-1-yl)phenyl]amino}methylene)-6-(phenylethynyl)-isoquinoline-1,3(2H,4H)-dione (22g).** A mixture of **18b** (0.10 g, 0.23 mmol), phenylacetylene (0.03 mL, 0.27 mmol), dichlorobis(triphenylphosphine)-palladium(II) (0.04 g, 0.046 mmol), CuI (0.0043 g, 0.023 mmol), and 0.12 mL (1.14 mmol) of triethylamine in 2 mL of *N,N*-dimethylformamide was added to a 10 mL round-bottom flask, sealed with a rubber septum, deaired, and backfilled with nitrogen gas three times and wrapped around with aluminum foil. The reaction mixture was stirred vigorously at 70 °C in an oil bath under nitrogen. Mass spectroscopy suggested the completion of reaction after 45 min. The reaction mixture was filtered through celite and subsequently evaporated under high-pressure vacuum to brown solid. The solid was dissolved in warm chloroform and ran through a pad of florisil, which is rinsed with 200 mL of warm chloroform. The collected organic portion was evaporated under vacuum and purified by HPLC to give 0.057 g (54.3%) of **22g** as a brown solid: mp 169–170 °C. MS (ESI) *m/z* 463.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.83–3.06 (m, 5H), 3.18 (s, 2H), 3.87 (m, 2H), 7.08 (d,  $J$  = 8.8 Hz, 2H), 7.38 (d,  $J$  = 8.3 Hz, 1H), 7.45–7.51 (m, 3H), 7.57 (d,  $J$  = 8.8 Hz, 1H), 7.63 (dd,  $J$  = 6.42, 2.90 Hz, 3H), 8.04 (d,  $J$  = 8.31 Hz, 1H), 8.40 (s, 1H), 8.95 (d,  $J$  = 12.8 Hz, 1H), 11.38 (s, 1H), 12.59 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>29</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>•2.6H<sub>2</sub>O•1.1TFA) C, N: H: calcd, 5.13; found 4.48.

**(4Z)-6-Phenyl-4-({[4-piperidin-1-ylmethyl]phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23a).** A mixture of 300 mg (0.68 mmol) of **19b**, tetrakis(triphenylphosphine)palladium(0) (118.0 mg, 0.102 mmol), saturated aqueous sodium carbonate (2 mL), and phenyl boronic acid (123.42 mg, 1.02 mmol) was placed in a three-neck flask. Under N<sub>2</sub>, *N,N*-dimethylformamide (8 mL) was added and the mixture was then placed in a preheated oil bath at 120 °C for 45 min. After cooling, the mixture was treated with CH<sub>2</sub>Cl<sub>2</sub> and filtered through celite. After evaporating all the solvents, the residue was dissolved in methylene chloride, washed three times with sodium bicarbonate solution, dried over magnesium sulfate, and evaporated. The yellow oily residue was purified by preparative thin layer chromatography (5:95 = methanol:methylene chloride), to give 90 mg (30% yield) of **23a** as a yellow solid: mp 214–215 °C. MS (ESI) *m/z* 437.54 (M + 1). HRMS (ESI) *m/e* calcd for C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub> 438.21761, found 438.21743 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.49 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 7.34 (d,  $J$  = 8.1 Hz, 2H), 7.45 (d,  $J$  = 7.3 Hz, 1H), 7.48–7.63 (m, 5H), 7.87 (d,  $J$  = 8.1 Hz, 2H), 8.10 (d,  $J$  = 7.3 Hz, 1H), 8.33 (s, 1H), 9.04 (d,  $J$  = 12.6 Hz, 1H), 11.33 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>•0.8H<sub>2</sub>O) C, N: H: calcd, 6.38; found 5.91.

**(4Z)-6-(3-Furyl)-4-({[4-piperidin-1-ylmethyl]phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23b).** By the procedure described above for **23a**, **19b** (0.16 g, 0.365 mmol) and 3-furanboronic acid (87 mg, 0.73 mmol) were reacted to give 50 mg (32%) of **23b** as a yellow solid: mp 204–205 °C. MS (ESI) *m/z* 427.50 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.24–1.40 (m, 2H), 1.44–1.74 (m, 4H), 2.34 (m, 4H), 3.44 (s, 2H), 7.26 (s, 1H), 7.36 (d,  $J$  = 8.4 Hz, 2H), 7.45–7.63 (m, 3H), 7.71–7.91 (m, 1H), 8.02 (d,  $J$  = 8.4 Hz, 1H), 8.22 (m, 1H), 8.44 (m, 1H), 8.97 (d,  $J$  = 12.6 Hz, 1H), 11.28 (s, 1H), 12.51 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>26</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>•0.6H<sub>2</sub>O) C, H, N.

**(4Z)-4-({[4-Piperidin-1-ylmethyl]phenyl]amino}methylene)-6-thien-3-ylisoquinoline-1,3(2H,4H)-dione (23c).** By the procedure described above for **23a**, **19b** (500 mg, 1.14 mmol) and 3-thiopheneboronic acid (300 mg, 2.28 mmol) were reacted to give 150 mg (30%) of **23c** as a yellow solid: mp 166–167 °C. MS (ESI) *m/z* 444.1 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.24–1.40 (m, 2H), 1.44–1.74 (m, 4H), 2.34 (m, 4H), 3.44 (s, 2H), 7.36 (d,  $J$  = 8.4 Hz, 2H), 7.45–7.63 (m, 3H), 7.71–7.91 (m, 2H), 8.02 (d,  $J$  = 8.3 Hz, 1H), 8.22 (s, 1H), 8.44 (s, 1H), 8.97 (d,  $J$  = 12.6 Hz, 1H), 11.28 (s, 1H), 12.51 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>26</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub>S•0.33H<sub>2</sub>O) C, H, N.

