

# Discovery of 4-(Benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-diones and 4-[(Pyridylmethyl)aminomethylene]isoquinoline-1,3-(2*H*,4*H*)-diones as Potent and Selective Inhibitors of the Cyclin-Dependent Kinase 4

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The series of 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-dione and 4-[(pyridylmethyl)aminomethylene]isoquinoline-1,3-(2*H*,4*H*)-dione derivatives reported here represents a novel class of potential antitumor agents, which potently and selectively inhibit CDK4 over CDK2 and CDK1. In the benzylamino headpiece, a 3-OH substituent is required on the phenyl ring for CDK4 inhibitory activity, which is further enhanced when an iodo, aryl, heteroaryl, *t*-butyl, or cyclopentyl substituent is introduced at the C-6 position of the isoquinoline-1,3-dione core. To circumvent the metabolic liability associated with the phenolic OH group on the 4-substituted 3-OH phenyl headpiece, we take two approaches: first, introduce a nitrogen *o*- or *p*- to the 3-OH group in the phenyl ring; second, replace the phenyl headpiece with N-substituted 2-pyridones. We present here the synthesis, SAR data, metabolic stability data, and a CDK4 mimic model that explains the binding, potency, and selectivity of our CDK4 selective inhibitors.

## Introduction

Progression of cells through the cell division cycle is regulated by a family of serine/threonine kinases (CDKs<sup>a</sup>) and their activating partners, the cyclins.<sup>1</sup> Different combinations of cyclin/CDK complexes regulate each of the cell cycle transitions. In the G1-S phase transition, the D-type cyclins (D1, D2, or D3) in complex with CDK4 (or CDK6 in hematopoietic cells), along with cyclin E/CDK2 complexes, cooperatively phosphorylate the retinoblastoma susceptibility gene family of proteins (Rb).<sup>1–3</sup> Phosphorylation of Rb converts it from a repressor to an activator of the E2F transcription factors, leading to the transcription of genes required for DNA synthesis.<sup>4</sup> The expression/activity of G1 cyclin/CDK complexes are positively regulated by the mitogenic signaling pathways and negatively regulated by the cyclin-dependent kinase inhibitors (CKIs), which include p16<sup>INK4a</sup>, p15<sup>INK4b</sup>, p21<sup>CIP1</sup>, and p27<sup>KIP1</sup>.<sup>2</sup> This coordinated regulation facilitates sequential activation of the G1 cyclin/CDK complexes required for orderly transition into S-phase.

Aberrant control of cell proliferation is a hallmark of cancer,<sup>5</sup> and the p16<sup>INK4a</sup>-cyclin D1/CDK4-Rb axis has been implicated in this dysregulation.<sup>6,7</sup> CDK4 mutants that are resistant to p16 have been identified in melanoma, while loss of p16 activity due to mutations, deletions, or gene silencing has been observed in glioblastoma, pancreatic cancers, and NSCLC.<sup>6</sup> Cyclin D1 is deregulated by chromosomal translocation in B-cell lymphoma and parathyroid adenomas, and is overexpressed in breast, esophagus, head and neck, and colon cancers.<sup>6,7</sup> CDK4 is overexpressed in osteosarcomas, and Rb is mutated in retinoblastomas and SCLC.<sup>6,7</sup> We recently reported that the

inhibition of endogenous cyclin D1 or CDK4 expression by RNA interference in MCF-7 breast cancer cells resulted in the inhibition of cell growth, hypophosphorylation of Rb, and accumulation of cells in the G1 phase.<sup>8</sup> This result supports the view that pharmacological inhibition of cyclin D1/CDK4 complexes is a useful strategy to inhibit the growth of tumors. Consistent with this, microinjection of cyclin D1 antibodies or antisense oligonucleotides in cells caused G1/S arrest and decreased tumorigenesis in nude mice.<sup>9–12</sup> The importance of deregulated cyclin D/CDK4 complexes in tumorigenesis is underscored by the observation that both cyclin D1 and CDK4 knockout mice are resistant to ErbB-2 induced tumorigenesis.<sup>13,14</sup> Importantly, this effect is mediated by the catalytic activity of CDK4 since mice expressing a mutant cyclin D1 that binds to, but cannot activate CDK4/6, are resistant to ErbB-2-mediated tumorigenesis.<sup>15</sup>

In the past few years, several small-molecule CDK inhibitors have been identified, but none have been approved for clinical use.<sup>16</sup> Flavopiridol and CYC-202 (Seliciclib), which inhibit multiple CDKs, are in clinical trials but have only shown modest activity.<sup>16a</sup> Until recently, flavopiridol was reported to demonstrate a marked clinical efficacy in treating patients with chronic lymphocytic leukemia by using a pharmacologically derived schedule for drug administration.<sup>16b</sup> Among the second generation of CDK inhibitors, a pyridopyrimidine (PD-0332991),<sup>17</sup> a 3-aminothioacridone,<sup>18</sup> a triaminopyrimidine derivative (CINK4),<sup>19</sup> a diarylurea,<sup>20</sup> and 3-( $\alpha$ -heteroarylaminobenzylidene)-2-indolinones<sup>21</sup> are reported to be selective for CDK4/6, as compared with other CDKs. Recently, we reported a novel series of 4-(phenylaminomethylene)isoquinoline-1,3-diones as potent and selective inhibitors of CDK4.<sup>22</sup> In that series, substitution with an *N*-methylpiperazine or a piperidinylmethyl group at the para position of the aniline headpiece is essential for CDK4 potency. Here, we report a new series, 4-[(3-hydroxybenzylamino)-methylene]-4*H*-isoquinoline-1,3-diones, as highly potent and selective CDK4 inhibitors. Compared to our earlier series where the headpiece was a substituted aniline, this new series

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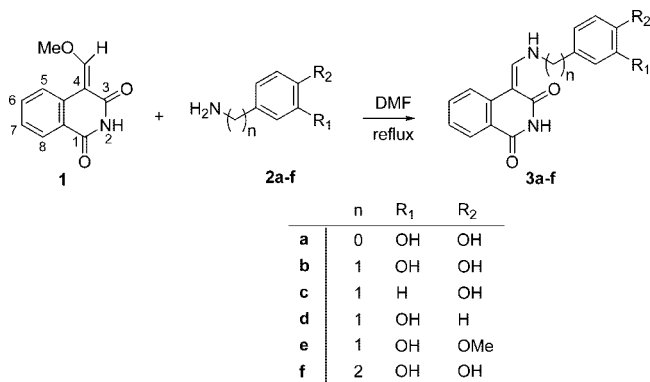
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<sup>a</sup> Abbreviations: CDK, cyclin-dependent kinase.

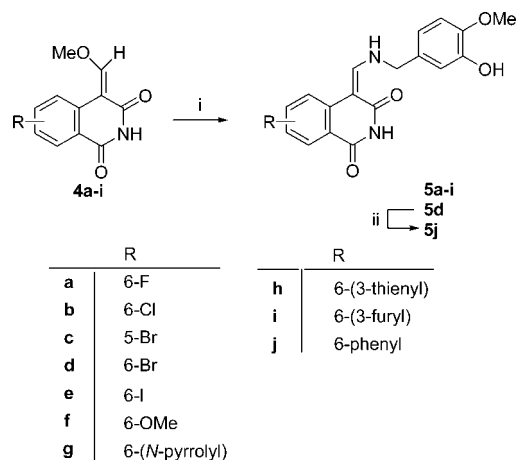
## Scheme 1



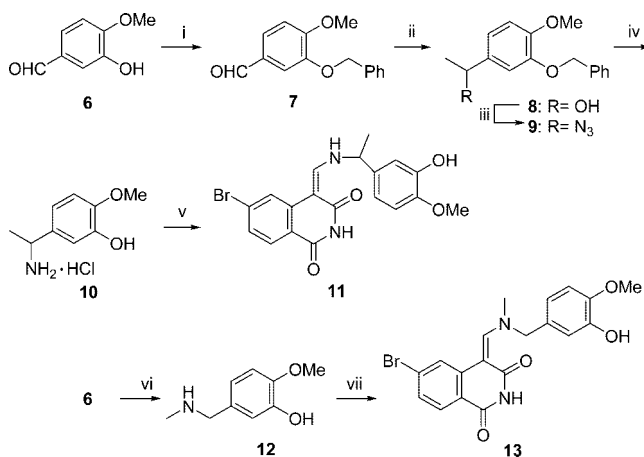
carried a substituted 3-hydroxybenzylamino headpiece and exhibited higher potency for CDK4 and higher activity in inhibiting cell proliferation. We describe the details of our optimization efforts and the structure–activity relationships relevant to the substituents at the C-4, C-5, and C-6 positions of the isoquinoline-1,3-dione core. Furthermore, we show a closely related new series, where the benzylamino headpiece was replaced with the pyridylmethylamino headpieces, to enhance metabolic stability while maintaining potency as CDK4 selective inhibitors. We also present one of our inhibitors docked in the ATP binding domain of the CDK4 mimic model, to show the critical hydrogen bond interactions that may explain the CDK4 selectivity as well as potency.

**Chemistry.** As shown in Scheme 1, 4-(methoxymethylene)isoquinoline-1,3-dione (**1**)<sup>22</sup> was reacted with 3,4-dihydroxyaniline (**2a**) or 3,4-dihydroxyphenethylamine (**2f**) to give the corresponding aniline derivative (**3a**) and phenethylamine derivative (**3f**), respectively, similar to the protocol used in our earlier paper.<sup>22</sup> The corresponding benzylamine derivatives (**3b–e**) were obtained from the reaction of **1** and benzylamines (**2b–e**). The early lead compound **3e** has a (3-hydroxy-4-methoxybenzyl)aminomethylene substituent at the C-4 of the isoquinoline-1,3-dione core, with no substituents on the rest of the core. We began to explore analogues carrying substituents at either the C-5 or the C-6 position. The 5-bromo and 6-halogen analogues, namely, **5c** and **5a,b,d,e**, were prepared from the corresponding 5-bromo intermediate (**4c**) and 6-halogen intermediates (**4a,b,d,e**),<sup>22</sup> respectively, as depicted in Scheme 2. Similarly, the 6-methoxy derivative (**5f**), 6-(*N*-pyrrolyl) derivative (**5g**), 6-(3-thienyl) derivative (**5h**), and 6-(3-furyl) derivative (**5i**) were prepared from the corresponding 6-methoxy, 6-(*N*-pyrrolyl), 6-(3-thienyl), and 6-(3-furyl) intermediates, namely, **4f–i**,<sup>22</sup> respectively. The 6-bromo derivative (**5d**) was further reacted with phenylboronic acid, under Suzuki coupling conditions, to yield the 6-phenyl derivative (**5j**).

The linker group at C-4 of **5d** was varied by introducing a methyl group at the methylene or the nitrogen of the benzylamine, namely, **11** and **13**, as shown in Scheme 3. The hydroxyl group of 3-hydroxy-4-methoxybenzaldehyde (**6**) was protected as the benzyl ether, and then methyllithium was added to the aldehyde group of **7** to form the secondary alcohol **8**. The alcohol **8** was treated with (PhO)<sub>2</sub>P(O)N<sub>3</sub> and DBU in toluene to yield the corresponding azide (**9**), which was reduced and debenzylated to yield the amine hydrochloride salt **10**. Coupling of **4d** with **10** afforded **11**. Derivative **13** was prepared by reacting **4d** with *N*-methyl-3-hydroxy-4-methoxybenzylamine (**12**), which was prepared by reductive amination of **6** with methylamine.

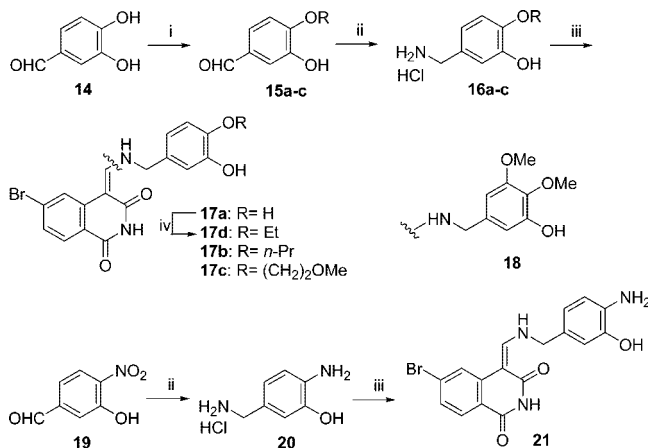
Scheme 2<sup>a</sup>

<sup>a</sup> (i) **2e**, DMF, Et<sub>3</sub>N, room temperature; (ii) PhB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, DMF, microwave, 150 °C.

Scheme 3<sup>a</sup>

<sup>a</sup> (i) PhCH<sub>2</sub>Br, K<sub>2</sub>CO<sub>3</sub>, DMF, room temperature; (ii) THF, MeLi in ethyl ether, -78 °C; (iii) (PhO)<sub>2</sub>P(O)N<sub>3</sub>, toluene, DBU, 0 °C; (iv) Pd/C, H<sub>2</sub>, EtOH, HCl; (v) **4d**, DMF, Et<sub>3</sub>N, room temperature; (vi) MeNH<sub>2</sub>, EtOH, room temperature; then NaBH<sub>4</sub>, MeOH; (vii) **4d**, THF, room temperature.

Early on, we observed that moving the OH group from C-3 (**3d**) to C-4 (**3c**) on the benzylamine, resulted in a dramatic loss of potency. Additional analogues also supported the importance of the OH group, at the C-3 position of benzylamine, for activity. Therefore, in the majority of our analoguing efforts, we kept this unique structural feature unchanged. We turned our attention to study the effect of the methoxy group at C-4 of the benzylamine in **5d**, by replacing it with a variety of other functional groups, as depicted in Scheme 4. Treatment of 3,4-dihydroxybenzaldehyde (**14**) and potassium carbonate in DMF, with 1-bromopropane, or 2-bromoethyl methyl ether, provided the corresponding 3-hydroxy-4-alkoxybenzaldehydes **15b,c**, after purification by column chromatography. Subsequent reaction of **15a–c** with methoxyamine hydrochloride in pyridine to form *O*-methyl-oximes, followed by catalytic hydrogenation, yielded the corresponding benzylamine hydrochlorides **16a–c**. Treatment of **4d** with **16a–c** gave the desired 4-alkoxy derivatives **17a–c**, respectively. Further reaction of **17a** with iodoethane and potassium carbonate in DMF yielded the ethoxy derivative **17d** after purification by high performance liquid chromatography. The 3-hydroxy-4,5-dimethoxy derivative **18** was prepared in a similar way from **4d** and 3-hydroxy-4,5-dimethoxybenzylamine. An additional analogue of **5d**, where the 4-methoxy

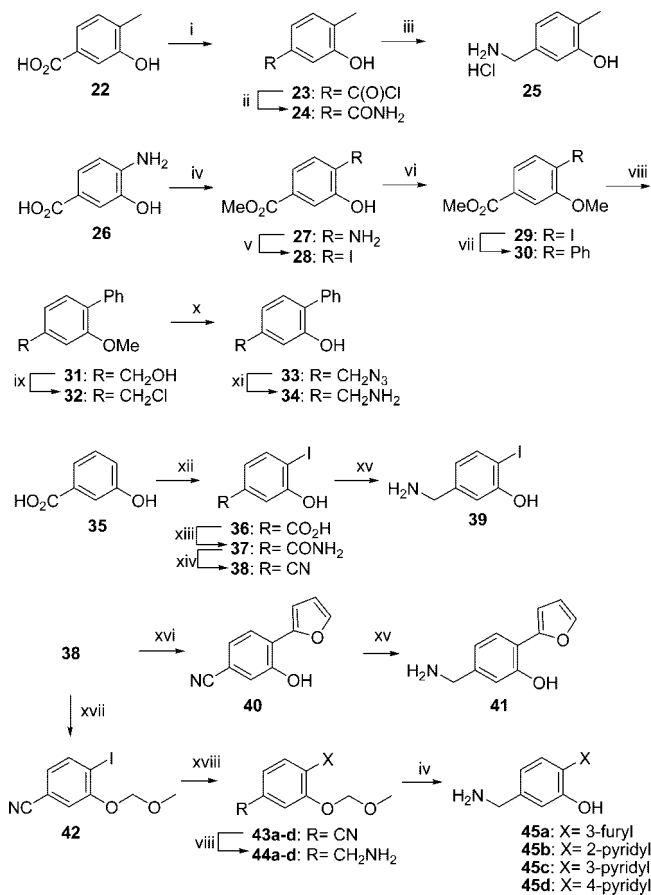
Scheme 4<sup>a</sup>

<sup>a</sup> (i) RBr, K<sub>2</sub>CO<sub>3</sub>, or Na<sub>2</sub>CO<sub>3</sub>, DMF, room temperature or 65 °C; (ii) MeONH<sub>2</sub> · HCl, pyridine, room temperature; then Pd/C, H<sub>2</sub>, EtOH, HCl; (iii) **4d**, DMF, room temperature; (iv) EtI, K<sub>2</sub>CO<sub>3</sub>, DMF, 65 °C.

group was replaced with an amine, was prepared starting from 3-hydroxy-4-nitrobenzaldehyde **19**. Hydrogenation of the oxime of **19** followed by reaction with **4d** provided the desired 4-amino analogue **21**.

Other methoxy replacements at C-4 of the benzylamine headpiece in **5d** included methyl and phenyl groups. Their preparations (**25** and **34**) are shown in Scheme 5. 3-Hydroxy-4-methylbenzoic acid **22**, was converted to the corresponding benzoyl chloride **23**, followed by the addition of ammonium hydroxide, to provide 3-hydroxy-4-methylbenzamide **24**. Reduction of **24** gave the desired 3-hydroxy-4-methylbenzylamine **25**. 3-Hydroxy-4-phenylbenzylamine **34** was prepared from 3-hydroxy-4-aminobenzoic acid **26**. After **26** was esterified, it was converted to the diazonium salt, and the latter was treated with potassium iodide to yield 3-hydroxy-4-iodobenzoate **28**. The 3-hydroxy group of **28** was protected as the methyl ether, then the resulting **29** was treated with phenylboronic acid under Suzuki coupling conditions to yield 3-methoxy-4-phenylbenzoate **30**. The benzoate group of **30** was reduced to the benzyl alcohol **31**,<sup>23</sup> which was then activated as the benzyl chloride **32**. Demethylation of **32** with boron tribromide, followed by treatment with sodium azide, yielded 3-hydroxy-4-phenylbenzylazide **33**, which was converted to the desired 3-hydroxy-4-phenylbenzylamine **34** under Staudinger reaction conditions.

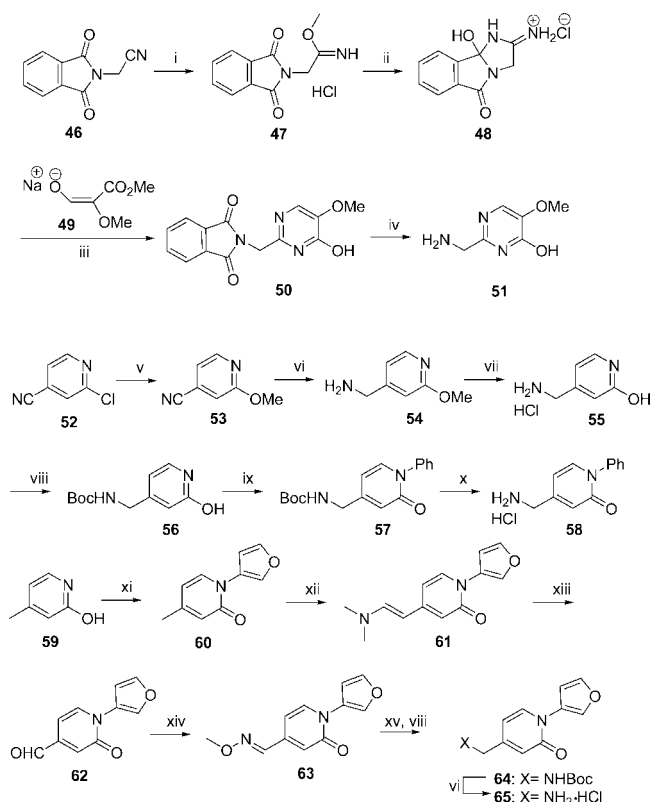
In addition, heteroaryl groups were introduced as methoxy replacements at the C-4 of the benzylamine headpiece in **5d**. Compounds **41** and **45a–d** were prepared via a common intermediate, 3-hydroxy-4-iodobenzonitrile **38**, which was readily synthesized from 3-hydroxybenzoic acid **35** using a procedure modified from Sagi's route.<sup>24a</sup> Treatment of **38** with 2-furyl-tributyltin under Stille coupling conditions, followed by borane reduction, gave 3-hydroxy-4-(2-furyl)benzylamine **41**. The corresponding 3-furyl derivative **45a** was prepared in a different way. The hydroxyl group of **38** was protected with MOMCl to provide **42**. The latter intermediate was then coupled with 3-furylboronic acid, followed by reduction of the nitrile group with lithium aluminum hydride, to yield 3-methoxymethoxy-4-(3-furyl)benzylamine **44a**. Refluxing of **44a** in methanolic hydrochloric acid removed the MOM group to give the desired 3-hydroxy-4-(3-furyl)benzylamine **45a**. The corresponding three 4-pyridyl regioisomers **45b–d** were prepared in a similar fashion, except that the introductions of the pyridyl groups were carried out via Stille coupling procedures instead of Suzuki coupling conditions.

Scheme 5<sup>a</sup>

<sup>a</sup> (i) (COCl)<sub>2</sub>, 60 °C; (ii) NH<sub>4</sub>OH, 0 °C to room temperature; (iii) BH<sub>3</sub>, THF, 0 °C; (iv) HCl, MeOH, reflux; (v) H<sub>2</sub>O, HCl, NaNO<sub>2</sub>, 0 °C; then KI, H<sub>2</sub>O, room temperature; (vi) NaH, DMF, then MeI, 0 °C; (vii) PhB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, DMF, 100 °C; (viii) LiAlH<sub>4</sub>, Et<sub>2</sub>O; (ix) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (x) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C; then NaN<sub>3</sub>, DMF, room temperature; (xi) PPh<sub>3</sub>, THF, H<sub>2</sub>O, room temperature; (xii) NH<sub>4</sub>OH, then I<sub>2</sub>, KI, H<sub>2</sub>O; (xiii) Et<sub>3</sub>N, THF, isobutylchloroformate, 0 °C; then NH<sub>3</sub> in THF, room temperature; (xiv) SOCl<sub>2</sub>, toluene, reflux; (xv) BH<sub>3</sub>, THF, room temperature; (xvi) PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, 2-furylSnBu<sub>3</sub>, CuI, DMF, 100 °C; (xvii) NaH, MOMCl, DMF, 0 °C; (xviii) (for **43a**) 3-furylboronic acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, DMF, 100 °C; (for **43b–d**) XSnBu<sub>3</sub>, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, CuI, DMF, 100 °C.

