

Synthesis and Biological Evaluation of Furo[2,3-*d*]pyrimidines as Akt1 Kinase Inhibitors

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Based on the hit compound **4** derived from focused library, a series of furo[2,3-*d*]pyrimidines were designed, synthesized and evaluated for the inhibitory activity against Akt1 kinase. And their structure-activity relationships were investigated. Of these compounds, **3a** having 2-thienyl and methyl groups at R₁ and R₂ showed the most potent activity with an IC₅₀ value of 24 μM. Introduction of the thienyl groups at C-5 and C-6 positions significantly improved potency compared to furyl and phenyl groups.

Key Words : Akt1 kinase, Inhibitory activity, Furo[2,3-*d*]pyrimidines

Introduction

Akt, a serine/threonine protein kinase as a viral oncogene, is a critical regulator of PI3K-mediated cell proliferation and survival.¹⁻³ Since the discovery of human Akt1 (PKB), two additional mammalian Akt isoforms, Akt2 and Akt3, have been identified.⁴ One of the intracellular signaling pathways that frequently are activated in cancer cells is the PI3K/Akt kinase pathways.⁵ On translocation, Akt is phosphorylated and activated, ultimately resulting in stimulation of cell growth and survival. As an attractive target for the potential treatment of cancer, a number of small molecule compounds have investigated as Akt1 kinase inhibitors.⁶⁻⁸

As a part of our program toward the novel Akt1 inhibitors with potent activity over PI3K signaling pathway, we found

hit compound **4** with an IC₅₀ value of 83 μM from focused library (Figure 1). This finding prompted us to synthesize a series of furo[2,3-*d*]pyrimidines by incorporating various moieties at C-4, C-5 and C-6 positions on the basis of chemical modification for the hit compound **4**. Various new furo[2,3-*d*]pyrimidines **1-3** were synthesized and tested for Akt1 inhibitory activity. And their effect of positional substituents on the biological activity was investigated.

Results and Discussion

Chemistry. 4-Halo-5,6-disubstituted furo[2,3-*d*]pyrimidines **8**, as key intermediates, were prepared by the sequence outlined in Scheme 1.

Hydroxyketones **5** were treated with malononitrile in

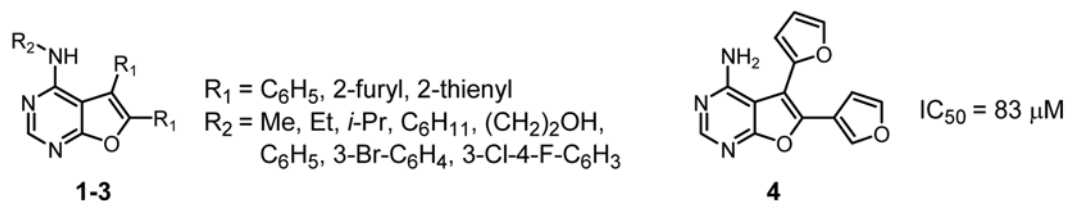
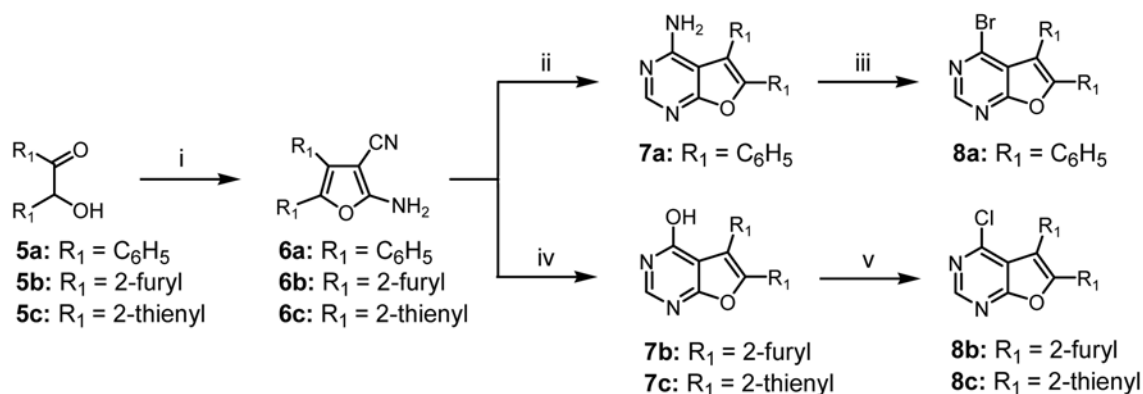


Figure 1. Structures of furo[2,3-*d*]pyrimidines.