**(4Z)-4-({[4-Piperidin-1-ylmethyl]phenyl}amino)methylene)-6-pyridin-3-ylisoquinoline-1,3(2H,4H)-dione (23d).** By the procedure described above for **23a**, **19b** (300 mg, 0.68 mmol) and 3-pyridylboronic acid (166.21 mg, 1.36 mmol) were reacted to give 100 mg (34%) of **23d** as a yellow solid: mp 247–248 °C. MS (ESI) *m/z* 438.53 (M + 1). HRMS (ESI) *m/e* calcd for C<sub>27</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub> 439.21286, found 439.21212 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.38 (m, 2H), 1.42–1.49 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 7.34 (d,  $J$  = 8.1 Hz, 2H), 7.54 (m, 3H), 7.61 (d,  $J$  = 8.1 Hz, 2H), 8.13 (d,  $J$  = 8.4 Hz, 1H), 8.27 (dd,  $J$  = 1.6, 8.1 Hz), 8.41 (m, 1H), 8.66 (d,  $J$  = 1.6 Hz 1H), 9.05 (d,  $J$  = 12.6 Hz, 1H), 9.11 (d,  $J$  = 1.6 Hz, 1H), 11.37 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>27</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>•1.2H<sub>2</sub>O) C, H, N.

**(4Z)-6-(4-Fluorophenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23e).** By the procedure described above for **22c** except replacing (di-*t*-butylphosphino)-biphenyl with tri-*t*-butylphosphine, and replacing sodium carbonate with cesium carbonate. **19b** (500 mg, 1.13 mmol) and 4-fluorophenyl boronic acid (395.27 mg, 2.83 mmol) were reacted to give 100 mg (20%) of **23e** as a yellow solid: mp 195–196 °C. MS (ESI) *m/z* 456.3 (M + 1)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.43–1.62 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 7.15–7.42 (m, 4H), 7.54 (d,  $J$  = 8.4 Hz, 3H), 7.91–7.95 (m, 2H), 8.09 (d,  $J$  = 8.4 Hz, 1H), 8.31 (s, 1H), 9.03 (d,  $J$  = 12.4 Hz, 1H), 11.32 (s, 1H), 12.52 (d,  $J$  = 12.4 Hz, 1H). Anal. (C<sub>28</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>2</sub>•0.25H<sub>2</sub>O) C, H, N.

**(4Z)-6-(4-Chlorophenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23f).** By the procedure described above for **23a**, using the procedure described for the preparation of **23a**, **19b** (300 mg, 0.68 mmol), and 4-chlorophenyl boronic acid (214.0 mg, 1.36 mmol) were reacted to give 250 mg (79%) of **23f** as a yellow solid: mp 204–205 °C. MS (ESI) *m/z* 464.90 (M + 1)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.44–1.60 (m, 4H), 2.32 (m, 4H), 3.43 (s, 2H), 7.34 (d,  $J$  = 8.3 Hz, 2H), 7.53 (d,  $J$  = 8.3 Hz, 2H), 7.57–7.71 (m, 3H), 7.91 (d,  $J$  = 8.8 Hz, 2H), 8.10 (d,  $J$  = 8.3 Hz, 1H), 8.33 (s, 1H), 9.03 (d,  $J$  = 12.8 Hz, 1H), 11.34 (s, 1H), 12.52 (d,  $J$  = 12.8 Hz, 1H). Anal. (C<sub>28</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>2</sub>•0.8H<sub>2</sub>O) C, H, N.

**(4Z)-6-(3-Hydroxyphenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23g).** By the procedure described above for **23e**, **19b** (300 mg, 0.68 mmol) and 3-(4,4,5,5-teramethyl-[1,3,2]dioxaborolan-2-yl)-phenol (300 mg, 1.36 mmol) were reacted to give 120 mg (39%) of **23g** as a yellow solid: mp 235–236 °C. MS (ESI) *m/z* 453.54 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.47–1.49 (m, 4H), 2.33 (m, 4H) 3.43 (s, 2H), 6.85 (d,  $J$  = 7.6 Hz, 1H), 7.2 (m, 1H), 7.26 (d,  $J$  = 7.6 Hz, 2H), 7.3–7.35 (m, 3H), 7.47 (d,  $J$  = 8.1 Hz, 1H), 7.54 (d,  $J$  = 8.3 Hz, 2H), 8.08 (m, 1H), 8.27 (s, 1H), 9.03 (d,  $J$  = 12.6 Hz, 1H), 9.59 (s, 1H), 11.31 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>•0.5H<sub>2</sub>O) C, H, N.

**(4Z)-6-(4-Hydroxyphenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23h).** By the procedure described above for **23e**, **19b** (300 mg, 0.68 mmol) and 4-(4,4,5,5-teramethyl-[1,3,2]dioxaborolan-2-yl)-phenol (300 mg, 1.36 mmol) were reacted to give 120 mg (39%) of **23h** as a yellow solid: mp 244–245 °C. MS (ESI) *m/z* 453.54 (M + 1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.43–1.64 (m, 4H), 2.32 (m, 4H), 3.42 (s, 2H), 6.90 (d,  $J$  = 8.4 Hz, 2H), 7.33 (d,  $J$  = 8.4 Hz, 2H), 7.47–7.54 (m, 3H), 7.73 (d,  $J$  = 8.4 Hz, 2H), 8.04 (d,  $J$  = 8.1 Hz, 1H), 8.23 (s, 1H), 8.61 (s, 1H), 9.00 (d,  $J$  = 12.6 Hz, 1H), 9.70 (s, 1H), 11.26 (s, 1H), 12.52 (d,  $J$  = 12.6 Hz, 1H). Anal. (C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>•0.1H<sub>2</sub>O•0.49TFA) C, H, N.

