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Rational design of 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidines as potent glycogen synthase kinase-3 inhibitors

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Abstract—4-Amino-5,6-diaryl-furo[2,3-*d*]pyrimidines have been identified as inhibitors of glycogen synthase kinase-3β (GSK-3β). One representative derivative, 4-amino-5-(4-(benzenesulfonylamino)-phenyl)-6-(3-pyridyl)-furo[2,3-*d*]pyrimidine (**12**) exhibited potent GSK-3β inhibitory activity in low nanomolar level of IC_{50}. The binding mode was proposed from a docking study.

Protein kinases are important intracellular enzymes mediating the signal transduction in the eukaryotic cells by transferring a phosphate from ATP to a tyrosine, serine or threonine residue on target protein substrates. This phosphorylation process controls a multitude of cellular processes, including gene transcription, metabolic pathways, cell growth and differentiation, and apoptosis.[1] Mutation and deregulation of protein kinases have been linked to many diseases such as cancer, inflammation and diabetes, affording the potential to develop inhibitors to regulate these enzymes for therapeutic intervention.[2]

To date well over 160 X-ray crystal structures of more than 40 different kinase catalytic domains have been deposited in the Protein Data Bank (PDB) either with or without bound ligands. This structural information provides a wealth of knowledge in aiding development of pharmacophore models of the kinase catalytic domain which is of immense value in the design and development of small molecule inhibitors.[3] Using such structural-based design approaches, a large number of kinase inhibitors have been reported to date in the literature.

In an earlier report we disclosed the identification and structure–activity relationship (SAR) of a series of 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidines **1** as Tie-2 and vascular endothelial growth factor-2 (VEGFR2) dual inhibitors whose activity was rationalized based on the crystal structure complexed with VEGFR2. As shown in Figure 1, in binding to VEGFR2 the aminopyrimidine moiety interacted with the upper portion of the kinase ATP-binding pocket hinge region via two hydrogen-bond interactions. Additionally, the two aryl rings at the 5- and 6-positions were located in the hydrophobic backpocket and phosphate-binding sites, respectively.[4] In comparison, our attention was drawn to the binding mode of two GSK-3 inhibitors, 4,5-diphenyl-pyrazolo[3,4-*c*]pyridazine **2** and 5-aryl-pyrazolo[3,4-*b*]pyridines **3**, reported by Witherington et al., which were studied in the GSK-3 homology protein. Those docking studies suggested that the compounds were bound with the hinge region via three hydrogen-bond interactions (Fig. 1). Although structurally similar to **1**, pyrazolopyridazine **2** binds with amine moiety in a flipped conformation facing out towards the pocket with the two aromatic rings of compound **2** located in the sugar pocket and lysine-interacting sites. The reported SAR and docking study of pyrazolopyridine **3** revealed