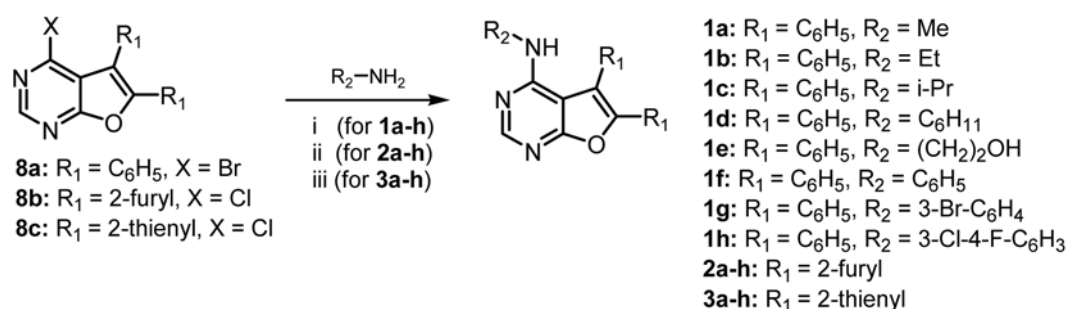


Scheme 1. Reagents and reaction conditions: (i) malononitrile, Et₂NH, DMF, 130-140 °C, 2 h (for **6a**) or 20 °C, 24 h (for **6b-c**); (ii) formamide, Ac₂O, 210 °C, 2 h; (iii) isoamyl nitrite, CH₂Br₂, 97 °C, 1 h; (iv) formic acid, Ac₂O, 100 °C, 5 h; (v) POCl₃, 105 °C, 2 h.

dimethylformamide in the presence of diethylamine to give 1-amino-2-cyano-3,4-disubstituted furans **6**.⁹ Ring closure by treatment of **6a** with acetic anhydride in formamide followed by bromination of the resulting 4-aminofuro[2,3-*d*]pyrimidine **7a** with isoamyl nitrite in methylene bromide provided 4-bromofuro[2,3-*d*]pyrimidine **8a**.^{10,11} On the other hand, 4-chlorofuro[2,3-*d*]pyrimidines **8b, c** were obtained from cyclization of **6b, c** with acetic anhydride in formic

acid and subsequent chlorination of **7b, c** with phosphorus oxychloride.^{12,13} In this case, bromination method as described above for the preparation of **7a** was not suitable due to side reactions toward the heteroaromatic ring system.

Finally, the amination of 4-halofuro[2,3-*d*]pyrimidines **8** was achieved by treatment with aliphatic and aromatic amines in ethanol, *i*-propanol and isoamyl alcohol to give the desired compounds **1-3**, respectively (Scheme 2).¹⁴



Scheme 2. Reagents and reaction conditions: (i) *i*-PrOH, 97 °C, 6-24 h (for **1a-f**) or EtOH, 78 °C, 8 h (for **1g, h**); (ii) *i*-PrOH, 97 °C, 4-24 h (for **2a-h**); (iii) *i*-PrOH, 97 °C, 7-10 h (for **3a-f**) or isoamyl alcohol, 130 °C, 25-30 h (for **3g, h**).