**(4Z)-6-(3-Methoxyphenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (23i).** By the procedure described above for **22c**, **19b** (0.5 g, 1.136 mmol) and 3-methoxyphenylboronic acid (0.26 g, 1.7 mmol) were reacted to give 0.096 g (18.1%) of **23i** as a yellow solid: mp 169–170 °C, MS (ESI) *m/z* 468.3 (M + H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (d,  $J$  = 4.28 Hz, 2H), 1.43–1.57 (m, 4H), 2.32 (s, 4H), 3.42 (s, 2H), 3.86 (s, 3H), 6.89–7.12 (m, 1H), 7.33 (d,  $J$  = 8.3 Hz, 2H), 7.36–7.48 (m, 3H), 7.49–7.62 (m, 3H), 8.09 (d,  $J$  =

8.1 Hz, 1H), 8.31 (s, 1H), 9.03 (d,  $J$  = 12.6 Hz, 1H), 11.32 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{29}H_{29}N_3O_3 \cdot 0.2H_2O$ ) C, H, N.

**(4Z)-6-(4-Methoxyphenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)-isoquinoline-1,3(2H,4H)-dione (23j).** By the procedure described above for **22c**, **19b** (0.5 g, 1.136 mmol) and 4-methoxyphenylboronic acid (0.26 g, 1.7 mmol) were reacted to give 0.12 g (22.6%) of **23j** as a yellow solid: mp 168–169 °C. MS (ESI)  $m/z$  468.2 (M + H)<sup>+</sup>.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (d,  $J$  = 4.28 Hz, 2H), 1.43–1.57 (m, 4H), 2.32 (s, 4H), 3.37–3.49 (m, 2H), 3.86 (s, 3H), 6.89–7.12 (m, 2H), 7.33 (d,  $J$  = 8.4 Hz, 2H), 7.36–7.48 (m, 2H), 7.49–7.62 (m, 3H), 8.09 (d,  $J$  = 8.31 Hz, 1H), 8.31 (s, 1H), 9.03 (d,  $J$  = 12.6 Hz, 1H), 11.32 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{29}H_{29}N_3O_3 \cdot 1.2H_2O$ ) C, H, N. H: calcd, 6.47; found 6.06.

**(4Z)-6-(4-Phenoxyphenyl)-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)-isoquinoline-1,3(2H,4H)-dione (23k).** By the procedure described above for **23a**, **19b** (300 mg, 0.68 mmol) and 4-phenoxyphenyl boronic acid (291.1 mg, 1.36 mmol) were reacted to give 70 mg (19%) of **23k** as a yellow solid: mp 132–133 °C. MS (ESI)  $m/z$  529.64 (M + 1).  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.47–1.49 (m 4H), 2.33 (m, 4H), 3.43 (s, 2H), 7.08–7.21 (m, 5H), 7.32 (d,  $J$  = 8.4 Hz, 2H), 7.44 (d,  $J$  = 8.3, 1H), 7.52–7.56 (m, 3H), 7.90 (d,  $J$  = 8.1 Hz, 2H) 8.09 (d,  $J$  = 8.3 Hz, 1H), 8.31 (m, 1H), 9.03 (d,  $J$  = 12.6 Hz, 1H), 11.34 (s, 1H), 12.53 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{34}H_{31}N_3O_3 \cdot 1.3H_2O$ ) C, H, N.

**(4Z)-1,3-Dioxo-4-({[4-(piperidin-1-ylmethyl)phenyl]amino}methylene)-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile (23l).** A mixture of 1.00 g (2.27 mmol) of **19b**, 239 mg (2.05 mmol) of Zn(CN)<sub>2</sub>, and 394 mg (0.341 mmol) of tetrakis(triphenylphosphine)-palladium(0) in 17 mL of DMF under N<sub>2</sub> was heated at 100 °C in the dark for 1.75 h. The reaction was then poured into 40 mL of ice–water and the product was collected, washed with H<sub>2</sub>O and Et<sub>2</sub>O, and dried. The crude product was boiled with 10% MeOH in CHCl<sub>3</sub> and filtered. The filtrate was washed with 2 M NH<sub>4</sub>OH and brine, dried, and evaporated. The residue was washed with boiling CH<sub>3</sub>CN and the insoluble material was dried to yield 200 mg (23%) of **23l** as yellow-orange crystals: mp 254–256 °C (dec). HRMS (ESI) *m/e* calcd for  $C_{23}H_{22}N_4O_2$  387.18156, found 387.18121 (M + H)<sup>+</sup>.  $^1H$  NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.39 (m, 2H), 1.49 (m, 4H), 2.33 (s, 4H), 3.43 (s, 2H), 7.35 (d,  $J$  = 6 Hz, 2H), 7.60 (m, 3H), 8.13 (d,  $J$  = 6 Hz, 1H), 8.76 (s, 1H), 9.01 (d,  $J$  = 9 Hz, 1H), 11.58 (s, 1H), 12.46 (d,  $J$  = 9 Hz, 1H). Anal. ( $C_{23}H_{22}N_4O_2 \cdot 0.3CHCl_3$ ) H, C: calcd, 66.27; found, 65.63; N: calcd, 13.27; found, 13.87.

**6-Iodo-4-{{[4-(5-methyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-phenylamino]methylene}-4H-isoquinoline-1,3-dione (34).** A solution of *N*-Boc-2,5-diaza-bicyclo[2.2.1]heptane (1 g, 5.04 mmol) and *p*-nitrobenzene (0.8 mL, 7.56 mmol) in 10 mL of acetonitrile was heated at 100 °C overnight. After the solvent was removed, the residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and evaporated to dryness. The oil was washed with hexane and recrystallized from ethyl acetate in hexane to give 0.83 g (52%) of 2-Boc-5-(4-nitrophenyl)-2,5-diaza-bicyclo[2.2.1]heptane (**24**) as a yellow solid: mp 193–194 °C.

A mixture of **24** (0.5 g, 1.566 mmol) in 20 mL of methanol and 1 mL of trifluoroacetic acid was stirred overnight. After the solvents were removed, the residue was treated with sodium bicarbonate solution and extracted with chloroform. The organic layer was dried over magnesium sulfate, filtered, and dried up to give a yellow solid, which upon recrystallization gave 0.251 (73%) of 5-(4-nitrophenyl)-2,5-diaza-bicyclo[2.2.1]heptane (**25**) as a yellow solid: mp 140–141 °C.