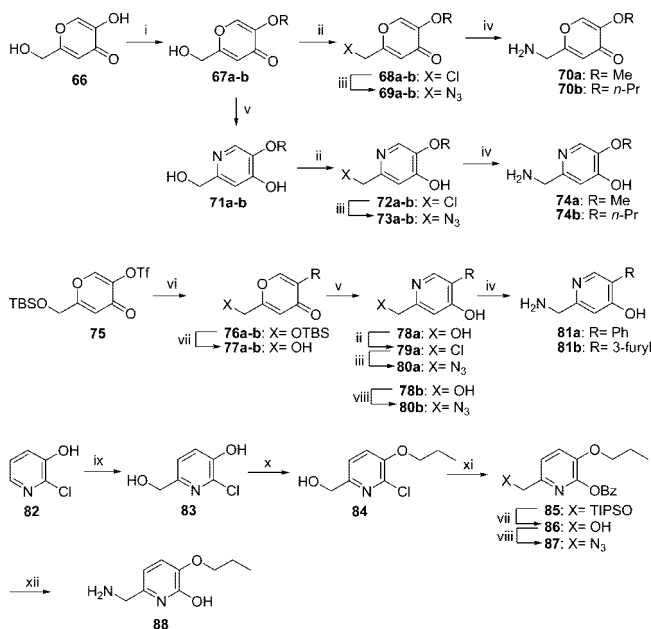
We then explored ways in our designs of new analogues to minimize the potential metabolic liability of the 3-OH group in the benzylamine headpiece while maintaining the potency and selectivity in the series. One way was to replace the phenyl ring of the 3-hydroxy-4-methoxybenzylamine headpiece with pyrimidine ring **51**, as shown in Scheme 6. Substituted acetone nitrile **46**<sup>24b</sup> was treated with hydrochloric acid in dioxane to form acetimidate hydrochloride **47**. Subsequent treatment of **47** with ammonia gave the tricyclic iminium salt **48**, which was then coupled with the sodium salt of 3-hydroxy-2-methoxyacrylic acid methyl ester **49**<sup>25</sup> to produce the pyrimidinol derivative **50**. Hydrazinolysis of **50** deprotected the phthalimide to yield the desired 2-aminomethyl-5-methoxy-pyrimidin-4-ol, **51**.

Other attempts to enhance metabolic stability of the phenolic OH in the headpiece involved the preparation of the corresponding 2-pyridinol **55**. Nucleophilic displacement of the chloro group of 2-chloro-4-cyanopyridine, **52**, with sodium methoxide,<sup>26</sup> followed by hydrogenation of the nitrile group of **53** with Raney Ni, in the presence of ammonia<sup>27</sup> gave 4-(2-methoxypyridyl)methylamine, **54**. Acid hydrolysis of the methoxy group of **54** provided the desired 2-pyridinol derivative **55**. To evaluate the contribution of potency from the 2-pyridinol

Scheme 6<sup>a</sup>

headpiece **55**, where N-1 is unsubstituted, we prepared the N-1 substituted 2-pyridones **58** and **65**, which no longer carry an OH group. Boc protection of the primary amine of **55** set the stage for installation of a phenyl substituent on N-1 of the pyridinol **56**, via treatment with trimethylphenyl tin in the presence of cupric acetate and *n*-tetrabutylammonium fluoride to generate the Boc protected 1-phenyl-2-pyridone derivative **57**. Acid treatment of **57** produced the desired 4-aminomethyl-1-phenyl-2-pyridone **58**. An alternate synthetic approach was used to synthesize the corresponding 1-(3-furyl)-2-pyridone derivative, **65**. 2-Hydroxy-4-methylpyridine **59** was N-arylated with 3-bromofuran via the copper-catalyzed Ullmann condensation<sup>28</sup> to yield 1-(3-furyl)-4-methyl-pyridone **60**. The 4-methyl group of **60** was extended to *N,N*-dimethylenamine **61**, which upon periodate oxidation produced the aldehyde **62**. The aldehyde **62** was then converted into its oxime **63**, which upon reduction with zinc in acetic acid yielded the desired, but impure, amine **65**. Because of the difficulty in purifying the free amine, we converted **65** into its Boc protected derivative **64**, which allowed easy purification. Final deprotection of the Boc group of **64** yielded pure 4-aminomethyl-1-(3-furyl)-2-pyridone, **65**.

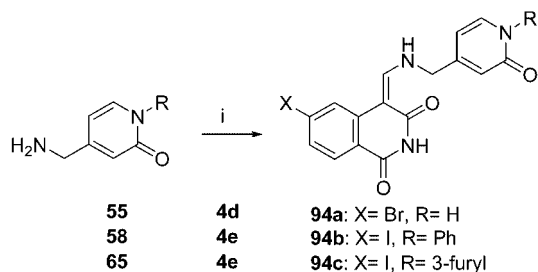
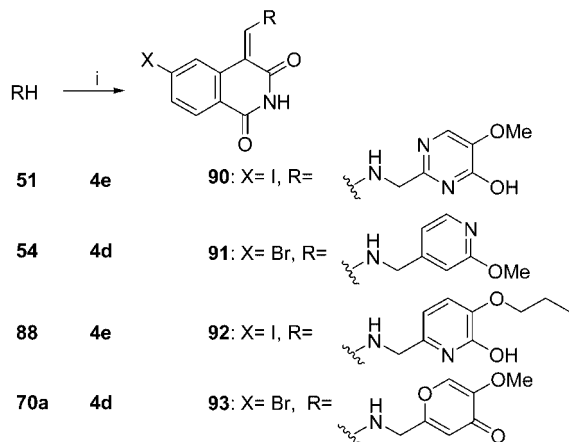
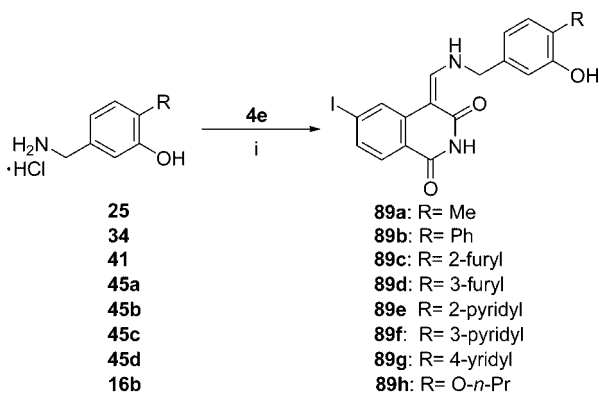
Additional headpieces, such as substituted 4-pyrones **70a–b**, 4-pyridinols **74a–b**, **81a–b**, and 6-pyridinol **88**, were prepared as shown in Scheme 7. Kojic acid **66** was alkylated,<sup>29</sup> and then the OH group was transformed into the NH<sub>2</sub> functionality,<sup>30</sup> under the same conditions used to prepare **34**, to yield the desired 2-aminomethyl-5-alkoxy-4-pyrones **70a–b**. The corresponding pyridinols **74a–b** were prepared starting from the

Scheme 7<sup>a</sup>

<sup>a</sup> (i) RBr, KI, K<sub>2</sub>CO<sub>3</sub>, DMF; (ii) SOCl<sub>2</sub>, CHCl<sub>3</sub>; (iii) NaN<sub>3</sub>, DMF, room temperature; (iv) Ph<sub>3</sub>P, THF, then H<sub>2</sub>O, room temperature; (v) conc. NH<sub>4</sub>OH or NH<sub>3</sub> in MeOH, 90 °C, sealed tube; (vi) RB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, KBr, dioxane, 60 °C; (vii) *n*-Bu<sub>4</sub>F, THF, room temperature; (viii) Ph<sub>3</sub>P, DMF, CBr<sub>4</sub>; then NaN<sub>3</sub>; (ix) (CH<sub>2</sub>O)<sub>n</sub>, NaHCO<sub>3</sub>; (x) *n*-PrI, K<sub>2</sub>CO<sub>3</sub>, 2-butanone; (xi) (*i*-Pr)<sub>3</sub>SiCl, imidazole, CH<sub>2</sub>Cl<sub>2</sub>, room temperature; NaOBz, BzOH, microwave, 120 °C; (xii) Ph<sub>3</sub>P, THF, H<sub>2</sub>O; then EtOH, Pd/C, H<sub>2</sub>.

4-pyrone intermediates **67a–b**. Treatment of **67a–b** with ammonia<sup>29</sup> in a sealed tube generated the corresponding 2-hydroxymethyl-5-alkoxy-4-pyridinols **71a–b**, which were then converted into **74a–b**, under the conditions described for the preparation of **70a–b**. Additional analogues of **74a–b** where the alkoxy group was replaced by aryl and heteroaryl groups were also prepared. 4-Pyrone-5-triflate **75**<sup>31</sup> was coupled with phenylboronic acid or furan-3-boronic acid under Suzuki coupling conditions, followed by the removal of the silyl protecting group to yield 5-phenyl-4-pyrone derivative **77a** and 5-(3-furyl)-4-pyrone derivative **77b**, respectively. The pyrones **77a–b** were then converted into pyridinols **78a–b**, followed by transformation of the hydroxymethyl group into the aminomethyl moiety, under the same conditions used to prepare **74a**, to yield the desired 5-phenyl- (**81a**) and 5-(3-furyl)-4-pyridinol (**81b**) derivatives. The 6-pyridinol isomer of **74b**, namely, **88**, was obtained from 2-chloro-3-pyridinol **82**. Treatment of **82** with paraformaldehyde in base<sup>32</sup> gave 6-hydroxymethyl-2-chloro-3-pyridinol **83**, followed by propylation of the phenolic group to give **84**. After the primary alcohol of **84** was protected as the triisopropylsilyl ether (TIPSO), it was reacted with sodium benzyloxide in benzyl alcohol under microwave conditions to give **85**. Removal of the TIPS group from the OH provided **86**, which was converted into the corresponding azide **87**. Treatment of **87** under Staudinger conditions, followed by catalytic hydrogenation, provided the desired 6-pyridinol derivative **88**.

Several analogues of **5e** were prepared with various alkyl, aryl, heteroaryl, and alkoxy substituents on the C-4 of the 3-hydroxybenzylamino headpiece. Compounds **89a–g** were prepared by the reaction of **4e** with the desired 3-hydroxybenzylamine hydrochloride in the presence of triethylamine and DMF at room temperature, as shown in Scheme 8. The 3-hydroxybenzylamines used included 4-methyl (**25**), 4-phenyl

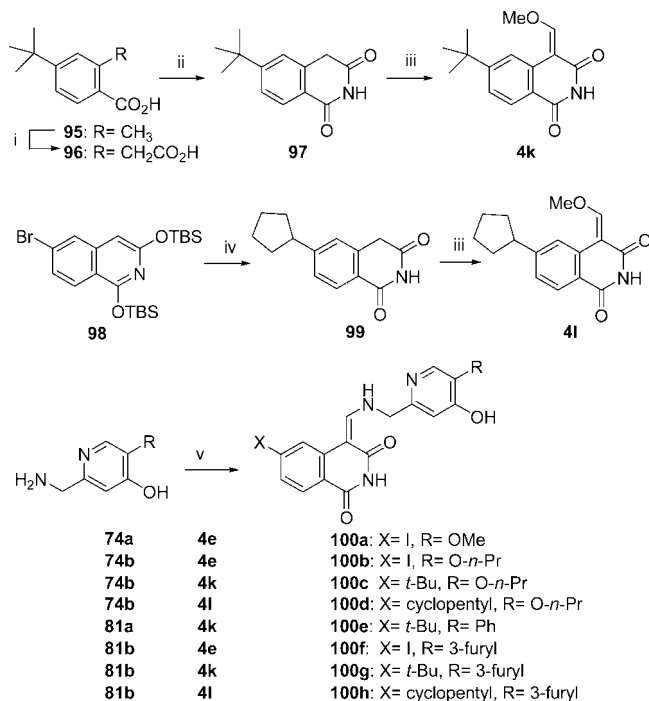
Scheme 8<sup>a</sup>

<sup>a</sup> (i) (For **89a**, **89d–h**, **94b–c**) DMF, Et<sub>3</sub>N, room temperature; (for **89b–c**, **90**, **92**, **93**) DMF, room temperature; (for **91**) THF, Et<sub>3</sub>N, room temperature; (for **94a**) THF, DMF, Et<sub>3</sub>N, room temperature.

(**34**), 2-furyl (**41**), 3-furyl (**45a**), the three possible pyridyl isomers (**45b–d**), and O-*n*-Pr (**16b**).

The analogue of **5e**, wherein the benzylamine was replaced with the pyrimidinylmethylamine, namely, **90**, was prepared by treatment of **4e** with the amine **51**. Analogue **92**, bearing a 2-aminomethyl-3-propoxy-6-pyridinol headpiece, was obtained by reacting **4e** with the amine **88**. Two analogues **91** and **93**, where no hydroxyl group was present in the headpiece, were prepared by reacting **4d** with 2-methoxypyridyl derivative **54** and 5-methoxy-4-pyrone derivative **70a**, respectively. The analogue of **91**, where the methoxy was replaced with a hydroxyl, namely **94a**, was obtained by treatment of 2-pyridone **55** with the 6-Br core **4d**. Other 6-iodo compounds carrying N-substituted 2-pyridones **94b–c** were formed by reacting **4e** with N-substituted 2-pyridones **58** and **65**, respectively.

Two cores **97** and **99**, carrying 6-*t*-butyl and 6-cyclopentyl substituents, respectively, were prepared, as depicted in Scheme 9. 2-Methyl-4-*t*-butylbenzoic acid **95**<sup>33</sup> was treated with lithium diisopropylamide at  $-78$  °C, followed by the addition of dimethylcarbonate to yield the diacid **96**, which was then

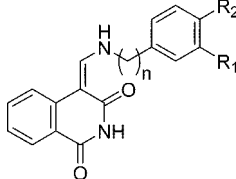
Scheme 9<sup>a</sup>

<sup>a</sup> (i) lithium diisopropylamide, (MeO)<sub>2</sub>CO,  $-78$  °C; (ii) urea, 150 °C; (iii) HC(OMe)<sub>3</sub>, HOAc, 90 °C; (iv) cyclopentylMgBr, Pd(PPh<sub>3</sub>)<sub>4</sub>, ethyl ether, microwave, 75 °C; then THF, H<sub>2</sub>O, HCl, room temperature; (v) DMF, room temperature.

cyclized as described before<sup>22</sup> to yield the 6-*t*-butyl core **97**. The corresponding 6-cyclopentyl core **99** was formed by reaction of bis-TBS protected 6-bromoisoquinoline-1,3-diol<sup>22</sup> with cyclopentyl magnesium bromide and tetrakis(triphenylphosphine)palladium under microwave conditions. Both **97** and **99** were then converted to the corresponding 4-methoxymethylene derivatives **4k** and **4l**, respectively, by treatment with trimethylorthoformate in acetic acid.<sup>22</sup> Further reaction of 6-*t*-butyl intermediate **4k** with 5-substituted 4-pyridinols **74b**, **81a**, and **81b** produced the final compounds **100c**, **100e**, and **100g**, respectively. Similarly, the 6-cyclopentyl intermediate **4l** was reacted with 5-substituted 4-pyridinols **74b** and **81b** to generate the final compounds **100d** and **100h**, respectively. 4-Methoxymethylene-6-iodo intermediate **4e** was also used to couple with 5-substituted 4-pyridinols **74a**, **74b**, and **81b** to yield **100a**, **100b**, and **100f**, respectively.

## Results and Discussion

**In Vitro Activity, and Metabolic Stability.** Our synthetic efforts on this series began with the discovery of **3f**, which carried a 4-(3,4-dihydroxyphenylethylamino)methylene headpiece and showed moderate CDK4 activity with an IC<sub>50</sub> value of 16.2 μM, as shown in Table 1. Further analogues were prepared by varying the chain length between the 3,4-dihydroxyphenyl ring and the aminomethylene moiety. When the 3,4-dihydroxyphenyl ring was directly linked to the aminomethylene moiety, as shown in **3a**, the CDK4 potency was not much improved (IC<sub>50</sub> = 7.8 μM). However, when the two groups were linked via a methylene bridge, the resulting compound **3b** showed a 10-fold enhancement in CDK4 potency. We then explored the importance of the two hydroxyl groups by preparing the two monohydroxyl isomers **3c** and **3d**. Compared to the 3,4-dihydroxy compound, **3b**, the 3-hydroxy derivative, **3d**, was equipotent and selective in inhibiting CDK4;

**Table 1.** Inhibition (IC<sub>50</sub>) of CDK4, CDK1, and CDK2 Activities


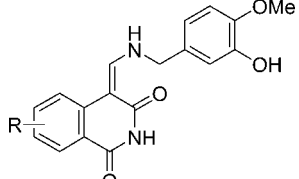
compd	<i>n</i>	R1	R2	kinase assays IC <sub>50</sub> (μM) <sup>a</sup>		
				CDK4	CDK1	CDK2
<b>3a</b>	0	OH	OH	7.8		
<b>3b</b>	1	OH	OH	1.2	36.6	12.5
<b>3c</b>	1	H	OH	<50	37.2	17.3
<b>3d</b>	1	OH	H	0.78	<50	16.9
<b>3e</b>	1	OH	OMe	0.19	48.7	6.9
<b>3f</b>	2	OH	OH	16.2		

<sup>a</sup> Concentration (μM) 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.

however, the 4-hydroxy derivative, **3c**, was at least 64-fold less potent. These results clearly show that the 3-hydroxyl moiety is critical for CDK4 potency and selectivity. Markwalder et al.<sup>34a</sup> and Rossi et al.<sup>34b</sup> reported a similar requirement of *m*-OH moiety on the 6-arylmethyl group of pyrazolopyrimidinone, a different core from ours, for enhancing CDK4 potency; however, the improvement was not sufficient enough to render their compound selective for CDK4. Our modeling studies revealed a key hydrogen bond interaction between the 3-OH group and the side chain of His 82 of the CDK4 enzyme, which will be elaborated below. Analogues of **3d** were then prepared by introducing a variety of substituents at the C-4 position next to the 3-OH group. One of them, the 3-hydroxy-4-methoxy analogue **3e**, showed a 4-fold increase in potency over **3d**, with an IC<sub>50</sub> value of 0.19 μM in inhibiting CDK4, with 256- and 36-fold selectivity over CDK1 and CDK2, respectively.

We then investigated substituent effects on the isoquinoline-1,3-dione core. Relative to **3e**, the corresponding 6-fluoro (**5a**) and 6-chloro (**5b**) derivatives showed comparable CDK4 inhibitory activity; however, the 6-bromo (**5d**) and 6-iodo (**5e**) derivatives exhibited enhanced potency in inhibiting CDK4 with IC<sub>50</sub> values of 30 nM and 10 nM, respectively, as shown in Table 2. In addition, **5d** is over 1600-fold selective for CDK4 over CDK1 and CDK2. The position of the substituent on the core seems to be important for activity. Moving the bromo substituent from C-6 (**5d**) to C-5 (**5c**) resulted in a 30-fold loss of CDK4 inhibitory activity. Therefore, our synthetic efforts focused on C-6 substituted analogues. We found that the 6-phenyl derivative (**5j**) was as potent as the 6-iodo derivative (**5e**) in inhibiting CDK4 (IC<sub>50</sub> = 10 nM); however, the former was more selective than the latter over CDK1 (3400-fold vs 600-fold) and CDK2 (2600-fold vs 80-fold). Compared to **5j**, the corresponding 6-heteroaryl derivatives such as *N*-pyrrolyl (**5g**), 3-thienyl (**5h**), and 3-furyl (**5i**) were more potent CDK4 inhibitors with IC<sub>50</sub> values of 4, 2, and 2 nM, respectively. In particular, **5h** showed excellent selectivity for CDK4 over CDK1 and CDK2 by 11,000- and 9,000-fold, respectively.

In the study of the 3-hydroxy-4-methoxybenzylamino group, shown in Table 3, the α-methylbenzylamine derivative **11** was an order of magnitude less potent than **5d**, but the *N*-methylbenzylamine derivative **13** showed a dramatic loss of activity. The importance of the NH group of the benzylamine for CDK4 inhibitory activity was substantiated by our molecular modeling studies, wherein a hydrogen bond interaction between

**Table 2.** Inhibition (IC<sub>50</sub>) of CDK4, CDK1, and CDK2 Activities


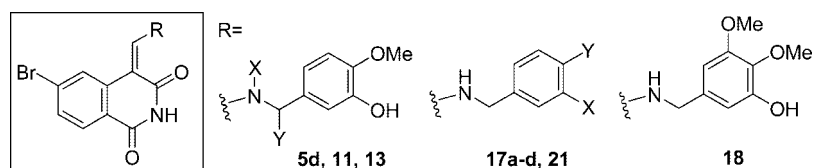
compd	R	kinase assays IC <sub>50</sub> (μM) <sup>a</sup>		
		CDK4	CDK1	CDK2
<b>3e</b>	H	0.19	48.7	6.9
<b>5a</b>	6-F	0.17	<50	6.6
<b>5b</b>	6-Cl	0.23	<50	<50
<b>5c</b>	5-Br	0.94	8.1	3.1
<b>5d</b>	6-Br	0.03	<50	<50
<b>5e</b>	6-I	0.01	6	0.87
<b>5f</b>	6-OMe	0.031	22.3	2.9
<b>5g</b>	6-( <i>N</i> -pyrrolyl)	0.004	9.7	3.1
<b>5h</b>	6-(3-thienyl)	0.002	23.3	18.3
<b>5i</b>	6-(3-furyl)	0.002	2.5	1.1
<b>5j</b>	6-phenyl	0.01	34.7	25.9

<sup>a</sup> Concentration (μM) 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.

this NH and the backbone carbonyl of Val 83 was shown. A detailed discussion on the modeling will be presented later. Introduction of an additional methoxy group, as in **18**, resulted in a 4-fold increase in potency compared to **5d**. We then investigated the effect of varying the 4-methoxy substituent of **5d**. Compared to **5d**, the 4-ethoxy derivative **17d** was equipotent, but the 4-*n*-propoxy derivative **17b** was about an order of magnitude less potent. However, the 4-methoxyethoxy derivative, **17c** was as active as **5d**. Interestingly, the 4-amino derivative **21** was 3-fold less potent than **5d**, but was much less selective versus CDK2, while selectivity over CDK1 was maintained.

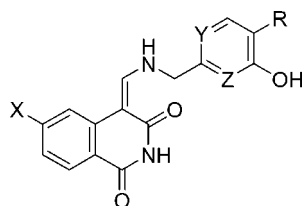
Table 4 shows the effect of varying the 4-methoxy substituent of **5e**. Compared to **5e**, the 4-methyl derivative **89a** was 3-fold less potent, whereas the 4-phenyl, 4-(2-furyl), and 4-(3-furyl) derivatives (**89b**, **89c**, and **89d**, respectively) were about 10-fold less potent. However, they all showed comparable selectivity for CDK4 over CDK1 and CDK2. One compound, **89d**, was further evaluated for its ability to inhibit a panel of protein kinases, as shown in Table 5. It is clear that **89d** was highly potent in inhibiting CDK4 among the serine–threonine kinases and tyrosine kinases tested. The CDK4 inhibitory activity for the three C-4 pyridyl isomers varied depending upon the position of the pyridyl nitrogen. Although the 4-(2-pyridyl) derivative **89e** was a poor CDK4 inhibitor, the 4-(3-pyridyl) and 4-(4-pyridyl) derivatives **89f** and **89g** were as potent as **5e**. Among these three C-4 pyridyl isomers, the pyridyl nitrogen atom from **89e** could potentially form a hydrogen bond interaction with the phenolic OH group, thereby interfering with this critical OH group interaction with His 82 of the CDK4 enzyme for activity. In terms of selectivity, both **89f** and **89g** showed higher selectivity for CDK4 over CDK1 and CDK2, relative to **5e**. Analogues **89f** demonstrated better cellular activity than **89g**.