Table 1. Physical and spectral data of furo[2,3-*d*]pyrimidines **1-3**

Comps	Y ^a (%)	Sol ^b	¹ H NMR (300 MHz, ^c CDCl ₃ , δ ppm)	¹³ C NMR (75 MHz, ^c CDCl ₃ , δ ppm)/FABHRMS <i>m/z</i>
1a	76.7	A	8.43 (s, 1H), 7.49 (d, <i>J</i> = 7.2 Hz, 7H), 7.24 (s, 3H), 4.63 (bs, 1H), 2.95 (s, 3H)	164.8, 158.2, 154.2, 146.6, 132.5, 129.9, 129.7, 129.5, 128.9, 128.5, 127.9, 126.4, 114.9, 103.3, 27.9
1b	96.9	A	8.40 (s, 1H), 7.52 (s, 7H), 7.24 (d, <i>J</i> = 2.5 Hz, 3H), 4.57 (s, 1H), 3.46-3.39 (m, 2H), 1.06 (dd, <i>J</i> = 2.4, 7.1 Hz, 3H)	164.8, 157.8, 157.5, 154.2, 146.5, 132.6, 129.8, 129.6, 128.9, 128.5, 128.4, 126.3, 114.9, 103.0, 35.7, 14.6
1c	83.8	A	8.34 (s, 1H), 7.50 (d, <i>J</i> = 6.6 Hz, 7H), 7.23 (s, 3H), 4.43 (bs, 1H), 4.25-4.23 (m, 1H), 1.04 (d, <i>J</i> = 6.3 Hz, 6H)	164.9, 156.9, 154.3, 146.3, 132.5, 129.9, 129.6, 129.5, 128.9, 128.5, 128.3, 126.3, 114.9, 102.9, 42.5, 29.7, 22.7
1d	70.1	A	8.39 (s, 1H), 7.55-7.48 (m, 7H), 7.28 (d, <i>J</i> = 9.4 Hz, 3H), 4.62 (d, <i>J</i> = 7.6 Hz, 1H), 4.09-4.01 (m, 1H), 1.85-1.81 (m, 2H), 1.49-1.31 (m, 6H), 1.06-0.96 (m, 2H)	164.8, 156.9, 154.3, 146.3, 132.6, 129.8, 129.6, 129.5, 128.9, 128.5, 128.3, 126.2, 114.9, 103.0, 48.5, 32.5, 25.5, 23.9
1e	90.0	A	8.36 (s, 1H), 7.50 (d, <i>J</i> = 4.3 Hz, 8H), 7.26 (s, 2H), 5.14 (bs, 1H), 3.98 (bs, 1H), 3.72 (d, <i>J</i> = 4.2 Hz, 2H), 3.57 (d, <i>J</i> = 3.9 Hz, 2H)	164.7, 158.1, 157.9, 153.7, 147.0, 132.2, 129.7, 129.3, 128.9, 128.5, 128.2, 126.4, 114.9, 103.4, 62.6, 44.2
1f	> 99.0	A	8.54 (s, 1H), 7.60-7.55 (m, 8H), 7.39-7.37 (m, 1H), 7.29-7.02 (m, 6H), 6.54 (s, 1H)	165.2, 155.0, 153.8, 147.5, 138.5, 132.3, 130.1, 129.9, 129.4, 129.3, 128.9, 128.8, 128.6, 126.5, 123.5, 119.9, 114.4, 104.3
1g	83.3	B	8.58 (s, 1H), 7.89 (d, <i>J</i> = 1.7 Hz, 1H), 7.66 (d, <i>J</i> = 6.0 Hz, 5H), 7.51-7.45 (m, 2H), 7.42-7.37 (m, 4H), 7.24-7.19 (m, 2H), 7.07 (m, 1H), 7.01 (s, 1H)	164.9, 154.3, 153.4, 147.0, 140.0, 131.0, 130.7, 129.9, 129.8, 129.4, 129.1, 128.9, 128.7, 126.3, 125.7, 122.0, 121.6, 118.6, 114.8, 104.3
1h	66.7	B	8.52 (s, 1H), 7.82 (dd, <i>J</i> = 2.6, 6.7 Hz, 1H), 7.61 (s, 5H), 7.48-7.44 (m, 2H), 7.37-7.35 (m, 3H), 7.3 (d, <i>J</i> = 9.0 Hz, 1H), 7.07-7.04 (m, 2H)	164.9, 154.8, 154.4, 153.3, 151.6, 147.0, 135.7, 130.9, 129.9, 129.7, 129.2, 129.1, 128.8, 128.7, 126.3, 121.8, 120.7, 120.6, 119.3, 119.0, 117.0, 116.7, 114.9, 104.0
2a	89.0	A	8.43 (s, 1H), 7.62 (s, 1H), 7.51 (s, 1H), 6.92 (t, <i>J</i> = 3.6 Hz, 2H), 6.62 (dd, <i>J</i> = 1.7, 3.2 Hz, 1H), 6.54 (dd, <i>J</i> = 1.7, 3.2 Hz, 1H), 6.35 (s, 1H), 3.14 (d, <i>J</i> = 4.8 Hz, 3H)	165.3, 158.0, 154.6, 144.9, 144.8, 143.5, 142.3, 139.5, 112.5, 111.9, 111.6, 111.1, 104.9, 101.3, 28.4 / Calcd for C ₁₅ H ₁₁ N ₃ O ₃ (M+H) ⁺ 282.0800, Found 282.0871
2b	97.1	A	8.41 (s, 1H), 7.61 (s, 1H), 7.51 (s, 1H), 6.92 (s, 2H), 6.63 (s, 1H), 6.54 (s, 1H), 6.28 (s, 1H), 3.61 (t, <i>J</i> = 6.2 Hz, 2H), 1.29 (t, <i>J</i> = 7.1 Hz, 3H)	165.4, 157.5, 154.8, 145.1, 145.1, 143.5, 142.1, 139.6, 112.6, 112.0, 111.6, 111.1, 105.0, 101.2, 36.2, 14.8
2c	87.1	A	8.39 (s, 1H), 7.61 (s, 1H), 7.51 (s, 1H), 6.92 (s, 2H), 6.64 (t, <i>J</i> = 1.7 Hz, 1H), 6.54 (t, <i>J</i> = 1.7 Hz, 1H), 6.32 (d, <i>J</i> = 6.9 Hz, 1H), 4.17 (t, <i>J</i> = 3.8 Hz, 1H), 1.28 (d, <i>J</i> = 12.4 Hz, 6H)	165.5, 156.9, 154.8, 145.2, 145.1, 143.4, 142.0, 139.5, 112.6, 111.9, 111.6, 111.0, 105.0, 101.2, 49.2, 32.9, 25.8
2d	68.4	A	8.39 (s, 1H), 7.60 (s, 1H), 7.51 (s, 1H), 6.92 (s, 1H), 6.64 (dd, <i>J</i> = 1.7, 3.3 Hz, 1H), 6.54 (dd, <i>J</i> = 1.7, 3.3 Hz, 1H), 6.22 (d, <i>J</i> = 6.5 Hz, 1H), 4.41 (dd, <i>J</i> = 6.5, 13.0 Hz, 1H), 1.28 (t, <i>J</i> = 6.3 Hz, 10H)	165.3, 156.7, 154.6, 145.0, 144.9, 143.3, 141.8, 139.3, 112.5, 111.8, 111.5, 110.9, 104.8, 101.1, 42.8, 29.7, 24.6, 22.8