A mixture of **25** (0.247 g, 1.127 mmol), paraformaldehyde (37% in water, 0.25 mL, 9 mmol), and formic acid (0.3 mL, 7.7 mmol) was heated at 80 °C for 3 h. It was basified with 5N NaOH solution to pH 10 and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and dried up to give a yellow solid, which upon recrystallization from ethyl

acetate and hexane to give 0.208 g (79%) of 2-methyl-5-(4-nitrophenyl)-2,5-diaza-bicyclo[2.2.1]heptane (**26**) as yellow crystals: mp 100–101 °C.

A mixture of **26** (0.19 g, 0.815 mmol) and a catalytic amount of Pd/C in ethanol (0.2 mL) was hydrogenated at 1 atm at room temperature overnight. It was filtered through Celite, and the organic solution was evaporated and then treated with 10 mL of methanol. It was then treated with hydrochloric acid in methanol, followed by ethyl ether, and filtered to give 0.107 g (65%) of 4-(5-methyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-phenylamine (**27**) as a bluish solid: MS (ESI)  $m/z$  204.2 (M – H)<sup>+</sup>.

A solution of **27** (0.1 g, 0.49 mmol), **17f** (0.2 g, 0.6 mmol), and *N,N*-dimethylformamide (0.2 mL) was heated at 90 °C overnight. After the solvent was removed, the residue was treated with ethyl ether and filtered to give crude product as a light-brown solid. It was purified by HPLC to yield 97 mg (39%) of **34** as a bright-orange solid: mp 210–211 °C. MS (ESI)  $m/z$  501.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.16 (d,  $J$  = 11.8 Hz, 1H), 2.39 (d,  $J$  = 11.8 Hz, 1H), 2.88 (s, 3H), 3.11 (d,  $J$  = 10.3 Hz, 1H), 3.31 (d,  $J$  = 8.8 Hz, 1H), 3.58 (d,  $J$  = 8.9 Hz, 1H), 3.68 (d,  $J$  = 10.3 Hz, 1H), 4.37 (s, 1H), 4.69 (s, 1H), 6.57 (d,  $J$  = 8.5 Hz, 2H), 7.51 (d,  $J$  = 8.5 Hz, 2H), 7.57 (dd,  $J$  = 8.3, 2.2 Hz, 1H), 7.72 (d,  $J$  = 8.3 Hz, 1H), 8.54 (s, 1H), 8.86 (d,  $J$  = 11.9 Hz, 1H), 11.31 (s, 1H), 12.63 (d,  $J$  = 12.6 Hz, 1H). Anal. calcd for  $C_{22}H_{21}IN_4O_2 \cdot 1.4C_2H_2F_2O_2$ .

**(4Z)-4-({[4-(3,5-Dimethylpiperazin-1-yl)phenyl]amino}methylene)-6-iodoisoquinoline-1,3(2H,4H)-dione (35).** By the procedure described above for **34**, **17f** 0.15 g (0.46 mmol) and **30** (0.44 g, 2.13 mmol) were reacted to give 0.15 g (63.5%) of **35** as a light-brown solid: mp 222–223 °C. MS (ESI)  $m/z$  503.1 (M + H)<sup>+</sup>.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.03 (d,  $J$  = 6.3 Hz, 6H), 2.12 (m, 2H), 2.72–3.03 (m, 3H), 3.54 (m, 2H), 6.98 (d,  $J$  = 8.8 Hz, 2H), 7.45 (d,  $J$  = 8.8 Hz, 2H), 7.56 (dd,  $J$  = 8.4, 1.13 Hz, 1H), 7.72 (d,  $J$  = 8.4 Hz, 1H), 8.55 (s, 1H), 8.86 (d,  $J$  = 12.6 Hz, 1H), 11.31 (s, 1H), 12.61 (d,  $J$  = 12.6 Hz, 1H). Anal. calcd for  $C_{22}H_{23}IN_4O_2$  C, H, N.

**(4Z)-6-Iodo-4-[[{4-[{(3R,5S)-3,4,5-trimethylpiperazin-1-yl}phenyl]amino}methylene]-isoquinoline-1,3(2H,4H)-dione (36).** By the procedure described above for **34**, **17f** (0.10 g, 0.30 mmol) and **31** (0.073 g, 0.33 mmol) were reacted. It was purified by column chromatography over silica gel using 5% MeOH/CHCl<sub>3</sub> as eluent to give 0.089 g (56.7%) of **36** as a light-brown solid: mp 224–225 °C. MS (ESI)  $m/z$  517.1 (M + H)<sup>+</sup>.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.08 (d,  $J$  = 6.0 Hz, 6H), 2.19 (s, 3H), 2.21–2.31 (m, 2H), 2.39 (m, 2H), 3.57 (m, 2H), 6.99 (d,  $J$  = 9.1 Hz, 2H), 7.46 (d,  $J$  = 9.1 Hz, 2H), 7.56 (dd,  $J$  = 8.3, 1.5 Hz, 1H), 7.72 (d,  $J$  = 8.3 Hz, 1H), 8.55 (d,  $J$  = 1.5 Hz, 1H), 8.86 (d,  $J$  = 12.6 Hz, 1H), 11.31 (s, 1H), 12.60 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{23}H_{25}IN_4O_2 \cdot 0.6H_2O$ ) C, H, N.

**(4Z)-4-({[4-(3,4-Dimethylpiperazin-1-yl)phenyl]amino}methylene)-6-iodoisoquinoline-1,3(2H,4H)-dione (37).** By the procedure described above for **34**, **17f** (0.15 g, 0.46 mmol) and **32** (0.11 g, 0.50 mmol) were reacted to give 0.052 g (22.6%) of **37** as a light-brown solid: mp 157–158 °C. MS (ESI)  $m/z$  503.1 (M + H)<sup>+</sup>.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.34 (d,  $J$  = 6.3 Hz, 3H), 2.68–2.83 (m, 2H), 2.89 (s, 3H), 3.57 (s, 2H), 3.78–4.02 (m, 2H), 7.08 (t,  $J$  = 8.7 Hz, 2H), 7.54 (d,  $J$  = 8.7 Hz, 2H), 7.58 (d,  $J$  = 8.3 Hz, 1H), 7.73 (d,  $J$  = 8.3 Hz, 1H), 8.56 (s, 1H), 8.87 (d,  $J$  = 12.6 Hz, 1H), 11.34 (s, 1H), 12.59 (d,  $J$  = 12.6 Hz, 1H). Anal. ( $C_{22}H_{23}IN_4O_2 \cdot 1.4TFA$ ) C, H, N.