Through our optimization efforts, we have synthesized selective CDK4 inhibitors with potencies as low as 2 nM. However, all of these inhibitors bear a phenolic OH group, a critical group for activity, but also a metabolic liability for the effectiveness of these inhibitors in vivo. It is well-known that phenolic OH groups<sup>34c</sup> are prone to glucuronidation in the endoplasmic reticulum and sulfation in the liver via phase II

**Table 3.** Inhibition (IC<sub>50</sub>) of CDK4, CDK1, and CDK2 Activities

compd	X	Y	kinase assays IC <sub>50</sub> (μM) <sup>a</sup>		
			CDK4	CDK1	CDK2
<b>5d</b>	H	H	0.03	<50	<50
<b>11</b>	H	Me	0.37	<50	<50
<b>13</b>	Me	H	<50	<50	44.4
<b>17a</b>	OH	OH	0.04	<50	<50
<b>17b</b>	OH	O- <i>n</i> -Pr	0.35	42.5	34.5
<b>17c</b>	OH	O-(CH <sub>2</sub> ) <sub>2</sub> OMe	0.04	<50	<50
<b>17d</b>	OH	OEt	0.03	<50	45.5
<b>18</b>			0.007	<50	<50
<b>21</b>	OH	NH <sub>2</sub>	0.08	<50	6.7

<sup>a</sup> Concentration (μM) 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.

**Table 4.** Inhibition (IC<sub>50</sub>) of CDK4, CDK1, and CDK2 Activities and Cell Proliferation

compd	X	Y	Z	R	kinase assays IC <sub>50</sub> (μM) <sup>a</sup>			cell-based assays IC <sub>50</sub> (μM) <sup>b</sup>	
					CDK4	CDK1	CDK2	HCT116	MCF-7
<b>5e</b>	I	CH	CH	OMe	0.01	6.00	0.87	2.00	3.70
<b>89a</b>	I	CH	CH	Me	0.03	>50	13.6	6.70	8.20
<b>89b</b>	I	CH	CH	Ph	0.13	47.8	15.1	0.42	0.88
<b>89c</b>	I	CH	CH	2-furyl	0.10	>50	8.40	0.38	0.70
<b>89d</b>	I	CH	CH	3-furyl	0.06	24.4	4.70	0.48	1.13
<b>89e</b>	I	CH	CH	2-pyridyl	19.30	>50	>50	0.25	0.54
<b>89f</b>	I	CH	CH	3-pyridyl	0.01	>50	3.60	0.44	1.10
<b>89g</b>	I	CH	CH	4-pyridyl	0.008	>50	>50	2.30	1.50
<b>89h</b>	I	CH	CH	O- <i>n</i> -Pr	0.02	>50	2.6	0.65	1.8
<b>90</b>	I	N	N	OMe	0.44	>50	>50	>50	>50
<b>92</b>	I	CH	N	O- <i>n</i> -Pr	0.02	10.1	0.70	0.67	1.40
<b>100a</b>	I	N	CH	OMe	0.01	14.7	0.50	1.30	1.00
<b>100b</b>	I	N	CH	O- <i>n</i> -Pr	0.03	25.3	1.60	0.73	0.88
<b>100c</b>	<i>t</i> -Bu	N	CH	O- <i>n</i> -Pr	0.02	6.20	0.96	0.43	0.70
<b>100d</b>	cyclopentyl	N	CH	O- <i>n</i> -Pr	<0.005	3.10	1.20	0.15	0.30
<b>100e</b>	<i>t</i> -Bu	N	CH	Ph	0.03	16.3	2.60	0.15	0.70
<b>100f</b>	I	N	CH	3-furyl	0.03	13.5	2.30	0.55	2.70
<b>100g</b>	<i>t</i> -Bu	N	CH	3-furyl	0.02	9.50	1.90	0.48	1.40
<b>100h</b>	cyclopentyl	N	CH	3-furyl	<0.005	3.10	1.30	0.81	0.83

<sup>a</sup> Concentration (μM) 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<sub>50</sub> (μM) values are the concentrations needed to inhibit cell growth by 50%, as determined from these curves.

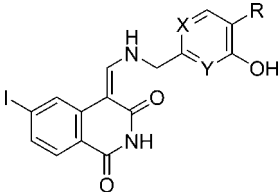
**Table 5.** Inhibitory Activity of **89d** against a Panel of Protein Kinases

kinase	IC <sub>50</sub> (μM)	kinase	IC <sub>50</sub> (μM)	kinase	IC <sub>50</sub> (μM)
CDK4	0.06	CDK1	24.4	CDK2	4.7
AKT	10.2	Braf	>10	EGFR	>10
IGFR	>10	IKK-2	>10	IR	>10
KDR	10	LCK	>30	LYN	8.5
MK2	19	mTOR	18	p70S6	>30
PDK1	17	PKCθ	25.9	Src	>30
Tpl2	>4	STAT3	1.7		

metabolism, leading to rapid excretion from the body. The rate of glucuronidation is dependent on the lipophilicity and the nucleophilicity of the phenols.<sup>35,36</sup> As the lipophilicity of phenols increases, the rate of glucuronidation increases. Electron-

donating substituents (higher pK<sub>a</sub>) facilitate the conversion of phenols to the corresponding glucuronides. As expected, 3-hydroxybenzylamine derivatives bearing 4-OMe, 4-(3-furyl), and 4-(O-*n*-Pr) substituents, namely, **5e**, **89d**, and **89h**, respectively, suffered reduced stability in phase II metabolism using rat microsomes, as depicted in Table 6.

To address the phase II metabolism issue associated with the phenolic OH, we introduced one or two nitrogen atoms in the phenyl ring of the benzylamine headpiece in **5e**, as shown in Table 4. We expect that electronic effects of the nitrogen in the dihydroxypyrimidine (**90**), 6-pyridinol (**92**), and 4-pyridinols (**100a–h**) would decrease the nucleophilicity of the phenols, thereby decreasing the nucleophilic attack on C-1 of the uridine

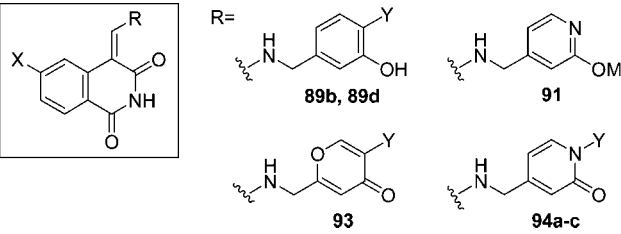
**Table 6.** Rat Microsomal Stability Studies


compd	X	Y	R	$t_{1/2}$ (min)	
				<sup>a</sup> phase I	<sup>b</sup> phase I/II
<b>5e</b>	CH	CH	OMe	18	6
<b>89d</b>	CH	CH	3-furyl	>30	18
<b>89h</b>	CH	CH	O- <i>n</i> -Pr	>30	15
<b>92</b>	CH	N	O- <i>n</i> -Pr	>30	>30
<b>100a</b>	N	CH	OMe	>30	>30
<b>100b</b>	N	CH	O- <i>n</i> -Pr	>30	>30
<b>100f</b>	N	CH	3-furyl	>30	22

<sup>a</sup> Substrate half-life (min) when incubated at 37 °C for 15 min with SD rat liver microsomes (0.5 mg/mL protein) and NADPH cofactor. <sup>b</sup> Substrate half-life (min) when incubated at 37 °C for 15 min with SD rat liver microsomes (0.5 mg/mL protein), NADPH, and UDPGA cofactors.

diphosphate glucuronic acid to generate glucuronidate metabolites. Furthermore, the 4-pyridinols and 6-pyridinols are expected to be in equilibrium with the corresponding 4-pyridones and 6-pyridones,<sup>37a,b</sup> which are devoid of the OH group, therefore reducing the extent of phase II metabolism. The keto-enol tautomeric equilibrium strongly depends on the substituent, on its ring position, and on the solvent dielectric constant.<sup>38</sup> However, in all cases, the solvent dielectric effect tends to increase the preponderance of the keto structure. In the physiological environment where water has a high dielectric constant, the equilibrium is expected to shift more toward the keto tautomeric form, leading to metabolically more stable molecules. We were pleased to see that the 5-(3-furyl)-4-pyridinol derivative **100f** showed improved phase II metabolic stability and that the 5-(O-*n*-Pr)-6-pyridinol derivative **92**, 5-OMe-4-pyridinol derivative **100a**, and 5-(O-*n*-Pr)-4-pyridinol derivative **100b** showed good stability with  $t_{1/2}$  values greater than 30 min (Table 6).

These metabolically more stable pyridinol derivatives are also potent CDK4 inhibitors. As shown in Table 4, the 4-pyridinol derivative **100a** was equipotent with **5e**, whereas the corresponding 4-pyrimidinol derivative **90** was 44-fold less active in inhibiting the CDK4 enzyme. Varying the C-5 substituent at the 4-pyridinol headpiece of **100a** from methoxy to *n*-propoxy in **100b** and 3-furyl in **100f** resulted in a 3–4-fold decrease in potency, but a 2-fold increase in cellular activities. Comparing the two pyridine isomers, the 6-pyridinol derivative **92** was as active and selective as the corresponding 4-pyridinol derivative **100b**, with comparable cellular activities. Additional substitution effects at the C-6 of the isoquinoline-1,3-dione core were evaluated. The 6-*t*-butyl derivatives (**100c** and **100g**) were equipotent with the corresponding 6-iodo derivatives (**100b** and **100f**), whereas the corresponding 6-cyclopentyl derivatives (**100d** and **100h**) showed at least 6-fold increase in CDK4 inhibitory activity, with IC<sub>50</sub> values below 5 nM. Furthermore, **100d** and **100h** showed good selectivity for CDK4 over CDK1 and CDK2, by more than 620-fold and 240-fold, respectively. Although both **100d** and **100h** showed equivalent potency against CDK4 and selectivity vs CDK1 and CDK2, **100d** was more active than **100h** in inhibiting HCT116 and MCF7 cells, with IC<sub>50</sub> values of 0.15 μM and 0.3 μM, respectively. Among a pair of 6-iodo and 6-*t*-butyl derivatives (**100b** and **100c**, respectively) where the headpiece is 5-propoxy-4-pyridinol, both showed comparable CDK4 potency (IC<sub>50</sub> values of 20–30 nM

**Table 7.** Inhibition (IC<sub>50</sub>) of CDK4, CDK1, and CDK2 Activities


compd	X	Y	kinase assays IC <sub>50</sub> (μM) <sup>a</sup>		
			CDK4	CDK1	CDK2
<b>89b</b>	I	Ph	0.13	47.8	15.1
<b>89d</b>	I	3-furyl	0.06	24.4	4.7
<b>91</b>	Br		31.7	>50	6.1
<b>93</b>	Br	OMe	1.3	>50	16.9
<b>94a</b>	Br	H	0.56	>5050	2.7
<b>94b</b>	I	Ph	0.27	29.6	41.5
<b>94c</b>	I	3-furyl	0.06	11.6	2.6

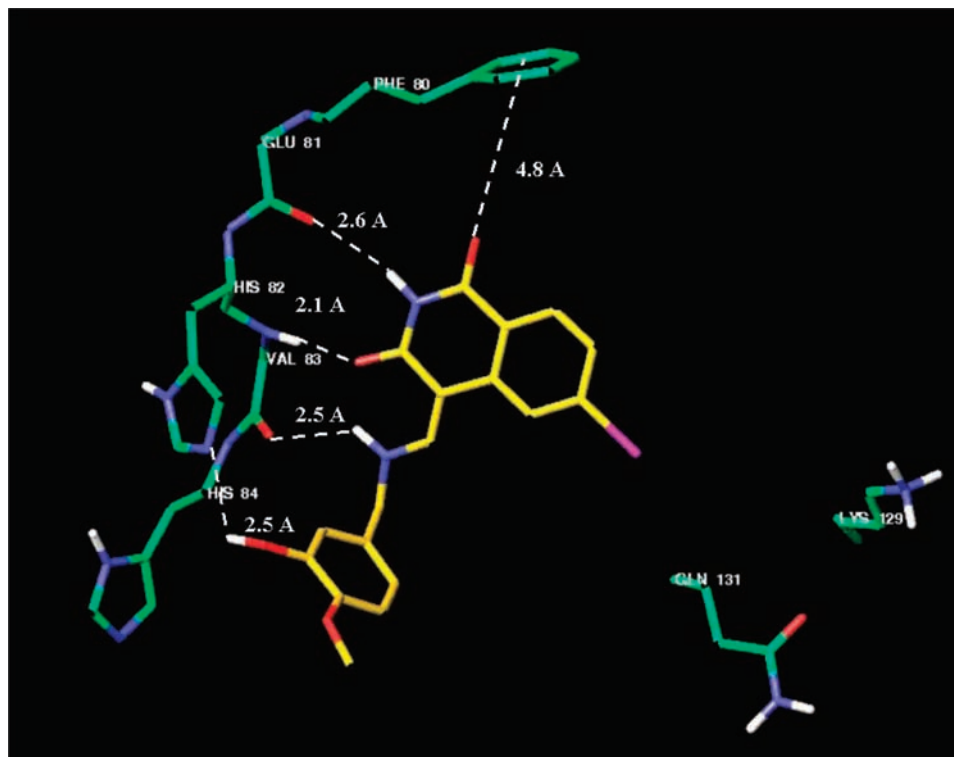
<sup>a</sup> Concentration (μM) needed to inhibit the Rb phosphorylation by 50%, as determined from the dose–response curve. Determinations were done in duplicate, and repeat values agreed, on average, with a mean 2-fold difference.

and selectivity, similar to the pairs of the corresponding 5-(3-furyl)-4-pyridinols (**100f** and **100g**). The three 6-*t*-butyl derivatives **100c**, **100e**, and **100g**, were equipotent against CDK4; however, **100e** was the most active one in inhibiting cells.

Additional derivatives were prepared where the 3-OH group in the headpiece of the isoquinoline-1,3-dione was replaced with a methoxy or a carbonyl group. On the basis of the importance of the 3-OH group on the headpiece for activity, we expected **91** would be a poor CDK4 inhibitor (IC<sub>50</sub> value of 31.7 μM) as shown in Table 7 since the 3-OH was replaced with a 3-OMe group. However, the 4-pyrone derivative **93** also lacks an OH group; to our surprise, its ability to inhibit CDK4 was 30-fold better than that of **91**. We were also intrigued to observe that N-substituted 2-pyridones **94b–c**, bearing no OH substituent, showed reasonably good potency in inhibiting CDK4. In fact, comparable potency was observed for the pair of *N*-phenyl-2-pyridone derivative **94b** and 4-phenyl-3-hydroxyphenyl derivatives **89b**, as well as for the pair of *N*-(3-furyl)-2-pyridone derivative **94c** and 4-(3-furyl)-3-hydroxyphenyl derivatives **89d**. This unexpected result seems to contradict what we observed earlier, i.e., that a 3-OH group on the headpiece is essential for activity. However, this puzzle was unraveled by our molecular modeling, as described below in the subsequent discussions.

**Molecular Modeling.** A binding model for compound **5e** at 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 amino acid numbering of the CDK4 mimic structure will therefore be that of CDK2, which is the parent structure. The inhibitor was docked using the GLIDE docking algorithm in the XP (extra precision) mode.<sup>39</sup> The resulting model successfully identifies key hydrogen bond interactions between the ligands and residues of the protein's ATP binding pocket. The binding model accounts for the nanomolar activity as well as the selectivity of the isoquinoline-1,3-dione inhibitor, in that significant and strong interactions with residues unique to CDK4 have been identified. The specific orientation of the ligand in the binding model exploits the range of residues unique to CDK4, namely, 82 and 83. In the most favored bound conformation, the NH of the isoquinoline-1,3-





**Figure 1.** Proposed binding model for **5e** in the ATP binding site of the CDK4 mimic model.

dione core is found to be 2.6 Å from the backbone carbonyl oxygen of Glu 81. The binding model places a carbonyl oxygen of the isoquinoline-1,3-dione core at 2.1 Å from the backbone NH of Val 83, a position in the binding cavity where the L83V mutation differentiates CDK4 from CDK2. Additionally, the amino NH of the benzylamino headpiece is found within 2.5 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, the gatekeeper residue. Short molecular dynamics runs resulted in the placement of this carbonyl oxygen within 3.5–4.0 Å of various aromatic hydrogens of the Phe 80 side chain. It is postulated here 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. These interactions of the core with Phe 80, Glu 81, and Val 83 of the CDK4 protein in the binding model are quite similar to the ones we observed in our previous series, the 4-(phenylaminomethylene)isoquinoline-1,3-diones.<sup>22</sup> However, in the headpiece, the binding interactions of the current series are different from those observed for our previous series. In the binding model of our previous series, the basic nitrogen atom of the headpiece was expected to interact with the flexible side chains of Asp 84 and His 82, two CDK4-unique amino acid residues, in the solvent exposed regions. In contrast, the current series bears a 3-hydroxyl substituent in the benzylamino headpiece. In the current binding model, this 3-hydroxyl group forms an H-bond with the imidazole nitrogen of the His 82 side chain, with an interaction distance of within 2.5 Å. This key interaction is not attainable by a 4-hydroxyl compound **3c**, a poor inhibitor for CDK4, compared to the corresponding 3-hydroxyl compound **3d**. Furthermore, placement of an electron

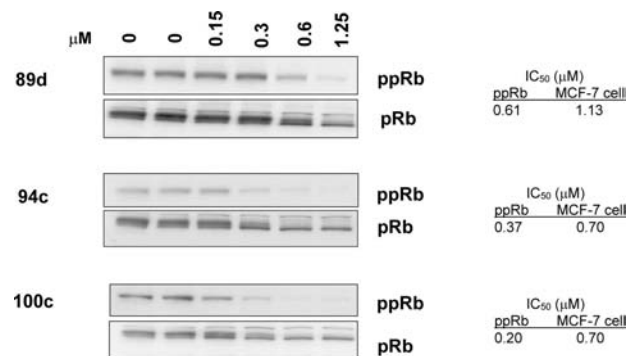
donating substituent such as methoxy (e.g., compound **3e** in Table 1 and compound **5e** in Table 2) is found to enhance this interaction.

In summary, our binding model identifies three key interactions with the backbone groups of hinge region residues as well as favorable, albeit supportive, electrostatic interactions with at least two of the aromatic hydrogens of the side chain of Phe 80, the gatekeeper residue. It is noted here that two of these three hydrogen bonds involve the backbone groups of Val 83, a residue unique to CDK4. Additionally, the hydrogen bond interaction between the 3-hydroxyl group on **5e** and the side chain of a CDK4-unique residue, His 82, contributes to and strengthens the cooperative hydrogen bond network between **5e** and CDK4 enzyme. Like **5e**, compound **5d** is a potent and selective CDK4 inhibitor. Addition of a methyl group at the  $\alpha$  position of the benzylamino headpiece in **5d** would perturb the overall binding to CDK4, and the resulting compound **11** showed a 10-fold loss of CDK4 potency, though it is still selective for CDK4. However, when the NH of the benzylamino headpiece in **5d** is methylated, the resulting compound **13** can no longer interact with the backbone carbonyl oxygen of Val 83. This led to such a disruption of the cooperative hydrogen bond network with CDK4 that **13** showed a dramatic loss of potency and selectivity for CDK4. Thus, having the 3-hydroxyl group is crucial but not sufficient for CDK4 potency and selectivity, both of which also depend on the optimal and unique orientation of these inhibitors in the CDK4 binding pocket, resulting in the formation of a strong and cooperative hydrogen bond network. In comparison, modeling studies of pyrazolopyrimidinone<sup>34a,b</sup> identify a hydrogen bond interaction between the meta-hydroxyl moiety of the 6-arylmethyl group and the side chain of His 82 of CDK4, in addition to interactions of the core with Val 83, a total of two hydrogen bond interactions with the backbone moieties of the hinge region of CDK4. Although improved potency for CDK4 was realized in these studies, no selectivity for CDK4 was achieved. The lack of CDK4 selectivity of the pyrazol-

opyrimidinone series may lie in the fact that its core is different from ours; therefore, the two series are expected to be oriented differently in the ATP-binding site of the CDK4 enzyme. In comparison to compound **5e**, the pyridone derivatives **94b,c** lack the critical 3-hydroxyl group. However, the pyridone moiety of **94b,c** (see Table 7) places the carbonyl oxygen within 3.5 Å of the flexible side chain of His 82 in the solvent exposed region. It is expected that the flexibility and the solvent exposed nature of this interaction could bring the carbonyl oxygen of pyridones closer to the NH of His 82 side chain and possibly a water-mediated interaction. There are precedents that showed H-bond interaction of NH of imidazole or His side chain with carbonyl oxygen.<sup>40a,b</sup> This favorable interaction may explain why the pyridones **94b,c** are as potent as the corresponding phenols **89b,d** in inhibiting CDK4. However, the His 82 side chain may be partially protonated. The extent of the protonation depends on the  $pK_a$  of the histidine side chain, which varies according to the environment surrounding the His residue.<sup>40c</sup> It is possible that the protonated nitrogen of imidazole could form an H-bond interaction with the carbonyl oxygen of the pyridones. But the nature of the interaction can be fully understood when X-ray crystal structure of CDK4 is available to enable cocrystal structures with our pyridone derivatives.

Other researchers such as McInnes,<sup>41</sup> Honma,<sup>42</sup> and Aubry<sup>43</sup> carried out computational studies using a crystal structure of inhibitor bound CDK2 and a homology model of CDK4, which was built using both 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>44</sup> by a value of 13 for the residues of the ATP binding site. Docking studies with CDK4 selective compounds were then used to elucidate binding features of the CDK4 selective compounds in the ATP binding pockets of both the CDK2 crystal structure and the CDK4 homology model. A key finding of their study is the presence of a positively charged moiety, proximal to the Asp and Glu residues that correspond to residues numbered 86 and 131, respectively, in the CDK2 structure used in our study (Figure 1). Although the 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-dione analogues of our study are not found to interact with Asp 86 (not shown) and are a little more than 4.0 Å distance from the Glu 131 side chain, our binding models place the phenol moiety of our inhibitors within 2.5 Å of the His 82 side chain, a residue unique to CDK4. The CDK4 mimic structure of Ikuta et al.<sup>20</sup> used in this study contains a Gln residue at position 131. The corresponding residue in CDK4 is Glu. Again, it should be noted that, in a previous study,<sup>22</sup> an *in silico* mutation of Gln 131 to Glu 131 with subsequent short molecular dynamics runs was carried out. Our binding models place the 6-iodo substituent of compound **5e** in close proximity to the Gln 131 residue. Specifically, Glu 131 is in a region of the binding pocket, where protein flexibility and the presence of solvent molecules are expected to be significant. Park et al.<sup>45</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 with the residue at position 131 mutated to Glu will identify the key role that Glu 131 plays in stabilizing the binding of protein to 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-dione and further enhance our understanding of the SAR of substituents at position 6 of the isoquinoline-1,3-dione core (Table 2).

The K89T mutation, which places a threonine at the entrance to the ATP binding cleft of CDK4, is singled out as a significant



**Figure 2.** Effect of **89d**, **94c**, and **100c** on pRb phosphorylation in MCF-7 cells. MCF-7 cells were incubated in the presence of **89d**, **94c**, or **100c** at the indicated concentrations at 37 °C for 24 h. Protein extracts were analyzed by SDS-PAGE, followed by immunoblotting using either phospho-Rb antibody to probe ppRb (Ser 807/811, upper panel) or Rb antibody to probe the total pRb (lower panel). Equivalent protein loading of lanes was confirmed by Ponceau S staining. Blots were scanned and quantified using the FluorS multi-image analyzer (BioRad). The results were normalized to the amount of total pRb, and the IC<sub>50</sub> values for ppRb were determined from inhibition plots (Kaleidagraph). The cellular IC<sub>50</sub> values were determined from inhibition curves (LSW Toolbox) using data points from the SRB cell proliferation assay.

source of CDK2/CDK4 selectivity by several investigators.<sup>20,44</sup> Our binding studies, however, do not indicate any involvement of the threonine residue in ligand binding. Pratt et al.<sup>44</sup> have pointed to the K89T mutation as being the primary source of CDK4 selectivity for the bisanilino pyrimidine analogues. We point to intrinsic differences in the shapes of the 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-dione analogues, which result in optimal orientations in the CDK4 binding cavity, that do not involve the threonine residue in question. Therefore, the 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-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 the side chain of His 82, a nonconserved amino acid residue 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 isoquinoline-1,3-dione analogues of this study.