Table 1. Continued

Comps	Y ^a (%)	Sol ^b	¹ H NMR (300 MHz, ^c CDCl ₃ , δ ppm)	¹³ C NMR (75 MHz, ^c CDCl ₃ , δ ppm)/FABHRMS <i>m/z</i>
2e	79.8	A	8.36 (s, 1H), 7.61 (s, 1H), 7.53 (s, 1H), 6.94 (d, <i>J</i> = 4.8 Hz, 3H), 6.61 (s, 1H), 6.55 (s, 1H), 3.89 (d, <i>J</i> = 4.0 Hz, 2H), 3.77 (s, 2H), 3.64 (d, <i>J</i> = 2.9 Hz, 1H)	165.3, 157.9, 154.3, 144.9, 144.8, 143.6, 142.4, 139.7, 112.4, 112.0, 111.6, 111.3, 105.0, 101.5, 62.8, 44.5
2f	87	A	8.53 (s, 1H), 8.34 (s, 1H), 7.72-7.68 (m, 3H), 7.55 (s, 1H), 7.39 (t, <i>J</i> = 7.0 Hz, 3H), 7.13 (t, <i>J</i> = 6.8 Hz, 1H), 7.0 (s, 1H), 6.70 (s, 1H), 6.57 (s, 1H)	165.6, 154.9, 154.2, 144.7, 144.6, 143.7, 142.3, 140.3, 138.7, 129.1, 123.8, 120.7, 112.7, 112.0, 111.9, 111.6, 104.4, 102.4
2g	54.3	A	8.57 (s, 1H), 8.40 (s, 1H), 8.06 (s, 1H), 7.74 (s, 1H), 7.56 (d, <i>J</i> = 6.0 Hz, 2H), 7.24 (d, <i>J</i> = 5.7 Hz, 2H), 7.01 (t, <i>J</i> = 7.9 Hz, 1H), 6.72 (s, 1H), 6.59 (d, <i>J</i> = 6.2 Hz, 2H)	165.7, 154.5, 154.1, 144.6, 144.5, 143.9, 142.3, 140.6, 140.1, 130.3, 126.6, 123.4, 123.1, 118.9, 112.9, 112.2, 112.1, 111.9, 104.2, 102.7 / Calcd for C ₂₀ H ₁₂ BrN ₃ O ₃ (M+H) ⁺ 422.0062, Found 422.0140
2h	66.1	A	8.53 (s, 1H), 8.33 (s, 1H), 7.93 (d, <i>J</i> = 4.3 Hz, 1H), 7.73 (s, 1H), 7.56 (s, 1H), 7.44 (d, <i>J</i> = 8.3 Hz, 1H), 7.14 (t, <i>J</i> = 8.6 Hz, 1H), 7.03-6.92 (m, 2H), 6.71 (s, 1H), 6.59 (s, 1H)	165.8, 154.7, 154.2, 144.8, 144.7, 144.0, 142.3, 140.7, 135.5, 122.9, 120.4, 120.4, 116.9, 116.6, 113.1, 112.3, 112.2, 112.0, 104.3, 102.6
3a	88.2	A	8.41 (s, 1H), 7.59 (dd, <i>J</i> = 2.2, 4.2 Hz, 1H), 7.35 (dd, <i>J</i> = 1.0, 3.7 Hz, 1H), 7.25 (t, <i>J</i> = 2.1 Hz, 3H), 6.98 (dd, <i>J</i> = 3.8, 5.0 Hz, 1H), 4.89 (d, <i>J</i> = 4.1 Hz, 1H), 3.01 (d, <i>J</i> = 4.9 Hz, 3H)	164.5, 157.8, 154.3, 145.3, 131.0, 130.8, 129.9, 128.7, 128.3, 127.4, 127.0, 126.5, 105.8, 103.3, 28.0 / Calcd for C ₁₅ H ₁₁ N ₃ OS ₂ (M+H) ⁺ 314.0344, Found 314.0418
3b	94.9	A	8.39 (s, 1H), 7.60 (dd, <i>J</i> = 1.1, 4.3 Hz, 1H), 7.36 (d, <i>J</i> = 3.6 Hz, 1H), 7.25 (d, <i>J</i> = 4.5 Hz, 3H), 7.00-6.97 (m, 1H), 4.82 (bs, 1H), 3.53-3.44 (m, 2H), 1.12 (t, <i>J</i> = 7.2 Hz, 3H)	164.6, 157.2, 154.4, 145.2, 131.0, 130.9, 129.9, 128.6, 128.2, 127.4, 126.9, 126.5, 105.9, 103.1, 35.8, 14.7