**(4Z)-6-Iodo-4-[[{4-[{(2S,5R)-2,4,5-trimethylpiperazin-1-yl}phenyl]amino}methylene]-isoquinoline-1,3(2H,4H)-dione (38).** By the procedure described above for **34**, **17f** (0.10 g, 0.30 mmol) and **33** (0.073 g, 0.33 mmol) were reacted. After column chromatography over silica gel using 5% MeOH/CHCl<sub>3</sub> as eluent, 0.078 g (49.7%) of **38** as a light-brown solid was obtained: mp 199–200 °C. MS (ESI)  $m/z$  517.1 (M + H)<sup>+</sup>.  $^1H$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: –0.07 to 0.09 (m, 3H), 0.90 (d,  $J$  = 4.78 Hz, 3H), 1.05–1.46 (m, 5H), 2.59–3.07 (m, 4H), 7.18 (d,  $J$  = 8.3 Hz, 2H), 7.60 (d,  $J$  = 8.31 Hz, 3H), 7.73 (d,  $J$  = 8.3 Hz, 1H), 8.58 (s, 1H),

8.89 (d,  $J = 12.6$  Hz, 1H), 11.38 (s, 1H), 12.54 (d,  $J = 12.6$  Hz, 1H). Anal. ( $C_{23}H_{25}BrN_4O_2 \cdot 0.6H_2O$ ) C, H, N.

**(4Z)-6-Bromo-4-({[5-(4-methylpiperazin-1-yl)pyridin-2-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (44a).** Using the procedure described for the preparation of **34**, 1.2 g (75%) of **44a** was obtained as a orange solid from 1.0 g (3.54 mmol) **17b** and 670 mg (3.54 mmol) of **41a**: mp 191–192 °C. MS (ESI)  $m/z$  444.0 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  ppm: 2.38 (s, 3H) 2.49–2.71 (m, 4H) 3.13–3.38 (m, 4H) 6.90 (d,  $J = 8.81$  Hz, 1H) 7.29 (d,  $J = 3.02$  Hz, 1H) 7.38 (dd,  $J = 8.56$ , 1.76 Hz, 1H) 7.91 (d,  $J = 1.51$  Hz, 1H) 8.03–8.14 (m, 2H) 8.53 (s, 1H) 9.15 (d,  $J = 12.09$  Hz, 1H) 12.34 (d,  $J = 12.09$  Hz, 1H). Anal. ( $C_{20}H_{20}BrN_5O_2 \cdot 0.7H_2O$ ) C, H, N.

**(4Z)-6-(3-Furyl)-4-({[6-(4-methylpiperazin-1-yl)pyridin-3-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (45b).** A mixture of 300 mg (0.68 mmol) of (4Z)-6-bromo-4-({[6-(4-methylpiperazin-1-yl)pyridin-3-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (**44b**), prepared using a similar procedure described for **18b**,  $Pd_2(dba)_3$  (125 mg, 0.136 mmol), tri-*tert*-butylphosphine (0.13 mL, 0.64 mmol), cesium carbonate (663 mg, 1.36 mmol), and 3-furan boronic acid (189.72 mg, 1.7 mmol) was placed in a three-neck flask under  $N_2$ , *N,N*-dimethylformamide (8 mL) was added, and the mixture was then stirred in a preheated oil bath 130 °C for 30 min. After cooling, the mixture was treated with  $CH_2Cl_2$  and filtered through celite. After evaporating all the solvents, the residue was dissolved in methylene chloride, washed three times with brine, dried over sodium sulfate, and evaporated. The orange oily residue was purified by silica gel chromatography (10:90 = methanol: methylene chloride) to give 130 mg (45%) of **45b** as a yellow solid: mp 200–201 °C. MS (ESI)  $m/z$  430.2 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ppm: 2.26 (s, 3H), 2.4–2.43 (m, 4H), 3.49–3.52 (m, 4H), 6.96 (d,  $J = 9.2$  Hz, 1H), 7.25 (m, 1H), 7.52 (dd,  $J = 8.4$ , 1.4 Hz, 1H), 7.82 (m, 1H), 7.90 (dd,  $J = 9.2$ , 2.0 Hz, 1H), 8.01 (d,  $J = 8.4$  Hz, 1H), 8.17 (m, 1H), 8.37 (d,  $J = 2.0$  Hz, 1H), 8.43 (s, 1H), 8.86 (d,  $J = 12.8$  Hz, 1H), 11.24 (s, 1H), 12.39 (d,  $J = 12.8$  Hz, 1H). Anal. ( $C_{24}H_{23}N_5O_3 \cdot 0.9H_2O$ ) C, H, N.

**(4Z)-6-(3-Furyl)-4-({[5-(4-methylpiperazin-1-yl)pyridin-2-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (45a).** By the procedure described for **45b**, **44a** (2.0g, 4.52 mmol) and 3-furan boronic acid (1.3 g, 11.3 mmol) were reacted to give 1.2g (63%) of **45a** as a yellow solid: mp 262–263 °C. MS (ESI)  $m/z$  430.1 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ppm: 2.23 (s, 3H), 2.45–2.49 (m, 4H), 3.08–3.25 (m, 4H), 7.21 (d,  $J = 2.4$  Hz, 1H), 7.53 (d,  $J = 2.4$  Hz, 1H), 7.55 (m, 2H), 7.83 (m, 1H), 8.03 (m, 1H), 8.11 (m, 2H), 8.44 (s, 1H), 9.13 (d,  $J = 12.8$  Hz, 1H), 11.30 (s, 1H), 12.45 (d,  $J = 12.8$  Hz, 1H). Anal. ( $C_{24}H_{23}N_5O_3 \cdot 1.13H_2O$ ) C, H, N: calcd, 15.58; found, 15.15.