**Inhibition of pRb Phosphorylation.** The biological activity of **89d**, **94c**, and **100c** was evaluated in MCF-7 cells, which overexpress cyclin D1 due to gene amplification. We examined the phosphorylation state of pRb, the physiological substrate for CDK4/cyclin D complexes, after overnight incubation with **89d**, **94c**, or **100c**. As shown in Figure 2, treatment of MCF-7 cells with each of these compounds resulted in a reduction of phosphorylation of pRb, as indicated by the decrease of the band corresponding to ppRb in polyacrylamide-SDS gels (upper panel). The inhibition was dose-dependent and could be detected at the lowest concentration tested (0.15 μM). The resulting IC<sub>50</sub> values are in agreement with those obtained in the cellular proliferation assays. Clearly, the biological activities of these compounds are consistent with the expected effects of CDK4 inhibition.

## Conclusions

We report here the discovery of 4-(benzylaminomethylene)isoquinoline-1,3-(2*H*,4*H*)-diones and 4-[(pyridylmethyl)aminomethylene]isoquinoline-1,3-(2*H*,4*H*)-diones as potent and selective inhibitors of the cyclin-dependent kinase 4 (CDK4). Compared to our earlier series<sup>22</sup> where the headpiece is a

substituted aniline, this new series carries substituted 3-hydroxybenzylamino or (hydroxypyridyl)methylamino headpieces and exhibits enhanced potency for CDK4 and higher activity in inhibiting cell proliferation. Furthermore, compounds bearing the (hydroxypyridyl)methylamino headpieces show good phase II metabolic stability. The inhibitory activity is enhanced when an iodo, aryl, heteroaryl, *t*-butyl, or cyclopentyl substituent is introduced at the C-6 position of the isoquinoline-1,3-dione core. We also present a proposed binding model for **5e** at the ATP binding site of a CDK4 mimic structure that contains three mutations at residues F82H, L83V, and K89T. This binding model shows a hydrogen bond interaction between the 3-hydroxyl group on **5e** and a CDK4-unique His 82 as well as interactions between the core and Phe 80, Glu 81, and Val 83, a residue unique to CDK4. The crucial interaction of the 3-hydroxyl group with CDK4 contributes to and strengthens the cooperative hydrogen bond network with CDK4, thus providing the potency and selectivity of this series of compounds for CDK4 over CDK2 and CDK1. In cells, selected compounds, **89d**, **94c**, and **100c**, from this series have demonstrated biological activity: they inhibit phosphorylation of pRb, the physiological substrate for CDK4/cyclin D1 at the IC<sub>50</sub> values consistent with their respective IC<sub>50</sub> values for inhibition of cell proliferation.

## Experimental Section

**Chemistry.** <sup>1</sup>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 Bruker 9.4T FTMS spectrometer. Chromatographic purifications were by flash chromatography using Baker 40  $\mu$ m 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  $\mu$ m), 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. LCMS1: Analytical HPLC was conducted on an HP 1100 liquid chromatography system over a 2.1 mm  $\times$  30 mm xterra C18 Luna column (5  $\mu$ m) 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 an additional 1.5 min) and a flow rate of 1 mL/min were employed. LCMS2: Analytical HPLC was conducted on an Agilent LC-1100-MSD liquid chromatography system over a 50  $\times$  2.1 mm Aquasil C18 column (Thermo Electron Corporation, 5  $\mu$ m) 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>46</sup> A public domain structure of CDK4<sup>20</sup> was used as the 3-D representation for molecular docking studies. The inhibitor structures were docked

using the GLIDE<sup>39</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>47</sup> empirical scoring function and the OPLS-AA<sup>48</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.

**Preparation of the Substituted Cores. 6-*tert*-Butylisoquinoline-1,3(2*H*,4*H*)-dione (**97**).** To a solution of *N,N*-diisopropylamine (1.06 g, 10.4 mmol) in THF (10 mL) at 0 °C was added *n*-butyllithium (4.2 mL, 2.5 M, 10.5 mmol). After it was stirred for 10 min, it was cooled to –78 °C, and then a solution of 4-*tert*-butyl-2-methylbenzoic acid (**95**) (500 mg, 2.6 mmol) and dimethylcarbonate (471 mg, 5 mmol) in THF (5 mL) was added. It was warmed up to room temperature and stirred overnight. The mixture was cooled, and water was added. The organic phase was extracted with water. The combined aqueous phase was washed with hexane to remove *N,N*-diisopropylamine. The water solution was acidified with conc. HCl to pH 2 and extracted with ethyl acetate. The ethyl acetate layer was dried and evaporated to give 552 mg (90%) of 4-*tert*-butyl-2-(carboxymethyl)benzoic acid (**96**) as a white solid: MS (ESI) *m/z* 237.1 (M + 1)<sup>+</sup>.

Compound **96** (90 mg, 0.38 mmol) and urea (46 mg, 0.76 mmol) were mixed and heated at 145 °C for 1 h. The solid was collected by filtration, washed with ethyl acetate, water, and methanol, and air-dried to give 45 mg (55%) of **97** as a solid: MS (ESI) *m/z* 216.1 (M – 1)<sup>–</sup>; HRMS (ESI) *m/z* calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>2</sub> 218.11756; found, 218.11765 (M + H)<sup>+</sup>.

**(4*E*)-6-*tert*-Butyl-4-(methoxymethylene)isoquinoline-1,3(2*H*,4*H*)-dione (**4k**).** A solution of **97** (45 mg, 0.20 mmol) in DMF (1 mL), acetic anhydride (2 mL), and trimethyl orthoformate (0.2 mL, 1.83 mmol) was heated at 120 °C for 1 h. After the solvents were evaporated, the precipitate was washed with methanol, and dried to give 44 mg (85%) of **4k** as a yellow solid: MS (ESI) *m/z* 260.2; HRMS (ESI) *m/z* calcd for C<sub>15</sub>H<sub>17</sub>NO<sub>3</sub> 260.12812; found, 260.12825 (M + H)<sup>+</sup>.

**6-Cyclopentyl-4*H*-isoquinoline-1,3-dione (**99**).** To 6-Bromo-1,3-bis-(*tert*-butyl-dimethyl-silyloxy)-isoquinoline<sup>22</sup> (1.76 g, 3.75 mmol) was added tetrakis(triphenylphosphine)palladium (110 mg, 0.095 mmol) and 2 M cyclopentyl magnesium bromide in ether (3.0 mL, 6.0 mmol). This mixture was at 75 °C under microwave conditions for 10 min. The mixture was cooled to room temperature, transferred to a flask with THF and water, then 2 M hydrochloric acid (20 mL) was added and stirred for 4 h at room temperature. The organic solvents were removed in vacuo, the mixture extracted with ethyl acetate, the organic layer dried over sodium sulfate, filtered, evaporated, and chromatographed with hexane-ethyl acetate on silica gel to give 268 mg (31%) of **99** as a white solid: MS (ESI) *m/z* 230.2 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.48 – 1.60 (m, 2H), 1.60 – 1.72 (m, 2H), 1.72 – 1.84 (m, 2H), 1.98 – 2.08 (m, 2H), 2.99 – 3.09 (m, 1H), 3.99 (s, 2H), 7.26 (bs, 1H), 7.34 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 11.20 (s, 1H).

**6-Cyclopentyl-4-methoxymethylene-4*H*-isoquinoline-1,3-dione (**4l**).** A mixture of **99** (222 mg, 0.97 mmol), 10 mL of acetic acid, and trimethyl orthoformate (212 mg, 2.0 mmol) was stirred and heated to 90 °C. After 2 h at that temperature, the reaction mixture was cooled, and the solvents were removed in vacuo, and the residue taken up in 4% methanol in dichloromethane, passed through a short pad of Florisil, and eluted with 4% methanol in dichloromethane. The eluate was evaporated, and the product was treated with 4:1 hexane-ethyl acetate and collected by filtration to give 165 mg (62%) of **4l** as a yellow solid: MS (ESI) *m/z* 272.2 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.48 – 1.62 (m, 2H), 1.63 – 1.73 (m, 2H), 1.73 – 1.85 (m, 2H), 1.99 – 2.11 (m, 2H), 3.00

– 3.12 (m, 1H), 4.23 (s, 3H), 7.32 (dd,  $J = 8.2, 1.6$  Hz, 1H), 7.99 (d,  $J = 8.3$  Hz, 1H), 8.01 (s, 1H), 8.14 (d,  $J = 1.4$  Hz, 1H), 11.18 (s, 1H).

**Preparation of the Headgroups. 3-Hydroxy-benzylamine Hydrogen Chloride (2d).** An amount of 2 g (16.79 mmol) of 3-cyanophenol was dissolved in THF (40 mL). After cooling to 0 °C, borane tetrahydrofuran complex (32.0 mL, 2.0 M) was added dropwise to the solution. It was allowed to stir at 0 °C for 15 min, then at room temperature for 25 min. After refluxing for 3 h, it was cooled to room temperature and then evaporated to dryness. Methanol (14 mL) was added and then evaporated to dryness. Subsequently conc. hydrogen chloride (155 mL) was added and then evaporated to dryness. The residue was recrystallized from ethyl acetate to give 2.66 g (100%) of **2d** as a white solid. MS (ESI)  $m/z$  123.15 ( $M + 1$ )<sup>+</sup>.

**3-Hydroxy-4-methoxy-benzylamine Hydrogen Chloride (2e).** An amount of 1.52 g (10.0 mmol) of 3-hydroxy-4-methoxy-benzylaldehyde was added to ethanol (20 mL) and pyridine (10 mL) at room temperature, followed by the addition of hydroxylamine hydrochloride (764.39 mg, 11.0 mmol). The mixture was stirred at ambient temperature for 24 h, and 200 mL of water was added. After the solvents were evaporated, the residue was dissolved in 400 mL of ethyl ether, washed successively with 100 mL of aqueous sodium bicarbonate, 100 mL of sodium bisulfite, and 100 mL of brine. It was dried over magnesium sulfate, filtered, and evaporated to give 1.39 g (83%) of 3-hydroxy-4-methoxy-benzylaldehyde oxime as a white solid.

A solution of 3-hydroxy-4-methoxy-benzylaldehyde oxime (1.0 g, 5.98 mmol) was dissolved in ethanol (50 mL). Then conc. hydrochloric acid (2 mL) was added, followed by 10% Pd/C (200 mg) and hydrogenated at 35 psi for 3 h. The mixture was filtered through celite and evaporated to dryness. The residue was recrystallized from ethyl acetate to give 1.13 g (91%) of **2e** as a white solid (HCl/hydrate salt): mp 160–161 °C; MS (ESI)  $m/z$  154.2 ( $M + 1$ )<sup>+</sup>.

**5-(1-Amino-ethyl)-2-methoxyphenol hydrochloride (10).** To a solution of 3-hydroxy-4-methoxybenzaldehyde (**6**) (5.0 g, 33 mmol) in DMF (165 mL) was added benzyl bromide (4.3 mL, 36 mmol), followed by potassium carbonate (~325 mesh, 14 g, 100 mmol). The reaction mixture was stirred for 4 h at room temperature and then partitioned between diethyl ether and water. The aqueous phase was extracted with ether (3 × 50 mL). The combined ethereal extracts were washed twice with water and once with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give a golden oil, which was crystallized upon standing to afford 3-benzyloxy-4-methoxybenzaldehyde (**7**) as a white solid. The material was used in the following step without further purification.

A solution of the crude **7** in THF (150 mL) was cooled to –78 °C. Methylolithium (1.6 M solution in diethyl ether, 31 mL, 49 mmol) was added dropwise via a syringe. Following completion of the addition, the cooling bath was removed, and the reaction mixture was allowed to warm to room temperature. After 90 min at that temperature, the mixture was cooled to 0 °C and treated with saturated aqueous sodium hydrogen carbonate. The quenched reaction mixture, after warming to room temperature, was acidified with 1 M hydrochloric acid solution to pH 1 and extracted thrice with diethyl ether. The combined extracts were washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give 1-(3-benzyloxy-4-methoxy-phenyl)-ethanol (**8**).

To a mixture of the crude **8** and diphenylphosphoryl azide (2.6 mL, 12 mmol) in toluene (18 mL) at 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (1.6 mL, 11 mmol). The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was washed with water and concentrated to give 4-(1-azido-ethyl)-2-benzyloxy-1-methoxybenzene (**9**), which was used in the next step without purification.

Crude **9** was hydrogenated at 50 psi, using 10% Pd/C in ethanolic hydrogen chloride solution to give a brown syrup, which, when was dried under vacuum, gave 0.53 g (8% over 4 steps) of 5-(1-

amino-ethyl)-2-methoxyphenol hydrochloride (**10**) as a tan foam: MS (ESI)  $m/z$  150.9 ( $M-NH_3 + 1$ )<sup>+</sup>.

**2-Methoxy-5-methylaminomethyl-phenol (12).** Methylamine (40% aqueous solution, 2.2 mL, 25 mmol) was added to a solution of 3-hydroxy-4-methoxybenzaldehyde (**6**) (3.0 g, 20 mmol) in ethanol (35 mL). The resulting slurry was stirred for 30 min at room temperature. Then the solvents were evaporated under reduced pressure. The resulting solid was dissolved in methanol (300 mL), and sodium borohydride (0.90 g, 24 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then concentrated under reduced pressure. The residue was partitioned between saturated aqueous potassium carbonate solution and ethyl acetate. The aqueous phase was extracted thrice with ethyl acetate, and the combined extracts were concentrated under reduced pressure to give 2.5 g (75%) of **12** as a granular white solid: MS (ESI)  $m/z$  168.2 ( $M + 1$ )<sup>+</sup>.

**3-Hydroxy-4-propoxybenzaldehyde (15b).** To a solution of 1-bromopropane (1.7 mL, 18.7 mmol) in anhydrous DMF (25 mL) was added potassium carbonate (5.7 g, 41.0 mmol). The mixture was stirred at room temperature, and 1,2-dihydroxy-4-benzaldehyde (**14**) (1.0 g, 7.2 mmol) was added. After the mixture was stirred at 65 °C for 30 min, the resulting mixture was concentrated, and the residue was then partitioned between water (25 mL) and ethyl acetate (25 mL). The organic layer was then dried and purified by flash chromatography to give 0.2 g (15%) of **15b** as a brown solid: MS (ESI)  $m/z$  181.2 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.99 (t,  $J = 7.3$  Hz, 3H), 1.77 (m, 2H), 4.03 (t,  $J = 6.6$  Hz, 2H), 7.11 (d,  $J = 8.3$  Hz, 1H), 7.26 (d,  $J = 2.0$  Hz, 1H), 7.38 (dd,  $J = 8.3, 2.0$  Hz, 1H), 9.49 (s, 1H), 9.76 (s, 1H).

**3-Hydroxy-4-(2-methoxyethoxy)benzaldehyde (15c).** An amount of **14** (5.0 g, 36.2 mmol) in 20 mL of DMF was added to 2-bromoethyl methyl ether (3.4 mL, 36.2 mmol) and sodium carbonate (5.0 g, 72.4 mmol). The reaction mixture was stirred at room temperature for 3 days. After removal of solids by filtration, the solution was subsequently evaporated under high-pressure vacuum to give a dark brown liquid. The residue was treated with water and acidified with 12 N HCl solution to pH ~2, then extracted with 4 × 100 mL of EtOAc. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, stirred in Darco, filtered, and evaporated to give a yellow liquid. Purification was performed by column chromatography over silica gel using 40% EtOAc/Hexane as eluent to give 2.13 g (30%) of **15c** as a colorless solid: mp 74–75 °C; MS (ESI)  $m/z$  195.1 ( $M + H$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 3.32 (s, 3 H), 3.62 – 3.79 (m, 2 H), 4.03 – 4.34 (m, 2 H), 7.13 (d,  $J = 8.3$  Hz, 1 H), 7.27 (d,  $J = 2.0$  Hz, 1 H), 7.38 (dd,  $J = 8.3, 2.0$  Hz, 1 H), 9.58 (s, 1 H), 9.77 (s, 1 H).

**5-(Aminomethyl)-2-propoxyphenol Hydrochloride (16b).** An amount of **15b** (150 mg, 0.83 mmol) was added to pyridine (4.0 mL) at room temperature, followed by the addition of methoxyamine hydrogen chloride (101.2 mg, 1.2 mmol). The mixture was stirred at ambient temperature for 24 h, and 4 mL of water was added. After the solvents were evaporated, the residue was dissolved in 8 mL of anhydrous ether and washed successively with 2 mL of aqueous sodium bicarbonate, 2 mL of sodium bisulfite, and 2 mL of brine. It was dried over magnesium sulfate, filtered, and evaporated to give 150 mg (86%) crude 3-hydroxy-4-propoxybenzaldehyde *O*-methyloxime as a yellow solid: MS (ESI)  $m/z$  210.5 ( $M + 1$ )<sup>+</sup>.

An amount of 3-hydroxy-4-propoxybenzaldehyde *O*-methyloxime (150 mg, 0.83 mmol) was dissolved in ethanol (4 mL). Then 12 N HCl (0.600 mL) was added, followed by 10% Pd/C (15.0 mg). After hydrogenation at 35 psi for 3 h, the solution was filtered through Celite and evaporated to dryness. The residue was recrystallized from ethyl acetate to give 112 mg (62%) of **16b** as a white solid: MS (ESI)  $m/z$  182.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.97 (t,  $J = 7.4$  Hz, 3H), 1.53 – 1.94 (m, 2H), 3.84 (s, 2H), 3.91 (t,  $J = 6.5$  Hz, 2H), 6.75 – 6.86 (m, 1H), 6.88 – 6.98 (m, 2H), 8.08 (s, 3H), 9.03 (s, 1H).

**5-(Aminomethyl)-2-(2-methoxyethoxy)phenol (16c).** Using the procedures described for the preparation of **16b**, **15c** (1.24 g, 6.32 mmol) was reacted to give a colorless oil, which upon recrystalli-

zation from MeOH/EtOAc yielded 0.45 g (55%) of **16c** as a colorless solid: mp: 89–90 °C; MS (ESI)  $m/z$  198.1 (M + H)<sup>+</sup>.

**2-Amino-5-(aminomethyl)phenol Hydrochloride (20)**. Using the procedure described for the preparation of **2e**, 4-nitro-3-hydroxybenzadehyde *O*-methyloxime (2.2 g, 16.1 mmol) was hydrogenated to give 2.5 g (89%) of **20** as a purple solid: MS (ESI)  $m/z$  138.9 (M + 1)<sup>+</sup>.

**5-Aminomethyl-2-methyl-phenol hydrogen chloride (25)**. A solution of 3-hydroxy-4-methylbenzoic acid (**22**) (2 g, 13.14 mmol) and oxalyl chloride (10 mL) was refluxed at 60 °C until all of the solid was in solution. After cooling, the mixture was evaporated to dryness to give 3-hydroxy-4-methylbenzoyl chloride (**23**). It was cooled to 0 °C, and ammonium hydroxide (20 mL) was added within 5 min. The mixture was allowed to stir at 0 °C for 30 min, then at room temperature for an additional 30 min. Water (30 mL) was added to the mixture. After the white precipitate was filtered, the aqueous solution was adjusted to pH 3 and then extracted with ethyl acetate. The organic solution was dried over MgSO<sub>4</sub>, filtered, and dried to give 1.4 mg (70%) of 3-hydroxy-4-methylbenzamide (**24**) as a white solid.

A solution of **24** (1.4 g, 9.65 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. A solution of borane tetrahydrofuran complex (25 mL, 2.0 M) was added dropwise over 15 min and maintained at 0 °C for an additional 10 min. The mixture was stirred at room temperature for 20 min, refluxed for 3 h, and then cooled to room temperature, and HCl (15 mL) was added and refluxed overnight. After cooling, the solvent was evaporated, and methanol (5 mL) was added. After evaporation to dryness, the residue was crystallized with ethyl acetate to provide 450 mg (28%) of the desired product **25** as a white solid: MS (ESI)  $m/z$  174.1 (M + 1)<sup>+</sup>.

**4-Aminomethyl-biphenyl-2-ol (34)**. (2-Methoxy-biphenyl-4-yl)-methanol (**31**) (170 mg, 0.79 mmol)<sup>23</sup> was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and cooled to 0 °C. Thionyl chloride (1 mL) was then added dropwise. The mixture was then allowed to stir till no starting material was left, ca. 1 h. The volatile was then removed, and the crude 4-chloromethyl-2-methoxy-biphenyl (**32**) was employed directly in the next step.

The crude product **32** was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to –78 °C. Boron tribromide (3 mL, 1 M solution in CH<sub>2</sub>Cl<sub>2</sub>, 3 mmol) was added. The cooling bath was then removed, and the mixture was allowed to stir at room temperature for 1 h. The mixture was quenched with ice water, and the methylene chloride layer was dried and concentrated. The residue was purified by column chromatography to obtain 4-chloromethyl-2-hydroxy-biphenyl. This product (170 mg) was then dissolved in DMF (5 mL) to which NaN<sub>3</sub> (100 mg, 1.53 mmol) was added at room temperature for 1 h. Ethyl ether and water were then added, and the ether layer was washed with water and dried over Na<sub>2</sub>SO<sub>4</sub>. Removal of ether provided 100 mg (53% over three steps starting from **31**) of the desired 4-azidomethyl-2-hydroxy-biphenyl (**33**).

Triphenylphosphine (100 mg, 0.38 mmol) was added to a solution of **33** (100 mg, 0.42 mmol) in THF (5 mL) and water (0.5 mL). The mixture was stirred at room temperature overnight. After the solvents were evaporated, the residue was purified by column chromatography to provide 84 mg (100%) of **34**: MS (ESI)  $m/z$  199.10 (M + 1)<sup>+</sup>.

**5-Aminomethyl-2-furan-2-yl-phenol (41)**. 3-Hydroxy-4-iodobenzonitrile (**38**) (120 mg, 0.49 mmol), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (35 mg, 0.049 mmol), and 2-furyltributyltin (200 g, 0.56 mmol) in DMF (5 mL) were degassed and heated at 100 °C for 30 min. After the mixture was cooled to room temperature, an aqueous work up was performed, and 80 mg (88%) of 4-furan-2-yl-3-hydroxy-benzonitrile (**40**) was isolated after column chromatography.

To a solution of **40** (180 mg, 0.97 mmol) in THF (5 mL) was added BH<sub>3</sub>·THF (5 mL, 1 M solution in THF, 5 mmol) under nitrogen. The mixture was then allowed to stir at room temperature for 24 h. The reaction was then quenched with 6 N HCl. THF was then removed, and the aqueous layer was then neutralized with ammonium hydroxide to pH 9. The mixture was then extracted with CHCl<sub>3</sub>/MeOH (9:1). The organic layer was dried and

evaporated, and the residue was chromatographed to provide 60 mg (33%) of **41**: MS (ESI)  $m/z$  190 (M + 1)<sup>+</sup>.