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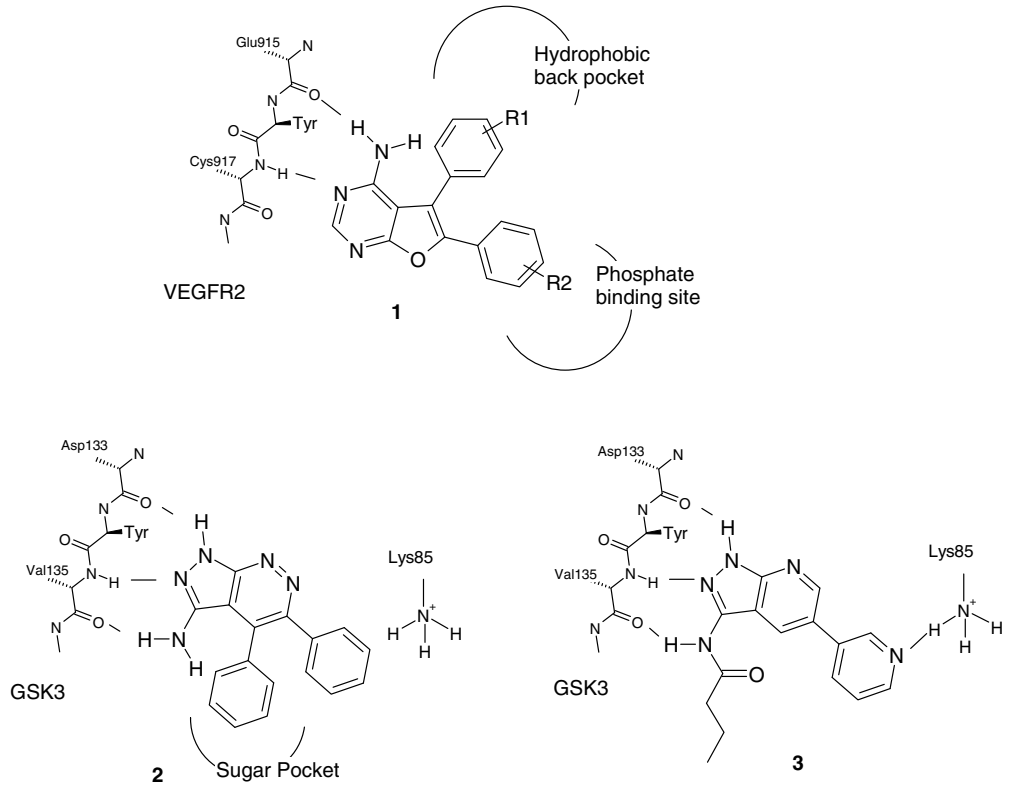


Figure 1. Binding modes of the VEGFR2/Tie-2 inhibitor 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidine **1**, and of GSK-3 inhibitors 4,5-diphenyl-pyrazolo[3,4-*c*]pyridazine **2** and 5-aryl-pyrazolo[3,4-*b*]pyridine **3**.

that the 3-pyridine at the 5-position is one of the key moieties to increase GSK-3 inhibitory activity via interaction with lysine 85 (Fig. 1).[5]

This information led us to postulate that the 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidines **1** could potentially be flipped to fit into the binding-pocket of GSK-3 similar to compounds **2** and **3** and hence the potential existed to alter the activity of this series away from Tie-2/VEGFR2 to target GSK-3. In a model of this flipped binding mode, both phenyl rings of furopyrimidine **1** appear to overlap well with those of pyrazolopyridazine **2** as shown in Figure 2. Additionally, the 3-pyridine of pyrazolopyridine **3** also overlaps well with 6-phenyl ring of furopyrimidine **1** (Fig. 2). Based on these docking studies, we decided to incorporate a 3-pyridine into the 6 position of the furopyrimidine scaffold to target the lysine 85 of GSK-3 to increase potency.[6]

In this article, we wish to report that alternative substitution at the 5- and 6-positions of the 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidine changed the kinase inhibitory profile to inhibit VEGFR2 and Tie-2 to GSK-3β.[7] Docking studies of the GSK-3β inhibitor have allowed us to envisage a different binding mode from that of the crystallized molecule complexed with VEGFR2, suggesting that kinase inhibitors can be designed for multiple targets via different binding modes from one chemical series by changing substitution in an effective way.

A synthetic method to prepare our modified furopyrimidines to test this hypothesis is shown in Scheme 1.