3c	88.3	A	8.38 (s, 1H), 7.62 (t, <i>J</i> = 3.1 Hz, 1H), 7.37 (d, <i>J</i> = 3.3 Hz, 1H), 7.26 (d, <i>J</i> = 3.1 Hz, 3H), 7.00-6.98 (m, 1H), 4.69 (d, <i>J</i> = 7.0 Hz, 1H), 4.31 (dd, <i>J</i> = 6.6, 12.4 Hz, 1H), 1.11 (d, <i>J</i> = 6.4 Hz, 6H)	164.6, 156.6, 154.4, 145.1, 131.0, 130.9, 129.9, 128.6, 128.2, 127.4, 126.9, 126.4, 105.9, 103.0, 42.5, 29.7, 22.7
3d	66.3	A	8.37 (s, 1H), 7.61 (t, <i>J</i> = 2.9 Hz, 1H), 7.37 (d, <i>J</i> = 3.3 Hz, 1H), 7.26-7.24 (m, 3H), 6.99 (t, <i>J</i> = 4.1 Hz, 1H), 4.85 (d, <i>J</i> = 7.3 Hz, 1H), 4.09-4.06 (m, 1H), 1.87 (d, <i>J</i> = 8.8 Hz, 2H), 1.52 (d, <i>J</i> = 8.2 Hz, 2H), 1.41-1.34 (m, 2H), 1.23-1.19 (m, 2H), 1.11 (t, <i>J</i> = 9.5 Hz, 2H)	164.6, 156.6, 154.4, 145.1, 131.1, 130.9, 129.9, 128.6, 128.2, 127.4, 126.9, 126.4, 105.9, 103.1, 48.5, 32.5, 25.5, 23.9
3e	96.8	A	8.35 (s, 1H), 7.59 (s, 1H), 7.36 (s, 1H), 7.27 (s, 2H), 7.00 (s, 1H), 5.36 (s, 1H), 4.22 (bs, 1H), 3.76 (s, 2H), 3.62 (s, 2H)	164.5, 157.6, 153.8, 146.9, 131.4, 130.6, 130.0, 128.8, 128.4, 127.4, 127.3, 126.7, 105.8, 103.5, 62.6, 44.3
3f	> 99.0	A	8.54 (s, 1H), 7.71 (d, <i>J</i> = 4.7 Hz, 1H), 7.48-7.29 (m, 8H), 7.10-7.02 (m, 2H), 6.77 (s, 1H)	164.9, 154.7, 153.9, 146.2, 138.3, 132.1, 130.6, 130.5, 130.4, 129.3, 129.0, 128.6, 127.6, 127.0, 123.7, 120.2, 105.3, 104.4
3g	66.7	C	8.57 (s, 1H), 7.82 (s, 1H), 7.73 (dd, <i>J</i> = 1.5, 4.6 Hz, 1H), 7.47 (d, <i>J</i> = 3.0 Hz, 1H), 7.36 (dd, <i>J</i> = 5.0, 8.9 Hz, 3H), 7.27 (d, <i>J</i> = 2.9 Hz, 1H), 7.21-7.13 (m, 2H), 7.07-7.04 (m, 1H), 6.75 (s, 1H)	165.0, 154.3, 153.8, 139.7, 137.2, 136.4, 130.5, 130.4, 130.2, 129.4, 128.6, 127.8, 127.6, 127.2, 126.4, 122.8, 122.6, 118.4, 105.0, 104.7 / Calcd for C ₂₀ H ₁₂ BrN ₃ OS ₂ (M+H) ⁺ 453.9605, Found 453.9653
3h	53.3	C	8.54 (s, 1H), 7.74 (dd, <i>J</i> = 2.5, 6.3 Hz, 2H), 7.46 (d, <i>J</i> = 3.4 Hz, 1H), 7.37-7.33 (m, 3H), 7.10-7.04 (m, 3H), 6.68 (s, 1H)	164.8, 154.3, 153.8, 135.5, 130.5, 129.4, 128.6, 127.8, 127.6, 127.2, 126.9, 126.8, 126.7, 122.2, 121.2, 119.8, 116.7, 116.4, 105.6, 103.6