**4-Iodo-3-methoxymethoxy-benzonitrile (42)**. 3-Hydroxy-4-iodobenzonitrile (**38**) (500 mg, 2.04 mmol) and MOMCl (350 mg, 4.37 mmol) were dissolved in anhydrous DMF (5 mL) and cooled to 0 °C. NaH (100 mg, 60% suspension in mineral oil, 2.5 mmol) was then added. The resulting mixture was allowed to stir at room temperature for 1 h. Ether was then added and washed with H<sub>2</sub>O (3 × 20 mL) and brine. After drying over Na<sub>2</sub>SO<sub>4</sub>, ether was removed, and the residue was purified by column chromatography to afford 570 mg (96%) of **42**: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.90 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 1.7 Hz, 1H), 7.03 (dd, *J* = 8.0 and 1.7 Hz, 1H), 5.27 (s, 2H), 3.51 (s, 3H).

**5-Aminomethyl-2-furan-3-yl-phenol (45a)**. 4-Iodo-3-methoxymethoxy-benzonitrile (**42**) (150 mg, 0.52 mmol), 3-furanboronic acid (96 mg, 0.86 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (60 mg, 0.052 mmol), and Cs<sub>2</sub>CO<sub>3</sub> (500 mg, 1.53 mmol) were mixed in DMF (10 mL), degassed, and then heated at 100 °C for 4 h. After the mixture was cooled to room temperature, an aqueous work up was performed, and the residue was purified to afford 100 mg (84%) of 4-furan-3-yl-3-methoxymethoxy-benzonitrile (**43a**).

Nitrile **43a** (120 mg, 0.52 mmol) was dissolved in ether (10 mL). The resulting solution was slowly added to a suspension of LiAlH<sub>4</sub> (100 mg, 2.6 mmol) in ether. After addition, the mixture was stirred for another 10 min before quenching with water and 5 N NaOH. It was extracted with ethyl acetate, and the organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and dried to provide 90 mg (74%) of the crude 4-furan-3-yl-3-methoxymethoxy-benzylamine (**44a**).

The MOM protected amine (**44a**) (90 mg, 0.39 mmol) was then dissolved in 2 N aq HCl/MeOH (1:1). The resulting solution was heated at reflux for 15 min. After cooling to room temperature, the mixture was basified with aqueous ammonium hydroxide and extracted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1). The organic layer was dried, filtered, and evaporated to yield a crude product **45a**, which was used directly in the next step. MS (ESI)  $m/z$  190 (M + 1)<sup>+</sup>.

**5-Aminomethyl-2-pyridin-2-yl-phenol (45b)**. 4-Iodo-3-methoxymethoxy-benzonitrile (**42**) (190 mg, 0.66 mmol), 2-pyridinyl tributyltin (370 mg, 1 mmol), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (60 mg, 0.084 mmol), and CuI (40 mg, 0.21 mmol) were mixed in DMF (10 mL). This mixture was then degassed and heated at 100 °C for 1 h. TLC suggested full conversion. After aqueous workup, the residue was purified by column chromatography to yield 162 mg (93%) of 3-methoxymethoxy-4-pyridin-2-yl-benzonitrile (**43b**): MS (ESI)  $m/z$  241 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.74 (m, 1H), 7.26–7.91 (m, 6H), 5.22 (s, 2H), 3.48 (s, 3H).

By the procedure described above for **44a**, 160 mg (0.67 mmol) of **43b** was reacted in two steps to give 63 mg (47%) of **45b**: MS (ESI)  $m/z$  201 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.50 (m, 1H), 7.76–7.92 (m, 3H), 7.24 (m, 1H), 6.86–6.97 (m, 2H), 3.87 (s, 2H).

**5-Aminomethyl-2-pyridin-3-yl-phenol (45c)**. By the procedure described above for **45b**, 200 mg (0.69 mmol) of **42** was reacted in three steps to give 53 mg (33%) of **45c**: MS (ESI)  $m/z$  201 (M + 1)<sup>+</sup>.

**5-Aminomethyl-2-pyridin-4-yl-phenol (45d)**. By the procedure described above for **45b**, 200 mg (0.69 mmol) of **42** was reacted in three steps to give 40 mg (25%) of **45d**: MS (ESI)  $m/z$  201 (M + 1)<sup>+</sup>.

**2-Aminomethyl-5-methoxy-pyrimidin-4-ol (51)**. 1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-acetonitrile (**46**)<sup>24</sup> (1 g, 5.34 mmol) was dissolved in 4 N HCl in dioxane (10 mL). The mixture was stirred at room temperature, and MeOH (1 mL) was added. The solution turned cloudy shortly. The precipitate was collected and washed with ether to provide 1 g (86%) of 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetimidic acid methyl ester hydrochloride (**47**): MS (ESI)  $m/z$  219 (M + 1)<sup>+</sup>.

A suspension of **47** (1 g, 4.58 mmol) in MeOH (15 mL) was added to a saturated NH<sub>3</sub>/MeOH solution. The mixture was stirred at 0 °C and then room temperature. After all the volatile was removed, the product 9b-hydroxy-2-imino-1,2,3,9b-tetrahydro-

imidazo[2,1-a]isoindol-5-one (**48**) was directly employed in the next step: MS (ESI)  $m/z$  204 ( $M + 1$ )<sup>+</sup>.

Compound **48** (500 mg, 2.09 mmol) and the sodium salt of 3-hydroxy-2-methoxy-acrylic acid methyl ester (**49**)<sup>25</sup> (500 mg, 3.24 mmol) were mixed in MeOH and stirred at room temperature for 2 h, and then heated at reflux overnight. The crude mixture was filtered. After the filtrate was concentrated, the resulting residue was purified by column chromatography to afford 100 mg (16%) of 2-(4-hydroxy-5-methoxy-pyrimidin-2-ylmethyl)-isoindole-1,3-dione (**50**): MS (ESI)  $m/z$  286 ( $M + 1$ )<sup>+</sup>.

Hydrazine (0.5 mL) was added to a suspension of **50** (50 mg, 0.18 mmol) in EtOH (2 mL), and the mixture was stirred till no starting material was left. The precipitate was filtered and the filtrate was concentrated to provide **51**, which was used directly without further purification: MS (ESI)  $m/z$  156 ( $M + 1$ )<sup>+</sup>.

**4-Aminomethyl-1H-pyridin-2-one hydrochloride (55)**. 2-Methoxy-4-cyanopyridine (**53**) was prepared from 2-chloro-4-cyanopyridine according to the procedure by Brown et al.<sup>26</sup> Reduction of **53** to 4-(2-methoxypyridinyl)methylamine (**54**) was achieved through the method of Walpole et al.<sup>27</sup>

A solution of **54** (0.19 g, 1.1 mmol) in water (50 mL) and 3 N aqueous hydrochloric acid (25 mL) was heated at reflux for 5 h. The solvents were evaporated to give 0.15 g (83%) of **55** as an off-white solid: MS (ESI)  $m/z$  125.1 ( $M + 1$ )<sup>+</sup>.

**2-Oxo-1-phenyl-1,2-dihydropyridin-4-ylmethylamine hydrochloride (58)**. To a solution of **55** (3.68 g, 23 mmol) in 50% aqueous dioxane (100 mL) was added sodium hydroxide (2.8 g, 69 mmol), followed by di-*tert*-butyldicarbonate (5.0 g, 23 mmol). After stirring overnight at room temperature, the mixture was neutralized with 5% aqueous potassium hydrogen sulfate solution. The mixture was extracted four times with ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. A sample of crude material was purified by reverse-phase HPLC to give *tert*-butyl (2-oxo-1,2-dihydropyridin-4-ylmethyl)-carbamate (**56**) as a straw colored foam: MS (ESI)  $m/z$  225.3 ( $M + 1$ )<sup>+</sup>.

To a solution of **56** (0.13 g, 0.58 mmol) in dichloromethane (5 mL) was added trimethylphenylstannane (210  $\mu$ L, 1.2 mmol), followed successively by copper (II) acetate (0.12 g, 0.64 mmol) and tetra-*n*-butylammonium fluoride (1.0 M solution in tetrahydrofuran, 1.2 mL). The reaction mixture was stirred for 2 days at room temperature and then was quenched by the addition of methanolic ammonia (2 M, 4 mL). The reaction mixture was concentrated and then purified by flash silica gel chromatography (methanol/chloroform) to give 88 mg (52%) of **57**: MS (ESI)  $m/z$  301.3 ( $M + 1$ )<sup>+</sup>.

Compound **57** (83 mg, 0.28 mmol) was treated with 4 N hydrogen chloride (2 mL) in dioxane to remove the Boc protecting group. The reaction solution was evaporated to dryness to give 66 mg (100%) of 4-aminomethyl-1-phenyl-1H-pyridin-2-one hydrochloride (**58**).

**1-Furan-3-yl-4-methyl-1H-pyridin-2-one (60)**. A mixture of 2-hydroxy-4-methylpyridine (**59**) (0.66 g, 6.0 mmol), 3-bromofuran (1.7 g, 12 mmol), copper (I) iodide (0.11 g, 0.60 mmol), and potassium carbonate (0.84 g, 6.0 mmol) in DMF (12 mL) was heated at 180 °C for 2 h in a 300 W microwave reactor. The reaction mixture was then diluted with 10% aqueous ammonium hydroxide solution and extracted thrice with ethyl acetate. The combined extracts were washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give 0.65 g (62%) of **60**: MS (ESI)  $m/z$  176.1 ( $M + 1$ )<sup>+</sup>.

**1-Furan-3-yl-2-oxo-1,2-dihydropyridine-4-carbaldehyde (62)**. A mixture of **60** (3.7 g, 21 mmol), *tert*-butoxybis(dimethylamino)methane (11 g, 63 mmol), and DMF (4 mL) was heated at 150 °C for 2.5 h and then concentrated to dryness under reduced pressure to yield crude 4-(2-dimethylamino-vinyl)-1-furan-3-yl-1H-pyridin-2-one (**61**), which was used without further purification: MS (ESI)  $m/z$  231.3 ( $M + 1$ )<sup>+</sup>.

To a solution of **61** (21 mmol) in 50% aqueous tetrahydrofuran (700 mL) was added sodium periodate (13 g, 63 mmol). After 6 h

of stirring at room temperature, the reaction mixture was filtered. The filtrate was washed thrice with saturated aqueous sodium hydrogen carbonate solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give 2.1 g (53% over 2 steps) of **62** as a yellow powder: MS (ESI)  $m/z$  190.3 ( $M + 1$ )<sup>+</sup>.

**4-Aminomethyl-1-furan-3-yl-1H-pyridin-2-one (65)**. A solution of **62** (1.4 g, 7.4 mmol) in pyridine (40 mL) was treated with methoxylamine hydrochloride (0.68 g, 8.1 mmol). After stirring overnight at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic phase was washed twice with water and once with saturated aqueous sodium chloride solution, dried, and concentrated under reduced pressure to provide 1-furan-3-yl-2-oxo-1,2-dihydro-pyridine-4-carbaldehyde *O*-methyl-oxime (**63**), which was used in the next step without further purification: MS (ESI)  $m/z$  219.3 ( $M + 1$ )<sup>+</sup>.

To a mixture of the crude **63** and glacial acetic acid (77 mL) was added zinc powder (3.1 g). The reaction mixture was heated for 45 min at 100 °C in an oil bath and then allowed to cool to room temperature. The mixture was filtered through a pad of diatomaceous earth. After the filtrate was concentrated, the residue was purified by reverse-phase HPLC to give 2.3 g of crude 4-aminomethyl-1-furan-3-yl-1H-pyridin-2-trifluoroacetic acid salt contaminated with zinc salt: MS (ESI)  $m/z$  191.3 ( $M + 1$ )<sup>+</sup>.

To a solution of 2.3 g of the crude 4-aminomethyl-1-furan-3-yl-1H-pyridin-2-one trifluoroacetic acid salt in 50% aqueous dioxane (20 mL) was added sodium hydroxide (800 mg), followed by an additional volume of aqueous dioxane (20 mL), and then the addition of di-*tert*-butyldicarbonate (600  $\mu$ L). After the reaction was completed, the reaction mixture was filtered. The filtrate was neutralized with 5% aqueous potassium hydrogen sulfate solution and extracted thrice with ethyl acetate. The combined extracts were washed with saturated aqueous sodium chloride solution, dried, and concentrated under reduced pressure to give 0.18 g (8% over 3 steps) of *tert*-butyl (1-furan-3-yl-2-oxo-1,2-dihydro-pyridin-4-ylmethyl)-carbamate (**64**): MS (ESI)  $m/z$  291.3 ( $M + 1$ )<sup>+</sup>.

*tert*-Butyl (1-furan-3-yl-2-oxo-1,2-dihydro-pyridin-4-ylmethyl)-carbamate (**64**) (0.20 g, 0.69 mmol) was treated with 4 N hydrogen chloride in dioxane (5 mL) at room temperature. After the deprotection was completed, the solution was concentrated to yield 43 mg (27%) of 4-aminomethyl-1-furan-3-yl-1H-pyridin-2-one (**65**): MS (ESI)  $m/z$  191.2 ( $M + 1$ )<sup>+</sup>.

**2-Hydroxymethyl-5-propoxy-pyran-4-one (67b)**. A mixture of kojic acid (**66**) (28.4 g, 0.20 mol), 120 mL of DMF, potassium carbonate powder (27.6 g, 0.20 mol), potassium iodide (1.66 g, 0.01 mol), and 1-bromopropane (24.6 g, 0.20 mol) was stirred for 15 min at ambient temperature and then stirred at 90 °C for 3 h. The reaction mixture was cooled, evaporated to dryness in vacuo, and then partitioned between water and chloroform. The aqueous layer was extracted with chloroform (3  $\times$  100 mL) and ethyl acetate (6  $\times$  100 mL). The combined organic solution was dried with sodium sulfate and passed through a pad of magnesol and silica gel eluting with ethyl acetate. The eluate was evaporated in vacuo and crystallized with hexane/ethyl acetate (2:1) to give 22.6 g (61%) of **67b** as an off-white solid: MS (ESI)  $m/z$  185.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.02 (t,  $J = 7.5$ , 3H), 1.78 – 1.89 (m, 2H), 3.57 (t,  $J = 6.5$ , 1H), 3.81 (t,  $J = 6.7$ , 2H), 4.48 (dd,  $J = 6.7$ , 0.7, 2H), 6.52 (m, 1H), 7.57 (s, 1H).

**2-Hydroxymethyl-5-propoxy-pyridin-4-ol (71b)**. A mixture of **67b** (30.0 g, 0.163 mol) and ammonium hydroxide (150 mL) was stirred and heated in a sealed vessel at 90 °C for 2 h. The reaction mixture was cooled and evaporated to dryness in vacuo. The residue was taken up in 15% methanol in chloroform and passed through a pad of magnesol and silica gel eluting with the same solvent. The eluate was evaporated, and the residue was treated with acetone, filtered, washed with acetone, and air-dried to give 8.03 g (80%) of **71b** as a gray solid: mp 159–160 °C; MS (ESI)  $m/z$  184.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.00 (t,  $J = 7.5$ , 3H), 1.73 – 1.83 (m, 2H), 4.08 (t,  $J = 6.6$ , 2H), 4.68 (s, 2H), 7.21 (s, 1H), 8.19 (s, 1H).

**2-Azidomethyl-5-propoxy-pyridin-4-ol (73b).** To a mixture of **71b** (5.56 g, 30.3 mmol) and chloroform (30 mL) cooled in an ice bath was added 30 mL of thionyl chloride. It was stirred for 15 min at ice bath temperature and then refluxed for 1 h. The reaction mixture was cooled, evaporated to dryness in vacuo, and then treated with isopropanol. The solid was filtered, washed with fresh isopropanol, then ether, and air-dried to give 3.4 g (48%) of 2-chloromethyl-5-propoxy-pyridin-4-ol (**72b**) as an off-white solid: mp 165–167 °C; MS (ESI)  $m/z$  202.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.99 (t,  $J = 7.5$ , 3H), 1.71–1.82 (m, 2H), 4.10 (t,  $J = 6.6$ , 2H), 4.95 (s, 2H), 7.83 (s, 1H), 8.34 (s, 1H).

To a suspension of sodium azide (2.82 g, 43.3 mmol) in 30 mL of DMF was added **72b** (8.74 g, 43.3 mmol). It was stirred overnight at ambient temperature and then quenched into ice water. The solid formed was filtered, washed with cold water, and dried to give 5.74 g (63%) of **73b** as an off white solid: MS (ESI)  $m/z$  209.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.00 (t,  $J = 7.5$ , 3H), 1.73–1.83 (m, 2H), 4.09 (t,  $J = 6.6$ , 2H), 4.76 (s, 2H), 7.26 (s, 1H), 8.35 (s, 1H).

**2-Aminomethyl-5-propoxy-pyridin-4-ol (74b).** Azide **73b** (9.70 g, 46.6 mol) was suspended in 120 mL of THF and treated with triphenylphosphine (12.22 g, 46.6 mmol). After stirring for 10 min at ambient temperature, water was added (12.6 mL, 700 mmol), and the reaction mixture was stirred at ambient temperature overnight. The solid was gradually dissolved, followed by the formation of a precipitate. The resulting solid was filtered, washed with THF/water (10:1), and dried to give 5.92 g (69%) of **74b**: mp 159–160 °C; MS (ESI)  $m/z$  183.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.00 (t,  $J = 7.5$ , 3H), 1.73–1.84 (m, 2H), 4.10 (t,  $J = 6.6$ , 2H), 4.24 (bs, 2H), 7.32 (s, 1H), 8.44 (s, 1H), 8.51 (bs, 3H).

**2-Azidomethyl-5-methoxy-pyridin-4-ol (73a).** Using the procedure described for **73b**, 2-chloromethyl-5-methoxy-pyridin-4-ol<sup>29</sup> (**72a**) (3.47 g, 20.0 mmol) and sodium azide (1.30 g, 20.0 mmol) were reacted to give 1.53 g (42%) of **73a** as a light brown solid: mp 111–114 °C (dec); MS (ESI)  $m/z$  179.3 ( $M - 1$ )<sup>-</sup>.

**2-Aminomethyl-5-methoxy-pyridin-4-ol (74a).** Using the procedure described for **74b**, **73a** (1.45 g, 8.05 mol) and triphenylphosphine (2.11 g, 8.05 mmol) were reacted to give 0.897 g (88%) of **74a**: mp 196–201 °C (dec); MS (ESI):  $m/z$  155.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>, w/TFA) δ 4.00 (s, 3H), 4.27 (s, 2H), 7.32 (s, 1H), 8.43 (s, 1H), 8.59 (bs, 3H).

**2-Hydroxymethyl-5-furan-3-yl-pyran-4-one (77b).** A mixture of trifluoro-methanesulfonic acid 6-(*tert*-butyl-dimethyl-silyloxy-methyl)-4-oxo-4H-pyran-3-yl ester<sup>31</sup> (**75**) (6.94 g, 17.8 mmol), furan-3-boronic acid (4.0 g, 35.7 mmol), tetrakis(triphenyl)phosphine palladium (1.024 g, 0.87 mmol), cesium carbonate (16.32 g, 50.1 mmol), and potassium bromide (10.63 g, 89.3 mmol) in (250 mL) dioxane (250 mL) was heated to 60 °C and stirred overnight. The reaction mixture was cooled to room temperature and diluted with saturated aqueous ammonium chloride and extracted with ethyl acetate. The combined ethyl acetate layers were washed with water, dried over sodium sulfate, filtered, diluted with an equal volume of hexane, and passed through a short column of magnesol and silica gel eluting with 1:1 hexane/ethyl acetate. The product was eluted with 2:1 ethyl acetate/hexane, the solvents were evaporated, triturated with 1:1 hexane/ethyl acetate, filtered, washed with the same solvent, and air-dried to give 2.58 g (47%) of **76b** as an off white solid: MS (ESI)  $m/z$  307.3 ( $M + 1$ ).

Compound **76b** was dissolved in tetrahydrofuran, and tetrabutylammonium fluoride solution in tetrahydrofuran (40.0 mL, 40.0 mmol) was added and the mixture stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The ethyl acetate layers were combined and washed with water, dried over sodium sulfate, filtered, diluted with an equal volume of hexane, and passed through a short column of magnesol and silica gel eluting with 1:1 hexane/ethyl acetate. The product was eluted with 2:1 ethyl acetate/hexane, and the solvents were evaporated, triturated with 1:1 hexane/ethyl acetate, filtered, washed with the same solvent, and air-dried to give 2.06 g (37%) of **77b** as an off white solid, MS (ESI)  $m/z$  193.3 ( $M +$

$1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.36(d,  $J = 5.9$ , 2H), 5.74 (t,  $J = 5.9$ , 1H), 6.39 (s, 1H), 6.93–6.97 (m, 1H), 7.72–7.56 (m, 1H), 8.40–8.43 (m, 1H), 8.70 (s, 1H).

**2-Hydroxymethyl-5-(3-furyl)-pyridin-4-ol (78b).** A mixture of 2-hydroxymethyl-5-furan-3-yl-pyran-4-one (**77b**) (1.92 g, 10.0 mmol) and 7 M ammonia in methanol (50.0 mL) was stirred and heated in a sealed vessel at 90 °C overnight. The reaction mixture was cooled, evaporated to dryness in vacuo, taken up in 15% methanol in chloroform, and passed through a pad of magnesol and silica gel eluting with the same solvent. The eluate was evaporated, treated with acetone, filtered, washed with acetone, and air-dried to give 1.21 g (63%) of **78b** as a gray solid: MS (ESI)  $m/z$  192.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>, w/TFA) δ 4.75 (s, 2H), 7.20–7.26 (m, 1H), 7.34 (s, 1H), 7.82–7.87 (m, 1H), 8.39 (s, 1H), 8.81 (s, 1H).

**2-Aminomethyl-5-furan-3-yl-pyridin-4-ol (81b).** To a mixture of **78b** (1.148 g, 6.00 mmol) and 30 mL of *N,N*-dimethylformamide stirred and cooled with an ice bath to 0 °C was added triphenylphosphine (2.361 g, 9 mmol) followed by carbon tetrabromide (2.988 g, 9 mmol). This was stirred for 15 min, maintaining the temperature between 0–5 °C. Sodium azide was then added (1.172 g, 18.03 mmol) and the reaction mixture stirred for 24 h at ambient temperature. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were dried over sodium sulfate, filtered, evaporated, and the residue chromatographed on silica gel eluting with ethyl acetate to give 0.417 g (32%) of 2-azidomethyl-5-furan-3-yl-pyridin-4-ol (**80b**) as a white solid: mp 198–200 °C (dec); MS (ESI)  $m/z$  217.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.87 (s, 2H), 7.23–7.25 (m, 1H), 7.40 (s, 1H), 7.81–7.83 (m, 1H), 8.42 (bs, 1H), 8.95 (s, 1H).

To a mixture of **80b** (216 mg, 1 mmol), 6 mL of tetrahydrofuran and triphenylphosphine (262 mg, 1 mmol) were added followed by water (270 μL, 15.0 mmol). It was stirred at ambient temperature overnight and then at 60 °C overnight. The reaction mixture was cooled, evaporated to dryness in vacuo, and then treated with warm toluene. This mixture was cooled and the solid was filtered, washed with toluene, and dried to give 0.170 g (89%) of **81b** as an off-white solid: mp 183–7 °C (dec); MS (ESI)  $m/z$  191.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>, w/TFA) δ 4.32 (s, 2H), 7.25 (s, 1H), 7.41 (s, 1H), 7.86 (s, 1H), 8.45 (s, 1H), 8.58 (bs, 3H), 9.00 (s, 1H).