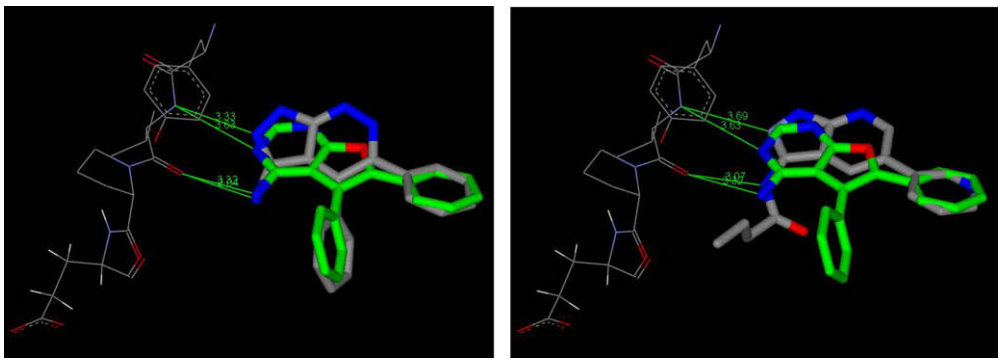
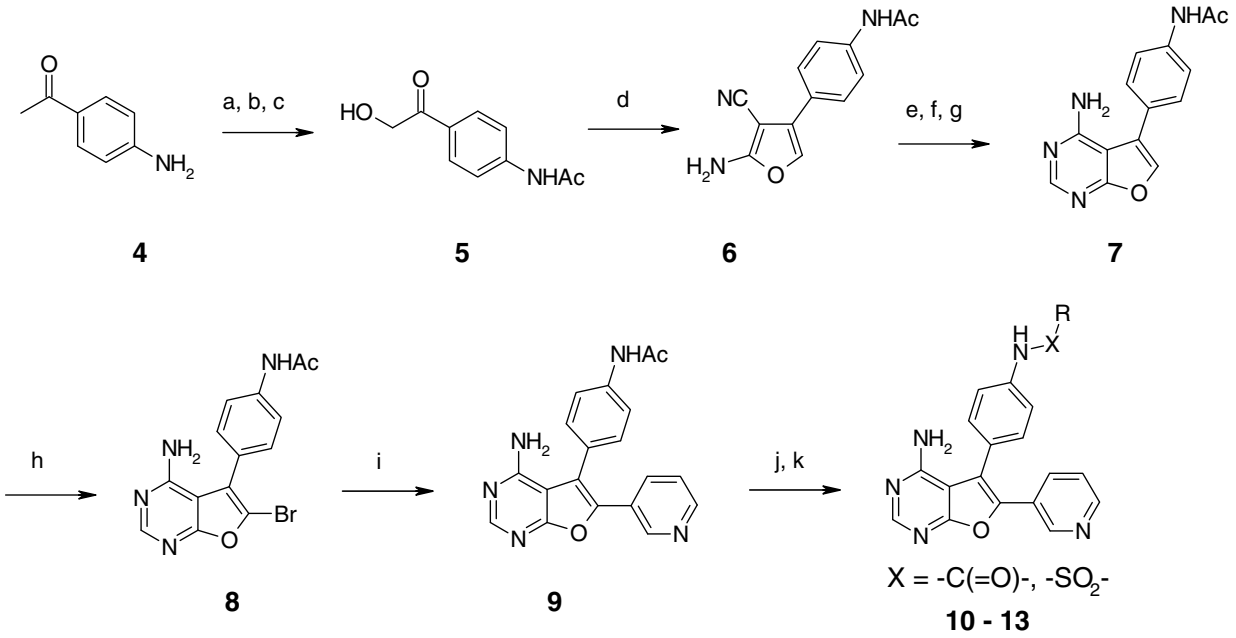


Figure 2. Overlay of the flipped binding mode of furopyrimidine **1** (green) with pyrazolopyridazine **2** (left) and pyrazolopyridine **3** (right) in GSK-3.