**(4Z)-6-(3-Furyl)-4-({[6-(4-methylpiperazin-1-yl)pyridazin-3-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (45c).** By the procedure described for **45b**, **44c** (400 mg, 0.9 mmol) and 3-furan boronic acid (252 mg, 2.25 mmol) were reacted to give 90 mg (23%) of **45c** as a yellow solid: mp 275–276 °C. MS (ESI)  $m/z$  431.1 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ppm: 2.23 (s, 3H), 2.37–2.47 (m, 4H), 3.45–3.66 (m, 4H), 7.22 (m, 1H), 7.51 (d,  $J = 10.0$  Hz, 1H), 7.58 (dd,  $J = 8.0$ , 1.2 Hz, 1H), 7.83 (m, 1H), 7.91 (d,  $J = 10.0$  Hz, 1H), 8.03 (d,  $J = 8.0$  Hz, 1H), 8.17 (m, 1H), 8.45 (s, 1H), 9.13 (d,  $J = 12.0$  Hz, 1H), 11.38 (s, 1H), 12.51 (d,  $J = 12.0$  Hz, 1H). Anal. ( $C_{23}H_{22}N_6O_3 \cdot 1.0H_2O \cdot 0.1TFA$ ) C, H, N.

**(4Z)-4-({[3-Fluoro-4-(4-methylpiperazin-1-yl)phenyl]amino}-methylene)-6-(3-furyl)isoquinoline-1,3(2H,4H)-dione (45d).** By the procedure described for **45b**, **44d** (0.5 g, 1.09 mmol) and 3-furan boronic acid (0.2 g, 1.78 mmol) were reacted to give 0.32 g (65.8%) of **45d** as a light-brown solid: mp 203–204 °C. MS (ESI)  $m/z$  447.2 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ppm: 2.23 (s, 3H), 2.48 (s, 4H), 2.90–3.09 (m, 4H), 7.07 (t,  $J = 9.19$  Hz, 1H), 7.21–7.37 (m, 2H), 7.54 (dd,  $J = 8.3$ , 1.3 Hz, 1H), 7.65 (m, 1H), 7.83 (m, 1H), 8.01 (d,  $J = 8.3$  Hz, 1H), 8.20 (s, 1H), 8.43 (s, 1H), 8.86 (d,  $J = 12.6$  Hz, 1H), 11.28 (s, 1H), 12.47 (d,  $J = 12.6$  Hz, 1H). Anal. ( $C_{25}H_{23}FN_4O_3 \cdot 1.0H_2O$ ) C, H, N.

**(4Z)-6-(3-Furyl)-4-({[2-(4-methylpiperazin-1-yl)pyrimidin-5-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (45e).** By the procedure described for **45b**, **44e** (73.5 mg, 0.15 mmol) and 3-furan boronic acid (33 mg, 0.3 mmol) were reacted to give 36 mg (55%) of **45e** as a yellow solid: MS (ESI)  $m/z$  431 ( $M + H$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ppm: 2.22 (s, 3H), 2.29–2.43 (m, 4H), 3.61–3.94 (m, 4H), 7.23 (d,  $J = 1.0$  Hz, 1H), 7.52 (dd,  $J = 8.3$ , 1.3 Hz, 1H), 7.82 (t,  $J = 1.8$  Hz, 1H), 8.00 (d,  $J = 8.3$  Hz, 1H), 8.14 (s, 1H), 8.41 (s, 1H), 8.68 (s, 2H), 8.78 (d,  $J = 12.6$  Hz, 1H), 11.24 (s, 1H), 12.09 (d,  $J = 12.6$  Hz, 1H). Anal. ( $C_{23}H_{22}N_6O_3 \cdot H_2O \cdot 0.3TFA$ ) C, H, N.

**(4Z)-6-(3-Furyl)-4-({[5-(4-methylpiperazin-1-yl)pyrazin-2-yl]amino}methylene)isoquinoline-1,3(2H,4H)-dione (45f).** By the procedure described for **45b**, **44f** (500 mg, 0.41 mmol) and 3-furan boronic acid (114 mg, 1.02 mmol) were reacted to give 40 mg (23%) of **45f** as a yellow solid: mp 226–227 °C. MS (ESI)  $m/z$  431.1 ( $M + 1$ )<sup>+</sup>.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  ppm: 2.37 (s, 3H), 2.50–2.64 (m, 4H), 3.35–3.74 (m, 4H), 7.41 (dd,  $J = 8.0$ , 1.6 Hz, 1H), 7.55 (m, 1H), 7.77 (d,  $J = 1.6$  Hz, 1H), 7.90 (s, 1H), 7.93 (s, 1H), 7.99 (d,  $J = 8.0$  Hz, 1H), 8.23 (s, 1H), 8.41 (s, 1H), 9.08 (d,  $J = 12.4$  Hz, 1H), 11.24 (s, 1H) 12.45 (d,  $J = 12.4$  Hz, 1H). Anal. ( $C_{23}H_{22}N_6O_3 \cdot 1.0DMF \cdot 0.6TFA$ ) C, H, N.