**2-Aminomethyl-5-phenyl-pyridin-4-ol (81a).** A mixture of 2-hydroxymethyl-5-phenyl-pyridin-4-ol (**78a**) (0.40 g, 2 mmol) and 5 mL of thionyl chloride was heated to a gentle reflux. After 4 h, the reaction mixture was cooled and evaporated to dryness in vacuo, and the residue was treated with water and neutralized with sodium bicarbonate. The resulting solid was collected by filtration, washed with water, and dried to give 256 mg (58%) of the 2-chloromethyl-5-phenyl-pyridin-4-ol (**79a**).

Compound **79a** (252 mg, 1.15 mmol) was then stirred with 3 mL of dimethyl formamide, and sodium azide was then added (75 mg, 1.15 mmol) and the reaction mixture stirred for 24 h at ambient temperature. The solvent was removed in vacuo and the residue treated with water, filtered, washed with water, and dried to give 231 mg (88%) of 2-azidomethyl-5-phenyl-pyridin-4-ol (**80a**).

Compound **80a** (228 mg, 1.01 mmol) was then suspended in 3 mL of tetrahydrofuran and treated with triphenylphosphine (264 mg, 1.01 mmol). After stirring for 10 min at ambient temperature, water was added (272 μL, 15 equivalents) and the reaction mixture warmed with an oil bath at 60 °C, and stirred at that temperature overnight. The reaction mixture was evaporated to dryness in vacuo and treated with a 2:1 mixture of ethyl acetate and hexane. The resulting solid was filtered, washed with fresh 2:1 ethyl acetate and hexane, and dried to give 116 mg (57%) of **81a** as a gray solid: MS (ESI)  $m/z$  201.1 ( $M + 1$ )<sup>+</sup>.

**(6-Chloro-5-propoxy-pyridin-2-yl)-methanol (84).** A mixture of 2-chloro-6-hydroxymethyl-pyridin-3-ol<sup>32</sup> (**83**) (27.92 g, 0.175 mol), 120 mL of 2-butanone, potassium carbonate powder (48.37 g, 0.35 mol), and 1-iodopropane (37.19 g, 0.219 mol) was stirred for 15 min at ambient temperature and then stirred at 90 °C for 3 h. The reaction mixture was cooled, evaporated to dryness in vacuo, and

then portioned between water and dichloromethane. The organic layer was dried, filtered, and crystallized with 2/1 hexane/ethyl acetate to give 5.875 g (16%) of **84** as a pale yellow solid: mp 59–60 °C; MS (ESI)  $m/z$  160.3, 162.3 ( $M + 1$ )<sup>+</sup>.

**2-Benzyloxy-3-propoxy-6-triisopropylsilyloxymethyl-pyridine (85)**. A mixture of **84** (0.93 g, 4.61 mmol), 20 mL of dichloromethane, tri(isopropyl)silyl chloride (1.0 g, 5.19 mmol), and imidazole (0.47 g, 6.9 mmol) was stirred overnight at ambient temperature. The reaction mixture was washed with water, dried over sodium sulfate, filtered, and evaporated in vacuo to give an oil which was chromatographed on silica gel with 10:1 hexane/ethyl acetate to give 2-chloro-3-propoxy-6-triisopropylsilyloxymethyl-pyridine as a clear liquid (1.06 g) (64%); MS (ESI):  $m/z$  358.2, 360.2 ( $M + 1$ ). A portion of this (716 mg, 2.0 mmol) and 5 mL of 1 M sodium benzyloxide in benzyl alcohol were heated at 120 °C under microwave conditions for 5 min. The reaction mixture was cooled, transferred to a separatory funnel with ethyl acetate and washed with water, and the organic layer was dried, filtered, evaporated, and chromatographed on silica gel with hexane/ethyl acetate to give 524 mg (61%) of **85** as a clear liquid; MS (ESI)  $m/z$  430.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.02 (t,  $J = 7.4$  Hz, 3H), 1.07–1.12 (m, 18H), 1.14–1.23 (m, 3H), 1.77 (m, 2H), 3.95 (t,  $J = 6.7$  Hz, 2H), 4.75 (s, 2H), 5.44 (s, 2H), 7.03 (d,  $J = 7.9$  Hz, 1H), 7.09 (d,  $J = 7.9$  Hz, 1H), 7.26–7.38 (m, 3H), 7.45–7.50 (m, 2H).

**(6-Benzyloxy-5-propoxy-pyridin-2-yl)-methanol (86)**. To a solution of **85** (860 mg, 2.0 mmol) was added 1 M tetrabutylammonium fluoride solution in tetrahydrofuran (4.0 mL, 4.0 mmol) and the mixture stirred at room temperature for 4 h. The reaction mixture was diluted with water and extracted with ether. The ether layers were combined and washed with water, dried over sodium sulfate, filtered, and chromatographed on silica gel eluting with 1:1 hexane/ether to give 355 mg (65%) of **86** as a white solid: mp 50–1 °C; MS (ESI)  $m/z$  274.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.04 (t,  $J = 7.5$  Hz, 3H), 1.79–1.89 (m, 2H), 2.90 (bs, 1H), 3.97 (t,  $J = 6.7$  Hz, 2H), 4.57 (s, 2H), 5.48 (s, 2H), 6.74 (d,  $J = 7.8$  Hz, 1H), 7.07 (d,  $J = 7.8$  Hz, 1H), 7.26–7.31 (m, 1H), 7.33–7.38 (m, 2H), 7.45–7.49 (m, 2H).

**6-Azidomethyl-2-benzyloxy-3-propoxy-pyridine (87)**. Using the procedure described for the preparation of **80b**, **86** (1.09 g, 4.0 mmol) was reacted with triphenylphosphine and carbon tetrabromide (1.99 g, 6.0 mmol) to give 1.05 g (88%) of **87** as a clear liquid: MS (ESI)  $m/z$  299.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.04 (t,  $J = 7.5$  Hz, 3H), 1.79–1.90 (m, 2H), 3.96 (t,  $J = 6.7$  Hz, 2H), 4.22 (s, 2H), 5.49 (s, 2H), 6.78 (d,  $J = 7.8$  Hz, 1H), 7.02 (d,  $J = 7.8$  Hz, 1H), 7.26–7.31 (m, 1H), 7.32–7.38 (m, 2H), 7.47–7.52 (m, 2H).

**6-Aminomethyl-3-propoxy-pyridin-2-ol (88)**. To a mixture of **87** (544 mg, 2.00 mmol), 12 mL of tetrahydrofuran and triphenylphosphine (524 mg, 2.00 mmol) were added, followed by water (540  $\mu$ L, 30.0 mmol). It was stirred at ambient temperature overnight. The reaction mixture was evaporated to dryness in vacuo and then washed with 2:1 hexane/ethyl acetate and filtered. This resulting solid was taken up in ethanol and hydrogenated over 10% palladium on carbon at 1 atm. The reaction mixture was filtered, washed with ethanol, and evaporated to give 93 mg (25%) of **88** as a brownish green solid: MS (ESI)  $m/z$  183.3 ( $M + 1$ )<sup>+</sup>.

**Preparation of the Final Compounds. (4Z)-6-Iodo-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (5e)**. A mixture of (4Z)-6-iodo-4-[(4-hydroxy-3-methoxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (**4e**) (0.3 g, 0.91 mmol), 4-methoxy-3-hydroxyl-benzylamine hydrochloride (**2e**) (0.19 g, 1 mmol), and triethylamine (0.14 mmol, 1.37 mmol) in DMF (3 mL) was stirred at room temperature overnight. The mixture was diluted with diethyl ether, and the solid was collected by filtration. The crude product was washed with water and methanol, followed by recrystallization from DMF in ether to give 0.28 g (68%) of **5e** as a tan solid: mp 209–210 °C; MS (ESI)  $m/z$  449.0 ( $M - H$ )<sup>-</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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, 8.5 Hz, 1H), 8.56 (s, 1H), 8.86 (d, 12.84 Hz, 1H), 11.31 (s, 1H), 12.59 (d, 12.84 Hz, 1H); Anal. (C<sub>18</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**4-[(3,4-Dihydroxy-phenylamino)-methylene]-4H-isoquinoline-1,3-dione (3a)**. The title compound was obtained by the procedure described above for **5e** except that no triethylamine was used, and the reaction was carried out at 115 °C for 1.5 h. 4-Methoxymethylene-4H-isoquinoline-1,3-dione (**1**) (40.6 mg, 0.2 mmol) and 3,4-dihydroxyaniline (**2a**) (25 mg, 0.2 mmol) were reacted to give 15.8 mg (27%) of **3a**; LC/MS1 *R*<sub>t</sub> 1.716 min,  $m/z$  297.1 ( $M + H$ )<sup>+</sup>; LC/MS2 *R*<sub>t</sub> 1.74 min,  $m/z$  295 ( $M - H$ )<sup>-</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 6.55 (s, 1H) 6.60 (d,  $J = 8.7$  Hz, 1H) 7.24 (t,  $J = 8.3$  Hz,  $J = 7.3$  Hz, 1H) 7.39 (d,  $J = 8.7$  Hz, 1H) 7.60 (t,  $J = 7.8$  Hz, 1H) 8.02 (d,  $J = 7.8$  Hz, 1H) 8.11 (d,  $J = 8.7$  Hz, 1H) 8.79 (d,  $J = 12.8$  Hz, 1H) 9.56 (br s, 2H) 11.24 (s, 1H) 12.41 (d,  $J = 12.8$  Hz, 1H).

**4-[(3,4-Dihydroxy-benzylamino)-methylene]-4H-isoquinoline-1,3-dione (3b)**. The title compound was obtained by the procedure described above for **5e** except that the reaction was carried out at 115 °C for 1.5 h. Compound **1** (20.3 mg, 0.1 mmol), 3,4-dihydroxybenzylamine hydrobromide (**2b**) (22 mg, 0.1 mmol), and triethylamine (21  $\mu$ L) were reacted to give 28.4 mg (91%) of **3b**. LC/MS1 *R*<sub>t</sub> 1.940 min,  $m/z$  311.0 ( $M + H$ ); LC/MS2 *R*<sub>t</sub> 1.67 min,  $m/z$  311 ( $M + H$ ); HRMS (ESI)  $m/z$  calcd for N<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub> 311.1031; found, 311.1026 ( $M + H$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.52–4.54 (m, 2H), 6.63 (d,  $J = 8.0$  Hz, 1H), 6.71 (s, 1H), 6.47 (d,  $J = 8.0$  Hz, 1H), 7.17 (t,  $J = 7.4$  Hz,  $J = 7.9$  Hz, 1H), 7.55 (t,  $J = 7.4$  Hz,  $J = 8.3$  Hz, 1H), 7.83 (d,  $J = 8.3$  Hz, 1H), 7.97 (d,  $J = 7.9$  Hz, 1H), 8.61 (d,  $J = 13.2$  Hz, 1H), 8.93 (s, 1H), 8.99 (s, H), 10.55–10.64 (m, 1H), 10.97 (s, 1H).

**(4Z)-4-[(4-Hydroxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (3c)**. Using the procedure described for the preparation of **5e** except without the addition of triethylamine, **1** (300 mg, 1.48 mmol) and 4-hydroxy-benzylamine (**2c**) (235 mg, 1.48 mmol) were reacted to give 200 mg (46%) of **3c** as a red-brown solid: mp 272–273 °C; MS (ESI)  $m/z$  294.31 ( $M - 1$ )<sup>-</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.58 (d,  $J = 6.0$  Hz, 2H), 6.76 (d,  $J = 8.3$  Hz, 2H), 6.99–7.26 (m, 3H), 7.55 (t,  $J = 7.6$  Hz, 1H), 7.83 (d,  $J = 8.3$  Hz, 1H), 7.97 (d,  $J = 7.8$  Hz, 1H), 8.62 (d,  $J = 13.3$  Hz, 1H), 9.44 (s, 1H), 10.00–10.77 (m, 1H), 10.96 (s, 1H); Anal. (C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>·0.2H<sub>2</sub>O) C, H, N.

**(4Z)-4-[(3-Hydroxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (3d)**. Using the procedure described for the preparation of **5e** except without the addition of triethylamine, **1** (300 mg, 1.48 mmol) and 3-hydroxy-benzylamine (**2d**) (235 mg, 1.48 mmol) were reacted to give 200 mg (46%) of **3d** as a reddish-brown solid: mp 261–262 °C; MS (ESI)  $m/z$  294.31 ( $M - 1$ ); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.63 (d,  $J = 6.0$  Hz, 2H), 6.69 (d,  $J = 8.0$  Hz, 1H), 6.77 (m, 2H), 7.16 (t,  $J = 8.0$  Hz, 2H), 7.56 (t,  $J = 7.3$  Hz, 1H), 7.83 (d,  $J = 7.3$  Hz, 1H), 7.98 (d,  $J = 7.3$  Hz, 1H), 8.63 (d,  $J = 13.3$  Hz, 1H), 9.48 (s, 1H), 10.64 (m, 1H), 11.0 (s, 1H); Anal. (C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>·0.2H<sub>2</sub>O) C, H, N.

**(4Z)-4-[(3-Hydroxy-4-methoxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (3e)**. Using the procedure described for the preparation of **5e**, **1** (0.2 g, 0.98 mmol) and 4-methoxy-3-hydroxyl-benzylamine hydrochloride (**2e**) (0.2 g, 1.08 mmol) were reacted to give 0.25 g (78%) of **3e** as a red-brown solid: mp 192–193 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.56 (d,  $J = 4.5$  Hz, 2H), 6.78 (m, 2H), 6.91 (d,  $J = 6$  Hz, 1H), 7.17 (dd,  $J = 6, 6$  Hz, 1H), 7.55 (dd,  $J = 6, 6$  Hz, 1H), 7.83 (d,  $J = 6$  Hz, 1H), 7.98 (d,  $J = 6$  Hz, 1H), 8.62 (d,  $J = 9$  Hz, 1H), 9.04 (s, 1H), 10.60 (m, 1H), 10.97 (s, 1H); MS (ESI)  $m/z$  325.1 ( $M + H$ )<sup>+</sup>; Anal. (C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

**4-[[2-(3,4-Dihydroxy-phenyl)-ethylamino]-methylene]-4H-isoquinoline-1,3-dione (3f)**. Using the procedure described for the preparation of **5e**, **1** (20.3 mg, 0.1 mmol) and 3-hydroxytyramine hydrochloride (**2f**) (19 mg, 0.1 mmol) were reacted to give 18.1 mg (56%) of **3f** as a red-brown solid; LC/MS1 *R*<sub>t</sub> 1.748 min,  $m/z$  325.0 ( $M + H$ )<sup>+</sup>; LC/MS2 *R*<sub>t</sub> 1.73 min,  $m/z$  325 ( $M + H$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.74 (t,  $J = 7.1$  Hz, 2H), 3.65 (q,  $J = 7.1$  Hz,  $J = 6.7$  Hz, 2H), 6.52 (dd,  $J = 9.6$  Hz,  $J =$



1.8 Hz, 1 H), 6.64 (d,  $J = 1.8$  Hz, 1 H), 6.66 (d,  $J = 9.6$  Hz, 1 H), 7.14 (t,  $J = 7.5$  Hz,  $J = 7.8$  Hz, 1 H), 7.51 (t,  $J = 8.4$  Hz, 1 H), 7.72 (d,  $J = 8.4$  Hz, 1 H), 7.95 (d,  $J = 7.8$  Hz, 1 H), 8.41 (d,  $J = 13.5$  Hz, 1 H), 8.77 (br s, 2 H), 10.37 – 10.45 (m, 1 H), 10.91 (s, 1 H).

**(4Z)-6-Fluoro-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (5a).** Using the procedure described for the preparation of **5e**, (4E)-6-fluoro-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4a**) (300 mg, 1.06 mmol) and 3-hydroxy-4-methoxy-benzylamine hydrogen chloride (**2e**) (201.2 mg, 1.06 mmol) were reacted to give 340 mg (94%) of **5a** as a light-brown solid: mp 200–201 °C; MS (ESI)  $m/z$  342.33 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.56 (m, 2H), 6.77 (dd,  $J = 8.0$ , 2.0 Hz, 1H), 6.81 (d,  $J = 2.0$  Hz, 1H), 6.91 (d,  $J = 8.0$  Hz, 1H), 6.95–7.0 (m, 1H), 7.68 (dd,  $J = 12.0$ , 2.0 Hz, 1H), 8.01 (m,  $J = 8.0$ , 1H), 8.65 (d,  $J = 12.0$  Hz, 1H), 9.05 (s, 1H), 10.62 – 10.65 (m, 1H), 11.01 (s, 1H); Anal. (C<sub>18</sub>H<sub>15</sub>FN<sub>2</sub>O<sub>4</sub>·0.3 H<sub>2</sub>O) C, H, N.

**(4Z)-6-Chloro-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (5b).** Using the procedure described for the preparation of **5e**, (4E)-6-chloro-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4b**) (0.15 g, 0.63 mmol) and **2e** (0.114 g, 0.756 mmol) were reacted to give 0.182 g (80.2%) of **5b** as a tan solid: mp 265–266 °C; MS (ESI)  $m/z$  357.5 (M – H)<sup>–</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.75 (s, 3 H), 4.57 (d,  $J = 6.0$  Hz, 2 H), 6.78 (d,  $J = 8.1$  Hz, 1 H), 6.82 (s, 1 H), 6.91 (d,  $J = 8.1$  Hz, 1 H), 7.17 (d,  $J = 8.3$  Hz, 1 H), 7.81 – 8.08 (m, 2 H), 8.71 (d,  $J = 13.4$  Hz, 1 H), 9.05 (s, 1 H), 10.45 – 10.85 (m, 1 H), 11.07 (s, 1 H); Anal. (C<sub>18</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub>·0.8 H<sub>2</sub>O) C, N, H: calcd, 4.48; found, 4.06.

**(4Z)-5-Bromo-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (5c).** Using the procedure described for the preparation of **5e**, (4E)-5-bromo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4c**) (132 mg, 0.468 mmol) and **2e** (88.5 mg, 0.468 mmol) were reacted to give 163 mg (86%) of **5c** as a pale yellow amorphous solid: mp 204–206 °C; HRMS (ESI)  $m/e$  calcd for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>3</sub> 431.20886; found, 431.20820 (M – H)<sup>–</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.49 (d, 2H,  $J = 5.4$  Hz), 6.78 (m, 2H), 6.91 (d, 1H,  $J = 8.1$  Hz), 7.09 (m, 1H), 7.87 (d, 1H,  $J = 6.9$  Hz), 8.05 (d, 1H,  $J = 6.9$  Hz), 8.93 (d, 1H,  $J = 13.6$  Hz), 9.08 (s, 1H), 10.56 (m, 1H), 11.11 (s, 1H); Anal. (C<sub>18</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>4</sub>·1.25 H<sub>2</sub>O) C, H, N.

**(4Z)-6-Bromo-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (5d).** Using the procedure described for the preparation of **5e**, (4E)-6-bromo-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4d**) (300 mg, 1.06 mmol) and **2e** (201.2 mg, 1.06 mmol) were reacted to give 340 mg (77%) of **5d** as a light-brown solid: mp 243–244 °C; MS (ESI)  $m/z$  413.23 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.56 (m, 2H), 6.77 (dd,  $J = 8.4$ , 2.0 Hz, 1H), 6.81 (d,  $J = 2.0$  Hz, 1H), 6.91 (d,  $J = 8.4$  Hz, 1H), 7.30 (dd,  $J = 8.4$ , 1.2 Hz, 1H), 7.87 (d,  $J = 8.4$  Hz, 1H), 8.10 (d,  $J = 1.2$  Hz, 1H), 8.71 (m, 1H), 9.05 (s, 1H), 10.56 – 10.75 (m, 1H), 11.07 (s, 1H); Anal. (C<sub>18</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>4</sub>·0.5 H<sub>2</sub>O) C, N, H: calcd, 3.99; found, 3.56.

**4-[(3-Hydroxy-4-methoxy-benzylamino)-methylene]-6-methoxy-4H-isoquinoline-1,3-dione (5f).** Using the procedure described for the preparation of **5e**, (4E)-6-methoxy-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4d**) (117 mg, 0.50 mmol) and **2e** (145 mg, 0.50 mmol) were reacted to give 161 mg (91%) of **5f** as a light beige solid: mp 240–242 °C (dec); MS (ESI)  $m/z$  355.2 (M – 1)<sup>–</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 3.88 (s, 3H), 4.58 (d,  $J = 6.0$  Hz, 2H), 6.72 – 6.79 (m, 2H), 6.80 (d,  $J = 2.0$  Hz, 1H), 6.91 (d,  $J = 8.2$  Hz, 1H), 7.27 (d,  $J = 2.01$  Hz, 1H), 7.90 (d,  $J = 9.1$  Hz, 1H), 8.63 (d,  $J = 13.2$  Hz, 1H), 9.07 (bs, 1H), 10.63 (dt,  $J = 13.3$ , 5.8 Hz, 1H), 10.78 (s, 1H); Anal. (C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>·0.25H<sub>2</sub>O) C, H, N.

**(4Z)-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-6-(1H-pyrrol-1-yl)isoquinoline-1,3(2H,4H)-dione (5g).** Using the procedure described for the preparation of **5e**, (4E)-4-(methoxymethylene)-6-(1H-pyrrol-1-yl)isoquinoline-1,3(2H,4H)-dione (**4g**) (100 mg, 0.38 mmol) and **2e** (60 mg, 0.35 mmol) were reacted to give 90 mg

(66%) of **5g** as a light brown solid: mp 252–253 °C; MS (ESI)  $m/z$  389.41 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.60 (m, 2H), 6.34 (t,  $J = 4.0$ , 2.0 Hz, 2H), 6.78 (dd,  $J = 8.4$ , 2.0 Hz, 1H), 6.82 (d,  $J = 2.0$  Hz, 1H), 6.92 (d,  $J = 8.4$  Hz, 1H), 7.41 (dd,  $J = 8.8$ , 2.0 Hz, 1H), 7.59 (t,  $J = 4.0$ , 2.0 Hz, 2H), 7.92 (d,  $J = 2.0$  Hz, 1H), 8.02 (d,  $J = 8.8$  Hz, 1H), 8.80 (m, 1H), 9.07 (s, 1H), 10.37 – 10.78 (m, 1H), 10.96 (s, 1H); Anal. (C<sub>22</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>·0.6H<sub>2</sub>O) C, H, N.

**(4Z)-4-[(3-Hydroxy-4-methoxybenzyl)amino]methylene-6-thien-3-ylisoquinoline-1,3(2H,4H)-dione (5h).** Using the procedure described for the preparation of **5e**, (4E)-4-(methoxymethylene)-6-(3-thienyl)isoquinoline-1,3(2H,4H)-dione (**4h**) (0.15 g, 0.53 mmol) and **2e** (0.11 g, 0.62 mmol) were reacted to give 0.18 g (86%) of **5h** as a tan solid: mp 219–220 °C; MS (ESI)  $m/z$  407.1 (M + H)<sup>+</sup>; Anal. (C<sub>22</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S·0.8H<sub>2</sub>O) C, H, N.