^aIsolated yields. ^bReaction solvents: A = *i*-PrOH, B = EtOH, C = isoamyl alcohol. ^c1f-h: DMSO-*d*₆.

Physical and spectral data of the title compounds **1-3** were listed in Table 1.

Biological Evaluation. The Akt1 kinase inhibitory activities of the tested compounds **1-3** were evaluated by the known method¹⁵ and listed in Table 2. The heterocyclic compounds **2, 3** at C-5 and C-6 positions were found to be of higher activity (IC₅₀ values of 24-97 μM) than phenyl compounds **1** (IC₅₀ values of 76- > 100 μM). The inhibitory activities of 2-thienyl derivatives **3a-h** ranged from 24 to 68 μM, whereas 2-furyl derivatives **2a-h** showed the activities of 59 to 97 μM. Among the 24 compounds prepared, **3a** having 2-thienyl (R₁) and methyl (R₂) groups was found to be the most active inhibitor with an IC₅₀ value of 24 μM against Akt1 kinase. Namely, the substitution by a thienyl group at R₁ resulted in a dramatic increase of activity.

Generally, replacement of aliphatic group by aromatic group at R₂ led to a slight decrease in the inhibitory activity.

Experimental

Melting points were measured with a Thomas Hoover capillary melting point apparatus and were uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz for ¹H and 75.5 MHz for ¹³C) using tetramethylsilane as an internal standard. Mass spectra were obtained on a Jeol SX-102. Column chromatography was carried out using silica gel (230-400 mesh). Solvents and liquid reagents were transferred using hypodermic syringes. All other reagents and solvents used were reagent grade.

Table 2. Inhibitory activity for Akt1 kinase of furo[2,3-*d*]pyrimidines 1-3

Comps	R ₁	R ₂	IC ₅₀ (μM)	Comps	R ₁	R ₂	IC ₅₀ (μM)
1a	C ₆ H ₅	Me	78	2e	2-furyl	(CH ₂) ₂ OH	59
1b	C ₆ H ₅	Et	84	2f	2-furyl	C ₆ H ₅	84
1c	C ₆ H ₅	<i>i</i> -Pr	97	2g	2-furyl	3-Br-C ₆ H ₄	78
1d	C ₆ H ₅	C ₆ H ₁₁	76	2h	2-furyl	3-Cl-4-F-C ₆ H ₃	84
1e	C ₆ H ₅	(CH ₂) ₂ OH	78	3a	2-thienyl	Me	24
1f	C ₆ H ₅	C ₆ H ₅	83	3b	2-thienyl	Et	37
1g	C ₆ H ₅	3-Br-C ₆ H ₄	> 100	3c	2-thienyl	<i>i</i> -Pr	34
1h	C ₆ H ₅	3-Cl-4-F-C ₆ H ₃	> 100	3d	2-thienyl	C ₆ H ₁₁	56
2a	2-furyl	Me	61	3e	2-thienyl	(CH ₂) ₂ OH	37
2b	2-furyl	Et	65	3f	2-thienyl	C ₆ H ₅	61
2c	2-furyl	<i>i</i> -Pr	97	3g	2-thienyl	3-Br-C ₆ H ₄	56
2d	2-furyl	C ₆ H ₁₁	76	3h	2-thienyl	3-Cl-4-F-C ₆ H ₃	68