## Biological Methods

**Enzyme Assay.** CDK4/cyclin D1, CDK6/cyclin D1, and CDK2/cyclin E were expressed in insect cells (Sf9) infected with recombinant baculovirus and partially purified using ammonium sulfate fractionation. CDK1/cyclin B1 was purchased from New England BioLabs (Beverly, MA). Test compounds were diluted in 20% DMSO/20 mM HEPES, pH 7.5, and serial dilutions were prepared (5 concentrations; 0.005–50  $\mu$ M). High-binding ELISA microtiter plates (Costar) were coated with the kinase substrate, glutathione-*S*-transferase (GST) fusion of C-terminal fragment of the retinoblastoma susceptibility gene product (Rb). Nonspecific binding sites were blocked with Superblock in Tris-buffered saline (TBS; Pierce). Kinase reactions contained the test inhibitor, 200  $\mu$ M ATP, 0.5 mg/mL bovine serum albumin (BSA; Sigma), and 0.1  $\mu$ L of enzyme. Reaction volumes were adjusted to 30  $\mu$ L with kinase assay buffer (50 mM HEPES, pH 7.5, 10 mM  $MgCl_2$ , 5% glycerol, 10 mM 2-mercaptoethanol), and plates were incubated at 30 °C for 1 h. Reactions were terminated by aspiration, and nonspecific sites were blocked with blocking buffer (TBS containing 0.1% Tween-20 and 5% nonfat dry milk). Phosphorylation of the substrate was detected using phospho-Rb specific antibodies (Ser 795) (Cell Signaling Technologies) and antirabbit IgG/horseradish peroxidase conjugates (Amersham Life Science) using TMB (3,3',5,5'-tetramethylbenzidine) as substrate. Colorimetric reactions were stopped with 2 N sulfuric acid, and the absorbance was measured at 450 nm.  $IC_{50}$  values were determined from inhibition plots.

**Cell Culture.** MCF-7 cells were cultured in RPMI medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen) and 50  $\mu$ g/mL gentamicin (Invitrogen) at 37 °C in a humidified incubator under 5–7%  $CO_2$ .

**Cell Proliferation Assay.** Cells were plated in 96-well plates and cell growth was determined using sulforhodamine B (SRB), a protein binding dye. Briefly, cells were fixed with TCA and rinsed extensively in water. Cells were stained with 0.4% sulforhodamine B (Sigma-Aldrich, St Louis, MO) and washed in 1% acetic acid. Protein-associated dye was solubilized in 10 mM Tris, and the absorbance was measured in a Victor fluorescence reader (Wallac/Perkin-Elmer Life Sciences, Boston, MA).

**Flow Cytometry.** Cells growing in 6-well tissue culture plates were pulse-labeled with 10  $\mu$ M bromo-deoxyuridine for 30 min. Cells were collected by trypsinization, and fixed in 80%

methanol at  $-20^{\circ}\text{C}$ . After acid denaturation and permeabilization, the cells were stained with antibromodeoxyuridine-FITC conjugates (BD Biosciences, Mountain View, CA), counterstained with propidium iodide, and analyzed by flow cytometry (FACSort, BD Biosciences). Data were analyzed using Lysis II or CELLQuest software (BD Biosciences).

**Preparation of Cell Extracts and Protein Immunoblotting.** Cell lysates were prepared in lysis buffer (25 mM Tris-Cl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate) supplemented with 0.2 mM PMSF, 1 mM DTT, 0.2 mM sodium fluoride, and 1 mM sodium vanadate (all chemical reagents were from Sigma-Aldrich, St Louis, MO). Protein concentrations were determined by the BioRad protein assay (BioRad, Hercules, CA). Proteins (10–20  $\mu\text{g}$ ) were separated by electrophoresis on 8 or 10% polyacrylamide-SDS gels (SDS-PAGE) and transferred to nitrocellulose. Blots were blocked in phosphate buffered saline (PBS) or tris-buffered saline (TBS) containing 5% skim milk (or 5% BSA) and 0.1% Tween-20 and incubated with antibodies in the same buffer. Blots were developed using enhanced chemiluminescence (ECL, Amersham/GE Healthcare, Piscataway, NJ). Antibodies used were: cyclin D1 and phospho-Rb (Ser 795) (Cell Signaling Technologies, Beverly, MA), cyclin E and cyclin B1 (Santa Cruz Biotechnologies), Rb (Santa Cruz Biotechnologies, Santa Cruz, CA or EMD Biosciences, Darmstadt, Germany), CDK4 (Upstate, Lake Placid, NY), CDK2, CDK1, and p27 (Santa Cruz Biotechnologies), actin (Chemicon, Temecula, CA), and rabbit and mouse IgG-horseradish peroxidase conjugates (Amersham).