**(4Z)-6-(3-Furyl)-4-[(3-hydroxy-4-methoxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (5i).** Using the procedure described for the preparation of **5e**, (4E)-6-(3-furyl)-4-(methoxymethylene)isoquinoline-1,3(2H,4H)-dione (**4h**) (160 mg, 0.59 mmol) and **2e** (94.6 mg, 0.59 mmol) were reacted to give 150 mg (65%) of **5i** as a brown solid: mp 254–255 °C; MS (ESI)  $m/z$  390.40 (M – 1)<sup>–</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.60 (m, 2H), 6.78 (dd,  $J = 8.0$ , 2.0 Hz, 1H), 6.82 (d,  $J = 2.0$  Hz, 1H), 6.92 (d,  $J = 8.0$  Hz, 1H), 7.17 (d,  $J = 1.2$  Hz, 1H), 7.44 (dd,  $J = 8.4$ , 1.2 Hz, 1H), 7.82 (m, 1H), 7.76 (d,  $J = 8.4$  Hz, 1H), 8.0 (m, 1H), 8.36 (m, 1H), 8.75 (m, 1H), 9.07 (s, 1H), 10.56 – 10.74 (m, 1H), 10.94 (s, 1H); Anal. (C<sub>22</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>·0.7H<sub>2</sub>O) C, H, N.

**4-[(3-Hydroxy-4-methoxy-benzylamino)-methylene]-6-phenyl-4H-isoquinoline-1,3-dione (5j).** To a suspension of **5d** (40 mg, 0.1 mmol) in DMF (1 mL) was added phenylboronic acid (14.6 mg, 0.12 mmol), followed by 60  $\mu$ L of 2 M aqueous cesium carbonate and tetrakis(triphenyl)phosphine palladium (6 mg, 0.005 mmol). The reaction mixture was subjected to microwave heating at 150 °C under microwave conditions for 5 min. The reaction mixture was then diluted to 2 mL with DMF and purified by C18 reverse phase HPLC. The pure fractions were combined and concentrated to yield 6.2 mg (15.5%) of **5j**; LC/MS1  $R_t$  2.620 min,  $m/z$  401.0 (M + H)<sup>+</sup>; LC/MS2  $R_t$  2.36 min,  $m/z$  401 (M + H)<sup>+</sup>; HRMS (ESI)  $m/z$  calcd for C<sub>24</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> 401.1498; found, 401.1496 (M + H)<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.81 (s, 3H), 4.59 – 4.62 (m, 2H), 6.78 (d,  $J = 8.13$  Hz, 1H), 6.82 (s, 1H), 6.91 (d,  $J = 8.1$  Hz, 1H), 7.44 – 7.55 (m, 4H), 7.82 (d,  $J = 7.2$  Hz, 2H), 8.05 (d,  $J = 8.2$  Hz, 1H), 8.09 (s, 1H), 8.83 (d,  $J = 13.2$  Hz, 1H), 9.06 (s, 1H), 10.61 – 10.71 (m, 1H), 10.99 (s, 1H).

**(4Z)-6-Bromo-4-[(1-(3-hydroxy-4-methoxyphenyl)ethyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (11).** Using the procedure described for the preparation of **5e**, **4d** (0.10 g, 0.35 mmol) and 5-(1-amino-ethyl)-2-methoxyphenol hydrochloride (**10**) (0.142, 0.70 mmol) were reacted to give 0.11 (73%) of **11** as a light tan powder: MS (ESI)  $m/z$  417.1 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.59 (d,  $J = 6.8$  Hz, 3H), 3.75 (s, 3H), 4.76 – 4.85 (m, 1H), 6.80 (dd,  $J = 8.4$ , 2.0 Hz, 1H), 6.83 (d,  $J = 2.0$  Hz, 1H), 6.93 (d,  $J = 8.4$  Hz, 1H), 7.30 (dd,  $J = 8.4$ , 1.6 Hz, 1H), 7.86 (d,  $J = 8.4$  Hz, 1H), 8.09 (d,  $J = 1.6$  Hz, 1H), 8.69 (d,  $J = 13.2$  Hz, 1H), 9.08 (s, 1H), 10.79 (dd,  $J = 13.2$ , 8.4 Hz, 1H), 11.13 (s, 1H); Anal. (C<sub>19</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>4</sub>) C, H, N.

**(4Z)-6-Bromo-4-[(3-hydroxy-4-methoxybenzyl)(methyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (13).** Using the procedure described for the preparation of **3c**, except for replacing DMF with tetrahydrofuran, **4d** (0.15 g, 0.53 mmol) and 2-methoxy-5-methylaminomethyl-phenol (**12**) (80 mg, 0.48 mmol) were reacted to give 0.19 g (95%) of **13** as a yellow solid: MS (ESI)  $m/z$  417.9, 419.9 (M + 1)<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.10 (s, 3H), 3.81 (s, 3H), 4.81 (s, 2H), 6.81 (d,  $J = 2.0$  Hz, 1H), 6.84 (d,  $J = 2.0$  Hz, 1H), 6.93 (d,  $J = 8.8$  Hz, 1H), 7.28 (dd,  $J = 8.4$ , 1.6 Hz, 1H), 7.83 (d,  $J = 1.6$  Hz, 1H), 7.91 (d,  $J = 8.4$  Hz, 1H), 8.50 (s, 1H), 9.10 (s, 1H), 10.73 (s, 1H); Anal. (C<sub>19</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>4</sub>) C, H, N.

**(4Z)-6-Bromo-4-[(3,4-dihydroxybenzyl)amino]methylene-isoquinoline-1,3(2H,4H)-dione (17a).** Using the procedure described for the preparation of **5e**, **4d** (1.21 g, 4.3 mmol) and 3,4-dihydroxybenzylamine (**16a**) (0.596 g, 4.3 mmol) were reacted, and

the crude product was purified by high performance liquid chromatography to give 910 mg (54%) of **17a** as a white solid: MS (ESI)  $m/z$  389.7 ( $M + 1$ );  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 4.53 (d,  $J = 6.3$  Hz, 2H), 6.63 (dd,  $J = 8.0, 1.2$  Hz, 1H), 6.72 (d,  $J = 7.5$  Hz, 1H), 6.76 (d,  $J = 1.7$  Hz, 1H), 7.30 (dd,  $J = 8.3, 1.6$  Hz, 1H), 7.87 (d,  $J = 8.3$  Hz, 1H), 8.11 (d,  $J = 1.5$  Hz, 1H), 8.71 (d,  $J = 13.3$  Hz, 1H), 8.91 (s, 1H), 8.97 (s, 1H), 10.64 (m, 1H), 11.07 (s, 1H); Anal. ( $\text{C}_{17}\text{H}_{13}\text{BrN}_2\text{O}_4 \cdot 0.5 \text{H}_2\text{O}$ ) C, H, N.

**(4Z)-6-Bromo-4-[[3-(3-hydroxy-4-propoxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (17b)**. Using the procedure described for the preparation of **5e**, **4d** (171 mg, 0.607 mmol) and 5-(aminomethyl)-2-propoxyphenol (**16b**) (100 mg, 0.55 mmol) were reacted, and the crude product was purified by high performance liquid chromatography to give 67 mg (28%) of **17b** as a white solid: MS (ESI)  $m/z$  430.8 ( $M + 1$ ) $^{+1}$ ; HRMS (ESI)  $m/z$  calcd for  $\text{C}_{20}\text{H}_{19}\text{BrN}_2\text{O}_4$  431.06010; found, 431.05984 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  0.97 (t,  $J = 7.4$  Hz, 3H), 1.56 – 1.91 (m, 2H), 3.89 (t,  $J = 6.6$  Hz, 2H), 4.56 (d,  $J = 6.0$  Hz, 2H), 6.64 – 6.77 (m, 1H), 6.82 (s, 1H), 6.89 (d,  $J = 8.3$  Hz, 1H), 7.30 (d,  $J = 8.7$  Hz, 1H), 7.87 (d,  $J = 8.7$  Hz, 1H), 8.11 (s, 1H), 8.72 (d,  $J = 13.2$  Hz, 1H), 8.97 (s, 1H), 10.41 – 10.89 (m, 1H), 11.10 (s, 1H); Anal. ( $\text{C}_{20}\text{H}_{19}\text{BrN}_2\text{O}_4$ ) C, H, N.

**(4Z)-6-Bromo-4-[[3-(3-hydroxy-4-(2-methoxyethoxy)benzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (17c)**. Using the procedure described for the preparation of **5e**, **4d** (400 mg, 1.42 mmol) and 5-(aminomethyl)-2-(methoxyethoxy)phenol (**16c**) (330 mg, 1.67 mmol) were reacted to give 480 mg (76%) of **17c** as a tan solid: mp 179–180 °C; MS (ESI)  $m/z$  447.0–449.0 ( $M + \text{H}$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 3.30 (s, 3H), 3.47 – 3.69 (m, 2H), 3.92 – 4.19 (m, 2H), 4.57 (d,  $J = 6.3$  Hz, 2H), 6.75 (dd,  $J = 8.3, 2.0$  Hz, 1H), 6.83 (d,  $J = 2.0$  Hz, 1H), 6.92 (d,  $J = 8.3$  Hz, 1H), 7.30 (dd,  $J = 8.4, 1.7$  Hz, 1H), 7.87 (d,  $J = 8.4$  Hz, 1H), 8.11 (d,  $J = 1.7$  Hz, 1H), 8.72 (d,  $J = 13.35$  Hz, 1H), 9.06 (s, 1H), 10.45 – 10.81 (m, 1H), 11.09 (s, 1H); Anal. ( $\text{C}_{20}\text{H}_{19}\text{BrN}_2\text{O}_5$ ) C, H, N.

**(4Z)-6-bromo-4-[[4-(4-ethoxy-3-hydroxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (17d)**. To a solution of 1-iodoethane (43.3  $\mu\text{L}$ , 0.28 mmol) in anhydrous *N,N*-dimethylformamide (2 mL) was added potassium carbonate (207 mg, 1.5 mmol). The mixture was stirred at room temperature, and **17a** (100 mg, 0.26 mmol) was added. After the mixture was stirred at 65 °C for 30 min, the resulting mixture was concentrated, and the residue was then partitioned between water (50 mL) and ethyl acetate (50 mL). The organic layer was then dried and purified by high performance liquid chromatography to give 26 mg (24%) of **17d** as a white solid. MS (ESI)  $m/z$  416.7 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  1.31 (t,  $J = 7.0$  Hz, 3H), 3.99 (q,  $J = 7.0$  Hz, 2H), 4.56 (d,  $J = 6.3$  Hz, 2H), 6.75 (dd,  $J = 8.3, 2.0$  Hz, 1H), 6.82 (d,  $J = 2.0$  Hz, 1H), 6.90 (d,  $J = 8.3$  Hz, 1H), 7.30 (dd,  $J = 8.5, 1.6$  Hz, 1H), 7.87 (d,  $J = 8.5$  Hz, 1H), 8.11 (d,  $J = 1.7$  Hz, 1H), 8.71 (d,  $J = 13.3$  Hz, 1H), 9.00 (s, 1H), 10.45 – 10.94 (m, 1H), 11.09 (s, 1H); Anal. ( $\text{C}_{19}\text{H}_{17}\text{BrN}_2\text{O}_4 \cdot 0.3\text{DMF} \cdot 0.5\text{TFA}$ ) C, H, N.

**(4Z)-6-Bromo-4-[[3-(3-hydroxy-4,5-dimethoxybenzyl)amino]methylene]isoquinoline-1,3(2H,4H)-dione (18)**. Using the procedure described for the preparation of **5e**, **4d** (400 mg, 1.42 mmol) and 3-hydroxy-4,5-dimethoxybenzylamine (272 mg, 1.15 mmol) were reacted to give 400 mg (65%) of **18** as a brown solid: mp 262–263 °C; MS (ESI)  $m/z$  433.26 ( $M - 1$ ) $^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  3.64 (s, 3H), 3.76 (s, 3H), 4.56 (m, 2H), 6.49 (d,  $J = 1.2$  Hz, 1H), 6.55 (m, 1H), 7.3 (dd,  $J = 8.4, 1.2$  Hz, 1H), 7.87 (d,  $J = 8.4$  Hz, 1H), 8.10 (m, 1H), 8.70 (m, 1H), 9.24 (s, 1H), 10.50 – 10.80 (m, 1H), 11.09 (s, 1H); Anal. ( $\text{C}_{19}\text{H}_{17}\text{BrN}_2\text{O}_5$ ) C, H, N.

**(4Z)-4-[[4-(4-Amino-3-hydroxybenzyl)amino]methylene]-6-bromoisoquinoline-1,3(2H,4H)-dione (21)**. Using the procedure described for the preparation of **5e**, **4d** (500 mg, 1.77 mmol) and 2-amino-5-(aminomethyl)phenol hydrochloride (**20**) (448 mg, 2.57 mmol) were reacted to give 700 mg (100%) of **21** as a yellowish brown solid: mp 245–246 °C; MS (ESI)  $m/z$  390 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.45 (m, 2H), 4.56 (m, 2H), 6.57 (m, 2H), 6.66 (m, 1H), 7.29 (dd,  $J = 8.4, 2.0$  Hz, 1H), 7.86 (d,  $J = 8.4$  Hz, 1H), 8.11 (d,  $J = 2.0$  Hz, 1H), 8.71 (m, 1H), 9.09 (s,

1H), 10.48 – 10.75 (m, 1H), 11.07 (s, 1H); Anal. ( $\text{C}_{17}\text{H}_{14}\text{BrN}_3\text{O}_3 \cdot 1.4 \text{H}_2\text{O}$ ) C, H, N.

**(4Z)-4-[[4-(4-Methyl-3-hydroxybenzyl)amino]methylene]-6-iodoisoquinoline-1,3(2H,4H)-dione (89a)**. Using the procedure described for the preparation of **5e**, **4e** (200 mg, 0.61 mmol) and 5-aminomethyl-2-methyl-phenol hydrogen chloride (**25**) (105.64 mg, 0.61 mmol) were reacted to give 120 mg (45%) of **89a** as an orange solid: mp 312–313 °C; MS (APCI); HRMS (ESI)  $m/z$  calcd for  $\text{C}_{18}\text{H}_{15}\text{IN}_2\text{O}_3$  435.02002; found, 435.02006 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  2.08 (s, 3H), 4.61 (m, 2H), 6.70 (d,  $J = 7.6$  Hz, 1H), 6.75 (m, 1H), 7.1 (d,  $J = 7.6$  Hz, 1H), 7.5 (dd,  $J = 8.0, 1.0$  Hz, 1H), 7.70 (d,  $J = 8.0$  Hz, 1H), 8.28 (d,  $J = 1.0$  Hz, 1H), 8.73 (m, 1H), 9.40 (s, 1H), 10.64 – 10.68 (m, 1H), 11.08 (s, 1H); Anal. ( $\text{C}_{18}\text{H}_{15}\text{IN}_2\text{O}_3$ ) C, H, N.

**4-[[2-(2-Hydroxy-biphenyl-4-ylmethyl)-amino]-methylene]-6-iodo-4H-isoquinoline-1,3-dione (89b)**. Using the procedure described for the preparation of **5e**, except without the addition of triethylamine, **4e** (110 mg, 0.34 mmol) and 4-aminomethyl-biphenyl-2-ol (**34**) (80 mg, 0.4 mmol) were reacted to give 151 mg (89%) of **89b**: MS (ESI)  $m/z$  495.1 ( $M - 1$ ) $^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.68 (d,  $J = 6.6$  Hz, 2H), 6.61 – 6.96 (m, 2H), 7.23 – 7.32 (m, 2H), 7.34 – 7.42 (m, 2H), 7.48 – 7.53 (m, 2H), 7.53 (d,  $J = 1.5$  Hz, 1H), 7.69 (d,  $J = 8.3$  Hz, 1H), 8.30 (d,  $J = 1.5$  Hz, 1H), 8.75 (d,  $J = 13.1$  Hz, 1H), 9.67 (s, 1H), 10.65 – 10.81 (m, 1H), 11.10 (s, 1H); Anal. ( $\text{C}_{23}\text{H}_{20}\text{IN}_3\text{O}_4 \cdot 0.7\text{H}_2\text{O}$ ) C, H, N.

**4-[(4-Furan-2-yl-3-hydroxy-benzylamino)-methylene]-6-iodo-4H-isoquinoline-1,3-dione (89c)**. Using the procedure described for the preparation of **89b**, **4e** (80 mg, 0.24 mmol) and 5-aminomethyl-2-furan-3-yl-phenol (**41**) (50 mg, 0.26 mmol) were reacted to give 62 mg (53%) of **89c**: MS (ESI)  $m/z$  485.1 ( $M - 1$ ) $^{-1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.67 (d,  $J = 6.3$  Hz, 2H), 6.56 (dd,  $J = 3.3, 1.8$  Hz, 1H), 6.88 (dd,  $J = 8.1, 1.5$  Hz, 1H), 6.90 – 6.94 (m, 2H), 7.50 (dd,  $J = 8.3, 1.3$  Hz, 1H), 7.57 – 7.79 (m, 3H), 8.16 – 8.35 (m, 1H), 8.73 (d,  $J = 13.4$  Hz, 1H), 10.23 (s, 1H), 10.47 – 10.82 (m, 1H), 11.08 (s, 1H); Anal. ( $\text{C}_{21}\text{H}_{15}\text{IN}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$ ) C, H, N; calcd, 5.66; found 5.23.

**4-[(4-Furan-3-yl-3-hydroxy-benzylamino)-methylene]-6-iodo-4H-isoquinoline-1,3-dione (89d)**. Using the procedure described for the preparation of **5e**, **4e** (85 mg, 0.26 mmol) and 5-aminomethyl-2-furan-3-yl-phenol (**45a**) (73 mg, 0.39 mmol) were reacted to give 70 mg (56%) of **89d**: MS (ESI)  $m/z$  487.1 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.66 (d,  $J = 6.6$  Hz, 2H), 6.85 (dd,  $J = 7.9, 1.6$  Hz, 1H), 6.90 (d,  $J = 1.6$  Hz, 1H), 6.99 (d,  $J = 1.3$  Hz, 1H), 7.49 (dd,  $J = 8.1, 1.5$  Hz, 1H), 7.54 (d,  $J = 8.1$  Hz, 1H), 7.67 – 7.72 (m, 2H), 8.12 (s, 1H), 8.24 – 8.37 (m, 1H), 8.74 (d,  $J = 13.4$  Hz, 1H), 10.06 (s, 1H), 10.56 – 10.78 (m, 1H), 11.08 (s, 1H); Anal. ( $\text{C}_{21}\text{H}_{15}\text{IN}_2\text{O}_4$ ) C, H, N.

**4-[(3-Hydroxy-4-pyridin-2-yl-benzylamino)-methylene]-6-iodo-4H-isoquinoline-1,3-dione (89e)**. Using the procedure described for the preparation of **5e**, **4e** (98 mg, 0.30 mmol) and 5-aminomethyl-2-pyridin-2-yl-phenol (**45b**) (60 mg, 0.30 mmol) were reacted to give 85 mg (57%) of **89e**: MS (ESI)  $m/z$  498.1 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.69 (d,  $J = 6.3$  Hz, 2H), 6.75 – 7.02 (m, 2H), 7.43 (dd,  $J = 7.1, 5.5$  Hz, 1H), 7.50 (d,  $J = 8.3$  Hz, 1H), 7.69 (d,  $J = 8.3$  Hz, 1H), 7.95 – 8.08 (m, 2H), 8.20 (d,  $J = 7.8$  Hz, 1H), 8.28 (s, 1H), 8.61 (d,  $J = 5.0$  Hz, 1H), 8.71 (d,  $J = 13.4$  Hz, 1H), 10.37 – 11.03 (m, 1H), 11.07 (s, 1H), 14.27 (s, 1H); Anal. ( $\text{C}_{22}\text{H}_{16}\text{IN}_3\text{O}_3 \cdot 0.3\text{TFA}$ ) C, H, N.

**4-[(3-Hydroxy-4-pyridin-3-yl-benzylamino)-methylene]-6-iodo-4H-isoquinoline-1,3-dione (89f)**. Using the procedure described for the preparation of **5e**, **4e** (50 mg, 0.15 mmol) and 5-aminomethyl-3-pyridin-2-yl-phenol (**45c**) (53 mg, 0.27 mmol) were reacted to give 57 mg (76%) of **89f**: MS (ESI)  $m/z$  498.0 ( $M + 1$ ) $^{+1}$ ;  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  4.68 (d,  $J = 6.3$  Hz, 2H), 6.58 – 7.06 (m, 2H), 7.33 (d,  $J = 7.6$  Hz, 1H), 7.42 (dd,  $J = 7.6, 4.0$  Hz, 1H), 7.50 (dd,  $J = 8.3, 1.3$  Hz, 1H), 7.69 (d,  $J = 8.3$  Hz, 1H), 7.86 – 8.00 (m, 1H), 8.27 (d,  $J = 1.3$  Hz, 1H), 8.47 (d,  $J = 4.0$  Hz, 1H), 8.58 – 8.79 (m, 2H), 9.96 (s, 1H), 10.53 – 10.79 (m, 1H), 11.07 (s, 1H); Anal. ( $\text{C}_{22}\text{H}_{16}\text{IN}_3\text{O}_3 \cdot 0.4\text{H}_2\text{O}$ ) C, H, N.

**4-[(3-Hydroxy-4-pyridin-4-yl-benzylamino)-methylene]-6-iodo-4*H*-isoquinoline-1,3-dione (89g).** Using the procedure described for the preparation of **5e**, **4e** (50 mg, 0.15 mmol) and 5-aminomethyl-4-pyridin-2-yl-phenol (**45d**) (40 mg, 0.20 mmol) were reacted to give 30 mg (40%) of **89g**: MS (ESI)  $m/z$  498.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.69 (d,  $J = 6.0$  Hz, 2H), 6.88 – 7.06 (m, 2H), 7.39 (d,  $J = 7.9$  Hz, 1H), 7.50 (d,  $J = 8.3$  Hz, 1H), 7.58 (d,  $J = 5.7$  Hz, 2H), 7.69 (d,  $J = 8.3$  Hz, 1H), 8.28 (s, 1H), 8.55 (d,  $J = 5.7$  Hz, 2H), 8.73 (d,  $J = 13.2$  Hz, 1H), 10.07 (s, 1H), 10.71 (d,  $J = 13.2$  Hz, 1H), 11.10 (s, 1H); Anal. (C<sub>22</sub>H<sub>16</sub>IN<sub>3</sub>O<sub>3</sub>·1.5H<sub>2</sub>O) C, H, N.

**(4*Z*)-4-[(3-Hydroxy-4-propoxybenzyl)amino]methylene]-6-iodoisoquinoline-1,3(2*H*,4*H*)-dione (89h).** Using the procedure described for the preparation of **5e**, **4e** (2.5 g, 7.6 mmol) and 5-(aminomethyl)-2-propoxyphenol hydrochloride (**16b**) (1.82 g, 8.36 mmol) were reacted to give 3.0 g (83%) of **89h** as a tan solid: MS (ESI)  $m/z$  479.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 0.97 (t,  $J = 6.9$  Hz, 3H), 1.71 (m, 2H), 3.90 (t,  $J = 6.9$  Hz, 2H), 4.57 (d,  $J = 8.6$  Hz, 2H), 6.74 (d,  $J = 8.8$  Hz, 1H), 6.81 (m, 1H), 6.90 (d,  $J = 7.5$  Hz, 1H), 7.48 (d,  $J = 8.8$  Hz, 1H), 7.68 (d,  $J = 8.8$  Hz, 1H), 8.27 (s, 1H), 8.73 (m, 1H), 8.94 (s, 1H), 10.66 (m, 1H), 11.05 (s, 1H); HRMS (ESI)  $m/e$  calcd for C<sub>20</sub>H<sub>19</sub>IN<sub>2</sub>O<sub>4</sub> 479.04623; found, 479.04449 ( $M + H$ )<sup>+</sup>; Anal. (C<sub>20</sub>H<sub>19</sub>IN<sub>2</sub>O<sub>4</sub>·0.2H<sub>2</sub>O) C, H, N.