General procedure for the synthesis of 1-amino-2-cyano-3,4-disubstituted furans 6. To a mixture of the appropriate hydroxyketone (**5**, 5.0 mmol) and malononitrile (0.40 g, 6.0 mmol) in dimethylformamide (2 mL) was added diethylamine (0.093 g, 1.3 mmol). The reaction mixture was stirred at 20-140 °C for 2-24 h, then left at room temperature over a period of 24 h, diluted with water. The resulting precipitate was collected by filtration and recrystallized from dioxane to give **6**. **6a**: yield 53.0%; ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.73 (s, 2H), 7.44 (t, *J* = 7.0 Hz, 3H), 7.37 (d, *J* = 7.0 Hz, 2H), 7.27 (d, *J* = 7.5 Hz, 2H), 7.19 (t, *J* = 8.0 Hz, 3H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 163.9, 137.0, 131.6, 129.7, 129.3, 129.3, 129.2, 128.9, 128.7, 127.2, 124.6, 122.1, 115.9, 69.6. **6b**: yield 66.0%; ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.85 (bs, 2H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.75 (d, *J* = 1.7 Hz, 1H), 6.84 (d, *J* = 3.3 Hz, 1H), 6.73 (d, *J* = 3.4 Hz, 1H), 6.64 (dd, *J* = 1.8, 3.3 Hz, 1H), 6.61 (dd, *J* = 1.8, 3.3 Hz, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.0, 144.1, 143.6, 143.2, 143.0, 129.4, 115.4, 111.8, 111.7, 111.5, 109.2, 108.1, 65.1. **6c**: yield 54.9%; ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.82 (s, 2H), 7.68 (d, *J* = 5.1 Hz, 1H), 7.48 (d, *J* = 5.0 Hz, 1H), 7.29 (d, *J* = 3.5 Hz, 1H), 7.18 (t, *J* = 3.6 Hz, 1H), 7.11 (d, *J* = 3.6 Hz, 1H), 7.04 (t, *J* = 3.7 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 182.4, 171.1, 138.6, 137.4, 137.2, 128.6, 60.4, 31.5, 29.7, 22.6, 21.0, 14.2, 14.1.

4-Amino-5,6-diphenylfuro[2,3-*d*]pyrimidine (7a). To a stirred solution of 1-amino-2-cyano-3,4-diphenylfuran (**6a**, 0.5 g, 2.0 mmol) in formamide (2.9 mL) was added acetic anhydride (1 drop) at 100 °C. After being stirred at reflux for 2 h, the reaction mixture was cooled to room temperature and diluted with water (20 mL). The crude product was collected by filtration and recrystallized from dioxane to give 0.48 g (86.7%) of **7a**; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.27 (s, 1H), 7.58-7.50 (m, 6H), 7.42 (d, *J* = 6.6 Hz, 2H), 7.37-7.32 (m, 4H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 165.8, 159.2, 154.9, 146.5, 132.4, 130.5, 130.4, 129.9, 129.8, 129.6, 129.5, 126.8, 116.3, 102.9.

4-Hydroxy-5,6-di(2-furyl)furo[2,3-*d*]pyrimidine (7b). To a stirred solution of formic acid (12 mL) and acetic anhydride (24 mL) was added 1-amino-2-cyano-5,6-di(2-furyl)furan (**6b**, 0.5 g, 2.0 mmol). After being stirred at

reflux for 5 h, excess formic acid and acetic anhydride were distilled off *in vacuo* and the resulting residue was treated with water. The crude product was collected by filtration and recrystallized from ethanol to give 0.50 g (93.5%) of **7b**; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.19 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.47 (d, *J* = 2.2 Hz, 1H), 7.10 (d, *J* = 2.5 Hz, 1H), 6.68 (s, 1H), 6.63 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.6, 158.6, 148.4, 144.9, 144.3, 144.2, 143.9, 138.1, 113.5, 112.6, 112.0, 108.9.

4-Hydroxy-5,6-di(2-thienyl)furo[2,3-*d*]pyrimidine (7c). This compound was prepared from **6c** in the same manner as described above for the preparation of **7b**. Yield 85.5%; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.17 (bs, 1H), 8.02 (s, 1H), 7.70-7.64 (m, 2H), 7.41-7.34 (m, 2H), 7.18-7.11 (m, 2H), 8.11 (s, 1H), 7.64 (d, *J* = 5.0 Hz, 1H), 7.59 (d, *J* = 5.1 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.29 (d, *J* = 2.6 Hz, 1H), 7.10 (dd, *J* = 3.5, 5.1 Hz, 2H), 7.06 (dd, *J* = 3.7, 5.0 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 164.0, 158.3, 147.9, 143.5, 130.6, 130.4, 130.2, 128.9, 128.4, 128.2, 127.7, 127.6, 111.4, 108.5.