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**Supporting Information Available:** Elementary analysis data for compounds **5a–5d, 18a, 18b, 18e–18i, 19b–19i, 20a, 21a–21d, 22c–22e, 22g, 23a–23l, 34–38, 44a, and 45a–45f**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Massague, J. G1 cell cycle and cancer. *Nature* **2004**, *432*, 298–306.
- Shapiro, G. I. Cyclin-Dependent Kinase Pathways As Targets for Cancer Treatment. *J. Clin. Oncol.* **2006**, *24* (11), 1770–1783.
- Dyson, N. The regulation of E2F by pRB-family proteins. *Gene Dev.* **1998**, *12*, 2245–2262.
- Hanahan, D.; Weinberg, R. A. The hallmarks of cancer. *Cell* **2000**, *100*, 57–70.
- Grillo, M.; Bott, M. J.; Khandke, N.; McGinnis, J. P.; Miranda, M.; Meyyappan, M.; Rosfjord, E. C.; Rabindran, S. K. Validation of cyclin D1/CDK4 as an anticancer drug target in MCF-7 breast cancer cells: Effect of regulated overexpression of cyclin D1 and siRNA-mediated inhibition of endogenous cyclin D1 and CDK4 expression. *Breast Cancer Res. Treat.* **2006**, *95*, 185–194.
- Malumbres, M.; Barbacid, M. Is Cyclin D1-CDK4 kinase a bona fide cancer target? *Cancer Cell* **2006**, *9*, 2–4.
- Tetsu, O.; McCormick, F. Proliferation of cancer cells despite cdk2 inhibition. *Cancer Cell* **2003**, *3*, 233–245.
- Ortega, S.; Prieto, I.; Odajima, J. Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat. Genet.* **2003**, *35*, 25–31. (a) Berthet, C.; Aleem, E.; Coppola, V. Cdk2 knockout mice are viable. *Curr. Biol.* **2003**, *13*, 1775–1785.
- Sanderwicz, A. M. Novel small molecule cyclin-dependent kinases modulators in human clinical trials. *Cancer Biol Ther.* **2003**, *2*, S84–S95.
- Fry, D. W.; Harvey, P. J.; Keller, P. R. Specific inhibition of cyclin-dependent kinase 4/6 by PD0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Ther.* **2004**, *3*, 1427–1438.
- McClue, S. J.; Blake, D.; Clarke, R. In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (Roscovitine). *Int. J. Cancer* **2002**, *102*, 463–468.
- Misra, R. N.; Xiao, H. Y.; Kim, K. S. *N*-(cycloalkylamino)acyl-2-aminothiazole inhibitors of cyclin-dependent kinase 2. *N*-[5-[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS-387032), a highly efficacious and selective antitumor agent. *J. Med. Chem.* **2004**, *47*, 1719–1728.
- Grell, W.; Walter, R.; Heckel, A.; Himmelsbach, F.; Wittneben, H.; van Meel, J.; Redemann, N.; Haigh, R. Preparation of 3-( $\alpha$ -heteroarylaminobenzylidene)-2-indolinones as cyclin dependent kinase inhibitors. German Patent DE 19815020 A1, October 7, 1999.
- Crockett, G. C.; Swanson, B. J.; Anderson, D. R.; Koch, T. H. A preferred method for imide preparation. *Synth. Commun.* **1981**, *11*, 447–454.
- Wolfbeis, O. S.; Trummer, I.; Knierzinger, A. 2-Substituierte pyran[2,3-c]isoquinolin-3,6-dione und merocyaninfarbstoffe aus homophthalosaureimiden. *Liebigs Ann. Chem.* **1981**, 811–818. (a) Perez, D.; Bures, G.; Guitian, E.; Castedo, L. Synthesis of antitumor lycorines by intramolecular Diels–Alder reaction. *J. Org. Chem.* **1996**, *61*, 1650–1654.
- Hauser, F. M.; Rhee, R. Carboxylation of dilithium *ortho*-toluates. *Synthesis* **1977**, 245–246.
- Young, S. D.; Wiggins, J. M.; Huff, J. R. 3,4-Dihydrobenz[f]isoquinoline and 3,4-dihydrobenz[g]isoquinoline. *J. Org. Chem.* **1988**, *53*, 1114–1116.
- Shtutman, M.; Zhurinsky, J.; Simcha, I.; Albanese, C.; D’Amico, M.; Pestell, R.; Ben-Ze’ev, A. The cyclin D1 gene is a target of the  $\beta$ -catenin/LEF-1 pathway. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5522–5527.
- Tam, S. W.; Theodoras, A. M.; Shay, J. W.; Draetta, G. F.; Pagano, M. Differential expression and regulation of cyclin D1 protein in normal and tumor human cells: association with cdk4 is required for cyclin D1 function in G1 progression. *Oncogene* **1994**, *9*, 2663–2674.
- Ikuta, M.; Kamata, K.; Fukasawa, K.; Honma, T.; Machida, T.; Hirai, H.; Suzuki-Takahashi, I.; Hayama, T.; Nishimura, S. Crystallographic approach to identification of cyclin-dependent kinase 4 (CDK4)-specific inhibitors by using CDK4 mimic CDK2 protein. *J. Biol. Chem.* **2001**, *276*, 27548–27554.
- GLIDE; Schrodinger Software: Portland, OR; [www.schrodinger.com](http://www.schrodinger.com).
- (a) McInnes, C.; Wang, S.; Anderson, S.; O’Boyle, J.; Jackson, W.; Kontopidis, G.; Meades, C.; Mezna, M.; Thomas, M.; Wood, G.; Lane, D. P.; Fischer, P. M. Structural Determinants of CDK4 Inhibition and Design of Selective ATP Competitive Inhibitors. *Chem. Biol.* **2004**, *11*, 525–534. (b) Honma, T.; Hayashi, K.; Aoyama, T.; Hashimoto, N.; Machida, T.; Fukasawa, K.; Iwama, T.; Ikeura, C.; Iduta, M.; Suzuki-Takahashi, I.; Iwasawa, Y.; Hayama, T.; Nishimura, S.; Morishima, H. Structure-Based Generation of a New Class of Potent cdk4 Inhibitors: New de Novo Design Strategy and Library Design. *J. Med. Chem.* **2001**, *44*, 4615–4627. (c) Aubry, C.; Wilson, A. J.; Jenkins, P. R.; Mahale, S.; Chaudhuri, B.; Marechal, J.; Sutcliffe, M. J. Design, synthesis and biological activity of new CDK4-specific inhibitors based on fascaplysin. *Org. Biomol. Chem.* **2006**, *4*, 787–801.
- Park, H.; Yeom, M. S.; Lee, S. Loop flexibility and solvent dynamics as determinants for the selective inhibition of cyclin-dependent kinase 4: Comparative molecular dynamics simulation studies of CDK2 and CDK4. *ChemBioChem.* **2004**, *5*, 1662–1672.
- Pratt, D. J.; Bentley, J.; Jewsbury, F.; Boyle, T.; Endicott, J. A.; Noble, M. E. M. Dissecting the Determinants of Cyclin-Dependent Kinase 2 and Cyclin-Dependent Kinase 4 Inhibitor Selectivity. *J. Med. Chem.* **2006**, *49*, 5470–5477.
- SYBYL, version 7.2; Tripos, Inc.: St. Louis, MO.
- Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J. Comput. Aided Mol. Des.* **1997**, *11*, 425–445.
- Jorgensen, W. L.; Maxwell, D. S.; Triado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energistics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996**, *118* (45), 11225–11236.
- Quallich, G. J.; Makowski, T. W.; Sanders, A. F.; Urban, F. J.; Vazquez, E. Synthesis of 1,2,3,4-Tetrahydroisoquinolines Containing Electron-Withdrawing Groups. *J. Org. Chem.* **1998**, *63*, 4116–4119.
- Hurst, D. T.; Christophides, J. The synthesis of some 2-(substituted) 5-nitropyrimidines. *Heterocycles* **1977**, *6* (12), 1999–2004.