**4-[(4-Hydroxy-5-methoxy-pyrimidin-2-ylmethyl)-amino]-methylene]-6-iodo-4*H*-isoquinoline-1,3-dione (90).** Using the procedure described for the preparation of **89b**, **4e** (50 mg, 0.15 mmol) and 2-aminomethyl-5-methoxy-pyrimidin-4-ol (**51**) (10 mg, 0.064 mmol) were reacted to give 10 mg (34%) of **90**: MS (ESI)  $m/z$  453 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 3.72 (s, 3H), 4.57 (d,  $J = 6.0$  Hz, 2H), 7.44 – 7.56 (m, 2H), 7.69 (d,  $J = 8.3$  Hz, 1H), 7.89 (s, 1H), 8.20 (s, 1H), 10.55 (d,  $J = 13.1$  Hz, 1H), 11.09 (s, 1H), 12.74 (s, 1H).

**(4*Z*)-6-Bromo-4-[(2-methoxypyridin-4-yl)methyl]amino)methylene]-isoquinoline-1,3(2*H*,4*H*)-dione (91).** Using the procedure described for the preparation of **5e**, **4d** (0.15 g, 0.53 mmol) and 4-(2-methoxypyridyl)methylamine hydrochloride (**54**) (93 mg, 0.53 mmol) were reacted to give 0.13 (62%) of **91** as a golden solid: MS (ESI)  $m/z$  388.0, 390.0 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 3.84 (s, 3H), 4.68 (d,  $J = 6.4$  Hz, 2H), 6.76 (s, 1H), 6.98 (dd,  $J = 5.2$ , 1.2 Hz, 1H), 7.32 (dd,  $J = 8.4$ , 1.6 Hz, 1H), 7.87 (d,  $J = 8.4$  Hz, 1H), 8.08 (d,  $J = 1.6$  Hz, 1H), 8.15 (d,  $J = 5.2$  Hz, 1H), 8.68 (d,  $J = 13.2$  Hz, 1H), 10.66 (dd,  $J = 13.2$ , 6.4 Hz, 1H), 11.12 (s, 1H); Anal. (C<sub>17</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>3</sub>·0.2TFA) C, H, N.

**4-[(6-Hydroxy-5-propoxy-pyridin-2-ylmethyl)-amino]-methylene]-6-iodo-4*H*-isoquinoline-1,3-dione (92).** Using the procedure described for the preparation of **89b**, **4e** (165 mg, 0.50 mmol) and 6-aminomethyl-3-propoxy-pyridin-2-ol (**88**) (91 mg, 0.50 mmol) were reacted to give 152 mg (63%) of **92** as a beige solid: mp 275–81 °C (dec); MS (ESI)  $m/z$  478.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.95 (t,  $J = 7.4$  Hz, 3H), 1.64 – 1.76 (m, 2H), 3.81 (t,  $J = 6.6$  Hz, 2H), 4.41 (d,  $J = 6.1$  Hz, 2H), 6.11 (d,  $J = 6.8$  Hz, 1H), 6.79 (d,  $J = 7.3$  Hz, 1H), 7.50 (d,  $J = 8.1$  Hz, 1H), 7.68 (d,  $J = 8.31$  Hz, 1H), 8.19 (s, 1H), 8.57 (d,  $J = 13.3$  Hz, 1H), 10.52 (dt,  $J = 13.0$ , 6.1 Hz, 1H), 11.09 (s, 1H), 11.84 (s, 1H); Anal. (C<sub>19</sub>H<sub>18</sub>IN<sub>3</sub>O<sub>4</sub>·0.3H<sub>2</sub>O) C, H, N.

**6-Bromo-4-[(5-methoxy-4-oxo-4*H*-pyran-2-ylmethyl)-amino]-methylene]-4*H*-isoquinoline-1,3-dione (93).** Using the procedure described for the preparation of **89b**, **4d** (212 mg, 0.75 mmol) and 2-aminomethyl-5-methoxy-pyran-4-one<sup>30</sup> (**70a**) (116 mg, 0.75 mmol) were reacted to give 152 mg (50%) of **93** as a light pink solid: mp 259–61 °C (dec); MS (ESI)  $m/z$  403 ( $M - 1$ )<sup>-</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 3.65 (s, 3H), 4.59 (d,  $J = 6.3$  Hz, 2H), 6.29 (s, 1H), 7.34 (dd,  $J = 8.5$ , 1.7 Hz, 1H), 7.88 (d,  $J = 8.6$  Hz, 1H), 8.05 (d,  $J = 1.7$  Hz, 1H), 8.17 (s, 1H), 8.61 (d,  $J = 13.2$  Hz, 1H), 10.47 (dt,  $J = 13.2$ , 6.3 Hz, 1H), 11.15 (s, 1H); Anal. (C<sub>17</sub>H<sub>13</sub>BrN<sub>2</sub>O<sub>5</sub>·0.1TFA) C, N, H: calcd, 3.18; found, 2.67.

**(4*Z*)-6-Bromo-4-[(2-oxo-1,2-dihydropyridin-4-yl)methyl]amino)methylene]isoquinoline-1,3(2*H*,4*H*)-dione (94a).** Using the procedure described for the preparation of **5e**, **4d** (0.26 g, 0.94 mmol) and 4-aminomethyl-1*H*-pyridin-2-one hydrochloride (**55**) (0.15 g, 0.94 mmol) were reacted to give 0.19 g (54%) of **94a** as a gray powder:

MS (ESI)  $m/z$  372.0, 347.0 ( $M - 1$ )<sup>-</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 4.51 (d,  $J = 6.4$  Hz, 2H), 6.17 (dd,  $J = 6.8$ , 2.0 Hz, 1H), 6.20 (s, 1H), 7.32 (dd,  $J = 8.4$ , 2.0 Hz, 1H), 7.36 (d,  $J = 6.8$  Hz, 1H), 7.87 (d,  $J = 8.4$  Hz, 1H), 8.07 (d,  $J = 2.0$  Hz, 1H), 8.63 (d,  $J = 13.2$  Hz, 1H), 10.59 (dt,  $J = 6.4$ , 13.2 Hz, 1H), 11.13 (s, 1H), 11.53 (s, 1H); Anal. (C<sub>16</sub>H<sub>12</sub>BrN<sub>3</sub>O<sub>3</sub>·0.22TFA) C, H, N.

**(4*Z*)-6-Iodo-4-[(2-oxo-1-phenyl-1,2-dihydropyridin-4-yl)methyl]amino)methylene]isoquinoline-1,3(2*H*,4*H*)-dione (94b).** Using the procedure described for the preparation of **5e**, **4e** (92 mg, 0.28 mmol) and 4-aminomethyl-1-phenyl-1*H*-pyridin-2-one hydrochloride (**58**) (66 mg, 0.28) were reacted to give 8.8 mg (6.3%) of **94b** as a pale yellow solid: MS (ESI)  $m/z$  498.2 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 4.59 (d,  $J = 6.4$  Hz, 2H), 6.35 (dd,  $J = 5.2$ , 1.6 Hz, 1H), 6.37 (s, 1H), 7.37 – 7.54 (m, 6H), 7.67 (dd,  $J = 6.8$ , 0.4 Hz, 1H), 7.69 (d,  $J = 8.4$  Hz, 1H), 8.26 (d,  $J = 1.2$  Hz, 1H), 8.65 (d,  $J = 13.2$  Hz, 1H), 10.62 (dt,  $J = 13.2$ , 6.4 Hz, 1H), 11.12 (s, 1H); Anal. (C<sub>22</sub>H<sub>16</sub>IN<sub>3</sub>O<sub>3</sub>·0.51TFA) C, H, N.

**(4*Z*)-4-[(1-(3-Furyl)-2-oxo-1,2-dihydropyridin-4-yl)methyl]amino)methylene]-6-iodoisoquinoline-1,3(2*H*,4*H*)-dione (94c).** Using the procedure described for the preparation of **5e**, **4e** (0.20 g, 0.62 mmol) and 4-aminomethyl-1-furan-3-yl-1*H*-pyridin-2-one hydrochloride (**65a**) (140 mg, 0.62) were reacted to give 0.12 g (40%) of **94c** as a brown powder: MS (ESI)  $m/z$  488.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 4.58 (d,  $J = 6.0$  Hz, 2H), 6.39 – 6.42 (m, 2H), 7.0 (d,  $J = 2.0$  Hz, 1H), 7.50 (dd,  $J = 8.4$ , 1.6 Hz, 1H), 7.69 (d,  $J = 8.4$  Hz, 1H), 7.78 (m, 1H), 7.94 (d,  $J = 8$  Hz, 1H), 8.25 (d,  $J = 0.8$  Hz, 1H), 8.38 (d,  $J = 0.8$  Hz, 1H), 8.64 (d,  $J = 13.2$  Hz, 1H), 10.60 (dt,  $J = 13.2$ , 6.0 Hz, 1H), 11.12 (s, 1H); Anal. (C<sub>20</sub>H<sub>14</sub>IN<sub>3</sub>O<sub>4</sub>) C, H, N.

**6-Iodo-4-[(4-hydroxy-5-methoxy-pyridin-2-ylmethyl)-amino]-methylene]-4*H*-isoquinoline-1,3-dione (100a).** Using the procedure described for the preparation of **89b**, **4e** (247 mg, 0.75 mmol) and 2-aminomethyl-5-methoxy-pyridin-4-ol (**74a**) (116 mg, 0.75 mmol) were reacted to give 293 mg (87%) of **100a** as a pink solid: MS (ESI)  $m/z$  451.9 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 3.97 (s, 3H), 4.89 (d,  $J = 6.0$  Hz, 2H), 7.25 (s, 1H), 7.55 (dd,  $J = 8.3$ , 1.2 Hz, 1H), 7.73 (d,  $J = 8.3$  Hz, 1H), 8.22 (s, 1H), 8.41 (s, 1H), 8.64 (d,  $J = 13.4$  Hz, 1H), 10.56 (dt,  $J = 12.8$ , 6.0 Hz, 1H), 11.16 (s, 1H); Anal. (C<sub>17</sub>H<sub>14</sub>IN<sub>3</sub>O<sub>4</sub>) C, H, N.

**4-[(4-Hydroxy-5-propoxy-pyridin-2-ylmethyl)-amino]-methylene]-6-iodo-4*H*-isoquinoline-1,3-dione (100b).** Using the procedure described for the preparation of **89b**, **4e** (247 mg, 0.75 mmol) and 2-aminomethyl-5-propoxy-pyridin-4-ol (**74b**) (137 mg, 0.75 mmol) were reacted to give 313 mg (87%) of **100b** as a pink solid: MS (ESI)  $m/z$  479.9 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.01 (t,  $J = 7.3$  Hz, 3H), 1.74 – 1.84 (m, 2H), 4.10 (t,  $J = 6.4$  Hz, 2H), 4.86 (d,  $J = 6.8$  Hz, 2H), 7.22 (s, 1H), 7.54 (dd,  $J = 8.4$ , 1.2 Hz, 1H), 7.72 (d,  $J = 8.4$  Hz, 1H), 8.20 (d,  $J = 1.2$  Hz, 1H), 8.61 (d,  $J = 12.9$  Hz, 1H), 10.54 (dt,  $J = 12.9$ , 6.8 Hz, 1H), 11.16 (s, 1H); Anal. (C<sub>19</sub>H<sub>18</sub>IN<sub>3</sub>O<sub>4</sub>·0.2TFA) C, H, N.

**6-*tert*-Butyl-4-[(4-hydroxy-5-propoxy-pyridin-2-ylmethyl)-amino]-methylene]-4*H*-isoquinoline-1,3-dione (100c).** Using the procedure described for the preparation of **89b**, (*E*)-6-*tert*-butyl-4-(methoxymethylene)isoquinoline-1,3(2*H*,4*H*)-dione (**4k**) (50.0 mg, 0.193 mmol) and 2-aminomethyl-5-propoxy-pyridin-4-ol (**74b**) (35 mg, 0.193 mmol) were reacted to give 52 mg (66%) of **100c** as a light pink solid: mp 162–178 °C (dec); MS (ESI)  $m/z$  410.4 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.02 (t,  $J = 7.4$  Hz, 3H), 1.37 (s, 9H), 1.75 – 1.87 (m, 2H), 4.12 (t,  $J = 6.6$  Hz, 2H), 4.95 (d,  $J = 6.1$  Hz, 2H), 7.26 (s, 1H), 7.28 (dd,  $J = 8.5$ , 1.51 Hz, 1H), 7.76 (bs, 1H), 7.94 (d,  $J = 8.5$  Hz, 1H), 8.40 (s, 1H), 8.59 (d,  $J = 12.9$  Hz, 1H), 10.60 (dt,  $J = 12.9$ , 5.9 Hz, 1H), 11.02 (s, 1H); Anal. (C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>4</sub>·1.0H<sub>2</sub>O) C, H, N.

**6-Cyclopentyl-4-[(4-hydroxy-5-propoxy-pyridin-2-ylmethyl)-amino]-methylene]-4*H*-isoquinoline-1,3-dione (100d).** Using the procedure described for the preparation of **89b**, 6-cyclopentyl-4-methoxymethylene-4*H*-isoquinoline-1,3-dione (**4l**) (110 mg, 0.405 mmol) and 2-aminomethyl-5-propoxy-pyridin-4-ol (**74b**) (74 mg, 0.405 mmol) were reacted to give 162 mg (94%) of **100d** as a pink solid: mp 263–6 °C (dec); MS (ESI)  $m/z$  422.2 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.99 (t,  $J = 7.4$  Hz, 3H), 1.56

– 1.72 (m, 4H), 1.72 – 1.87 (m, 4H), 1.98 – 2.10 (m, 2H), 2.96 – 3.08 (m, 1H), 4.09 (t,  $J = 6.5$  Hz, 2H), 4.87 (d,  $J = 6.3$  Hz, 2H), 7.12 (d,  $J = 8.0$  Hz, 1H), 7.20 (bd,  $J = 1.5$  Hz, 1H), 7.63 (s, 1H), 7.92 (d,  $J = 8.0$  Hz, 1H), 8.39 (s, 1H), 8.56 (d,  $J = 12.8$  Hz, 1H), 10.49 (dt,  $J = 12.8, 6.3$  Hz, 1H), 10.99 (s, 1H); Anal. ( $C_{24}H_{27}N_3O_4 \cdot 1.0H_2O$ ) C, H, N.

**6-tert-Butyl-4-[[4-hydroxy-5-phenyl-pyridin-2-ylmethyl]-amino]-methylene]-4H-isoquinoline-1,3-dione (100e).** Using the procedure described for the preparation of **89b**, **4k** (40.0 mg, 0.154 mmol) and 2-aminomethyl-5-phenyl-pyridin-4-ol (**81a**) (31 mg, 0.154 mmol) were reacted to give 44 mg (66%) of **100e** as a pink solid: mp 195–218 °C (dec); MS (ESI)  $m/z$  428.4 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (s, 9H), 5.01 (d,  $J = 6.3$  Hz, 2H), 7.26 – 7.30 (m, 2H), 7.45 – 7.56 (m, 3H), 7.65 (d,  $J = 6.8$  Hz, 2H), 7.75 (s, 1H), 7.95 (d,  $J = 8.3$  Hz, 1H), 8.63 (d,  $J = 12.8$  Hz, 1H), 8.76 (s, 1H), 10.60 (dt,  $J = 12.7, 6.3$  Hz, 1H), 11.04 (s, 1H); Anal. ( $C_{26}H_{25}N_3O_3 \cdot 1.0H_2O$ ) C, H, N.

**(4Z)-[[5-Furan-3-yl-4-hydroxy-pyridin-2-ylmethyl]-amino]-methylene]-6-iodo-4H-isoquinoline-1,3-dione (100f).** Using the procedure described for the preparation of **89b**, **4e** (110 mg, 0.33 mmol) and 2-aminomethyl-5-furan-3-yl-pyridin-4-ol (**81b**) (64 mg, 0.33 mmol) were reacted to give 109 mg (67%) of **100f** as a light beige solid: mp 213–228 °C (dec); MS (ESI)  $m/z$  488.0 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.87 (d,  $J = 6.2$  Hz, 2H), 7.11 (s, 1H), 7.18 (d,  $J = 1.3$  Hz, 1H), 7.54 (dd,  $J = 8.3, 1.3$  Hz, 1H), 7.71 (d,  $J = 8.3$  Hz, 1H), 7.84 (t,  $J = 1.8$  Hz, 1H), 7.95 (s, 1H), 8.21 (d,  $J = 1.1$  Hz, 1H), 8.39 (s, 1H), 8.63 (d,  $J = 13.1$  Hz, 1H), 8.82 (s, 1H), 10.56 (dt,  $J = 13.1, 6.2$  Hz, 1H), 11.18 (s, 1H); Anal. ( $C_{20}H_{14}IN_3O_4 \cdot 1.5H_2O$ ) C, H, N.

**6-tert-Butyl-4-[[5-furan-3-yl-4-hydroxy-pyridin-2-ylmethyl]-amino]-methylene]-4H-isoquinoline-1,3-dione (100g).** Using the procedure described for the preparation of **89b**, **4k** (40.0 mg, 0.154 mmol) and 2-aminomethyl-5-furan-3-yl-pyridin-4-ol (**81b**) (29 mg, 0.154 mmol) were reacted to give 41 mg (64%) of **100g** as a pink solid: mp 215–230 °C (dec); MS (ESI)  $m/z$  418.3 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (s, 9H), 5.01 (d,  $J = 6.1$  Hz, 2H), 7.23 (s, 1H), 7.26 – 7.34 (m, 2H), 7.76 (s, 1H), 7.85 (s, 1H), 7.96 (d,  $J = 8.3$  Hz, 1H), 8.39 (s, 1H), 8.63 (d,  $J = 12.8$  Hz, 1H), 8.97 (s, 1H), 10.60 (dt,  $J = 12.5, 6.4$  Hz, 1H), 11.04 (s, 1H); Anal. ( $C_{24}H_{23}N_3O_4 \cdot 0.3TFA$ ) C, H, N.

**6-Cyclopentyl-4-[[5-furan-3-yl-4-hydroxy-pyridin-2-ylmethyl]-amino]-methylene]-4H-isoquinoline-1,3-dione (100h).** Using the procedure described for the preparation of **89b**, **4l** (110 mg, 0.405 mmol) and 2-aminomethyl-5-furan-3-yl-pyridin-4-ol (**81b**) (77 mg, 0.405 mmol) were reacted to give 152 mg (87%) of **100h** as a pink solid: mp 279–283 °C (dec); MS (ESI)  $m/z$  430.1 ( $M + 1$ )<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.55 – 1.74 (m, 4H), 1.74 – 1.88 (m, 2H), 1.98 – 2.10 (m, 2H), 2.96 – 3.09 (m, 1H), 4.95 (d,  $J = 6.3$  Hz, 2H), 7.13 (d,  $J = 8.6$  Hz, 1H), 7.21 – 7.24 (m, 1H), 7.24 – 7.29 (bm, 1H), 7.65 (s, 1H), 7.86 (d,  $J = 1.5$  Hz, 1H), 7.93 (d,  $J = 8.0$  Hz, 1H), 8.39 (s, 1H), 8.59 (d,  $J = 13.2$  Hz, 1H), 8.95 (s, 1H), 10.53 (dt,  $J = 13.2, 6.3$  Hz, 1H), 11.02 (s, 1H); Anal. ( $C_{25}H_{23}N_3O_4 \cdot 0.5H_2O$ ) C, H, N.

**Biological Methods. Enzyme Assay.** Cyclin D1/CDK4 and cyclin E/CDK2 were expressed in insect cells (Sf9) infected with recombinant baculovirus and partially purified using ammonium sulfate fractionation. Cyclin B1/CDC2 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<sub>2</sub>, 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 as substrate. Colorimetric reactions were stopped with 2 N sulfuric acid, and the absorbance was measured at 450 nm. IC<sub>50</sub> values were determined from inhibition plots.

**Cell Culture and Proliferation Assay.** HCT-116, LoVo, BT-474, and MCF-7 cells were cultured in RPMI medium (Invitrogen) 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<sub>2</sub>. Cells were seeded in RPMI medium supplemented with 5% fetal bovine serum in 96 well plates at a density of 5000 to 10000 cells per well depending on the cell line. The following day, 11 point serial dilutions of the test compound (3-fold increments) were added to each well. Cells were incubated with the compound for 3 days (6 days for BT-474) at 37 °C and cell growth was determined using sulforhodamine B (SRB), a protein binding dye. Briefly, the surviving cells were fixed with TCA, and rinsed extensively in water. Cells were stained with 0.4% sulforhodamine B (Sigma-Aldrich) 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).

**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  $\mu$ g) were separated by electrophoresis on 8% 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). The antibodies used were RB (Santa Cruz Biotechnologies, Santa Cruz, CA or EMD Biosciences, Darmstadt, Germany) and phospho-RB (Ser 807/811) (Cell Signaling Technologies, Beverly, MA).

**Metabolic Stability Assays.** Substrates were initially dissolved in HPLC grade DMSO to prepare a 0.5 mM solution, which was subsequently added to 4 equivalent volumes of acetonitrile. The resulting 0.1 mM substrate solution in DMSO/acetonitrile was added to 0.1 M potassium phosphate buffer at pH 7.4 containing 0.5 mg/mL male SD rat liver microsome protein (BD Gentest). Samples were preincubated at 37 °C for 10 min and diluted to 1  $\mu$ M by the addition of 1 mM NADPH regeneration solution (BD Gentest) for phase I metabolic stability screening or 1 mM NADPH regeneration solution containing 2 mM UDPGA (BD Gentest) for phase I and II metabolic stability screening. The microsomal solution was incubated at 37 °C for 15 min followed by the addition of 2 equivalent volumes of cold acetonitrile to quench the enzyme reaction. Nonincubated samples were prepared by adding cold acetonitrile before adding the regeneration solutions without incubation. Samples were analyzed by HPLC-MS/MS with a Waters Quattro Micro mass spectrometer coupled to an Agilent 1100 HPLC. The Agilent 1100 HPLC was equipped with an Aquasil C<sub>18</sub> 2.1  $\times$  50 mm, 5  $\mu$ m column. Samples were injected with a LEAP Technologies HTS PAL autosampler at 15  $\mu$ L. The mobile phase flow rate was 1.0 mL/min with a 3:1 post column split. The HPLC mobile phase gradient was initiated at 5% eluent B, held at 5% eluent B for 0.1 min, followed by a linear increase to 95% eluent B over 0.5 min, held at 95% eluent B for 0.4 min, and reconditioned at 5% eluent B for 0.5 min. Eluent A was 0.1% formic acid in water and eluent B was 0.1% formic acid in acetonitrile. HPLC column temperature was maintained at 40 °C. Compounds were detected by HPLC-MS/MS using multiple reaction monitoring (MRM) transitions. Automated determination of MRM transitions

were generated using Waters QuanOptimize software. The HPLC-MS/MS substrate peak area at 15 min was compared to the nonincubated substrate providing a relative percent remaining from which an experimental half-life could be calculated assuming pseudo-first-order reaction rate kinetics.

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**Supporting Information Available:** Elementary analysis data for compounds **3c–3e**, **5a–5i**, **11**, **13**, **17a–17d**, **18**, **21**, **89a–89h**, **91–93**, **94a–94c**, and **100a–100 h**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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