4-Bromo-5,6-diphenylfuro[2,3-*d*]pyrimidine (8a). Isoamyl nitrite (1 mL, 7.7 mmol) was added to a solution of 4-amino-5,6-diphenylfuro[2,3-*d*]pyrimidine (**7a**, 1.0 g, 3.5 mmol) in methylene bromide (4 mL) maintained at 80-85 °C. After being stirred at reflux for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 5:1) to give 0.40 g (33.3%) of **8a**; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.79 (s, 1H), 7.53-7.38 (m, 10H); ¹³C NMR (75 MHz, CDCl₃) δ 164.6, 152.6, 151.8, 143.6, 131.0, 129.8, 129.6, 128.9, 128.8, 128.4, 127.3, 121.6, 115.7.

4-Chloro-5,6-di(2-furyl)furo[2,3-*d*]pyrimidine (8b). 4-Hydroxy-5,6-di(2-furyl)furo[2,3-*d*]pyrimidine (**7b**, 0.2 g, 0.75 mmol) was added to phosphorus oxychloride (2.0 mL) at 0 °C. After being stirred at reflux for 2 h, excess phosphorus oxychloride was distilled off *in vacuo*. The resulting residue was treated with cold water, neutralized and purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 5:1) to give 0.1 g (50%) of **8b**; ¹H NMR (300 MHz, CDCl₃) δ 8.77 (s, 1H), 7.66 (s, 1H), 7.58 (s, 1H), 6.95 (d, *J* = 3.5 Hz, 1H), 6.77 (d, *J* = 3.3 Hz, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 6.56 (t, *J* = 1.7 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ

153.0, 152.8, 145.3, 143.4, 142.4, 130.7, 129.6, 128.9, 128.8, 127.7, 113.5, 113.1, 112.2, 111.4.

4-Chloro-5,6-di(2-thienyl)furo[2,3-*d*]pyrimidine (8c). This compound was prepared from **7c** in the same manner as described above for the preparation of **8b**. Yield 62.1%; ¹H NMR (300 MHz, CDCl₃) δ 8.70 (s, 1H), 7.60-7.58 (m, 1H), 7.48 (d, *J* = 2.8 Hz, 1H), 7.37 (d, *J* = 4.8 Hz, 1H), 7.21 (d, *J* = 2.6 Hz, 2H), 7.04-7.01 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 165.5, 152.8, 152.4, 150.2, 130.7, 129.7, 129.6, 128.8, 128.7, 127.8, 127.7, 119.1, 105.9.

General procedure for the synthesis of 4-*N*-substituted amino-5,6-disubstituted furo[2,3-*d*]pyrimidines 1-3. The appropriate amine (2.1 eq.) was added to a solution of the corresponding 4-halo-5,6-disubstituted furo[2,3-*d*]pyrimidine (**8**, 1.0 eq.) in alcohol and the reaction mixture was stirred under reflux. The progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give the title compound.

Inhibition assay for Akt1 kinase activity. Activated recombinant Akt1 kinase protein tagged with Glutathione S transferase (GST) in the *N*-terminal was obtained as described previously.¹⁵ Briefly, full length mouse Akt1 c-DNA was subcloned into pBacPAK8 baculoviral expression vector and baculovirus carrying Akt1 gene was generated using a baculovirus generation kit purchased from ClonTech according to the manufacturer's protocol. The viral stock was amplified to a titer of approximately 10⁸ pfu/mL. sf21 cells were infected with MOI of 10 and left for 48 hours, followed by treatment of 100 nM okadaic acid (from Sigma) for 4 hours before harvest. Subsequently GST tagged Akt1 was purified using glutathione agarose bead affinity column chromatography (Amersham) according to the manufacturer's protocol. Inhibition assay of Akt1 kinase activity was performed using 50 ng of the purified kinase in 20 μL of reaction mixture containing 20 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 0.5 mM dithiothreitol, 0.01 mM ATP, 4 μg of histone H2B (Sigma) as a peptide substrate and 0.2 μCi of ³²P-ATP in the presence of various concentrations of inhibitors. After 15 min incubation at 30 °C, the reaction was stopped by adding a half volume of 30% phosphoric acid. The reaction

mixture was spotted on p81 paper (Millipore) and washed with 0.1 M Tris-HCl (pH 8.0) five times for 10 min each and the radioactivity of each spot was quantitated using BAS image analyzer (Kodak). IC₅₀ value for each inhibitor was defined as the concentration of the inhibitor where the kinase activity is inhibited by 50%.

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