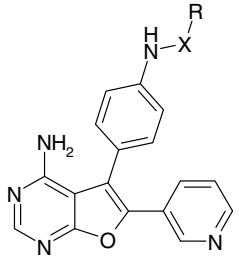
Scheme 1. Reagents and conditions: (a) Ac_2O, toluene, rt, 96%; (b) Br_2, AcOH, 60 °C, 63%; (c) potassium formate, $NaHCO_3$, EtOH–H_2O, 40 °C, 50%; (d) malononitrile, Et_2NH, DMF, rt, 81%; (e) $HC(OEt)_3$, Ac_2O, 100 °C; (f) NH_3, EtOH–THF, rt; (g) NaOEt, EtOH–THF, rt, 54% for three steps; (h) NBS, CCl_4–DMF, rt, 85%; (i) 3-(1,3,2-dioxaborinan-2-yl)pyridine, $Pd(PPh_3)_4$, K_3PO_4, DMF–H_2O, 80 °C, 85%; (j) 2 M KOH, EtOH–H_2O, 60 °C, 68–90%; (k) sulfonyl chloride, 4-DMAP, pyridine, rt, 57–88%; acyl chloride, 4-DMAP, pyridine, rt, 67–77%.

4-Aminoacetophenone **4** was converted to the α-hydroxyketone **5** via sequential acetylation, bromination and hydroxylation with potassium formate. Treatment of the α-hydroxyketone **5** with malononitrile in the presence of diethylamine provided 2-amino-3-cyano-furan **6**. The furan **6** was treated with triethylorthoformate, followed by amination and cyclization in the presence of sodium ethoxide, affording 4-amino-furo[2,3-*d*]pyrimidine **7**.[4,13] After bromination at the 6-position with NBS, Suzuki coupling was conducted with 3-pyridineboronic acid pinacol ester to provide furopyrimidine **9**, followed by deprotection of the acetyl group and reaction of the resulting amine with either sulfonyl or acid chlorides to yield the corresponding sulfonamide and amides **10**–**13**.

GSK-3β kinase inhibitory activity of furopyrimidines **9**–**13** was determined using a fluorescence anisotropy binding assay.[8] As indicated in Table 1, introduction of the 3-pyridine moiety at the 6-position and various sulfonamides and amides at the para position of the 5-phenyl ring led to a range of GSK-3 activities from approximately 1.6 μM to 23 nM.[9] The amides **9** and **10** showed moderate potencies with similar activities for both alkyl and aryl substituents. However, with the sulfonamides although the methylsulfonamide **11** showed moderate activity, the aryl sulfonamides **12** and **13** displayed potent GSK-3 inhibitory activity of 23–30 nM.[10]

Furopyrimidine **12** was docked into the ATP-binding site of GSK-3β available from crystal structures. Using the original binding mode we observed from co-crystal structures of furopyrimidine bound to VEGFR2, the aryl ring of the sulfonamide at the 5-position would appear to clash with residues Met101, Leu112 and Leu130

Table 1. Enzyme inhibitory activity of 4-amino-5,6-diaryl-furo[2,3-*d*]pyrimidines against GSK-3β



Compound	X	R	IC_{50} (nM)	SD
9	—(C=O)—	Methyl	1628	2259, $n = 3$
10	—(C=O)—	3-Fluorophenyl	1585	1645, $n = 2$
11	—SO_2—	Methyl	475	169, $n = 3$
12	—SO_2—	Phenyl	30	47, $n = 3$
13	—SO_2—	2-Thiophen	23	50, $n = 2$

of GSK-3, as shown in Figure 3. Contrary to this, in the flipped binding mode, furopyrimidine **12** looks much more likely to be accommodated in the pocket. The N3 nitrogen and NH_2 of aminopyrimidine are anchored with the carbonyl moiety and NH of Val135, respectively, via hydrogen bond interactions. Additionally, the 3-pyridine moiety at the 6-position is close to Lys85 of the conserved salt bridge (Lys85/Glu97). This observation was consistent with the SAR of the 3-pyridine moiety at the 5-position of the pyrazolopyridine **2**. Furthermore, the flipped binding mode places the sulfonamide in the sugar pocket where interactions with hydrophilic residues would be expected to assist in tight binding. Thus, furopyrimidine **12** is speculated to bind to GSK-3 in this flipped binding mode, different from

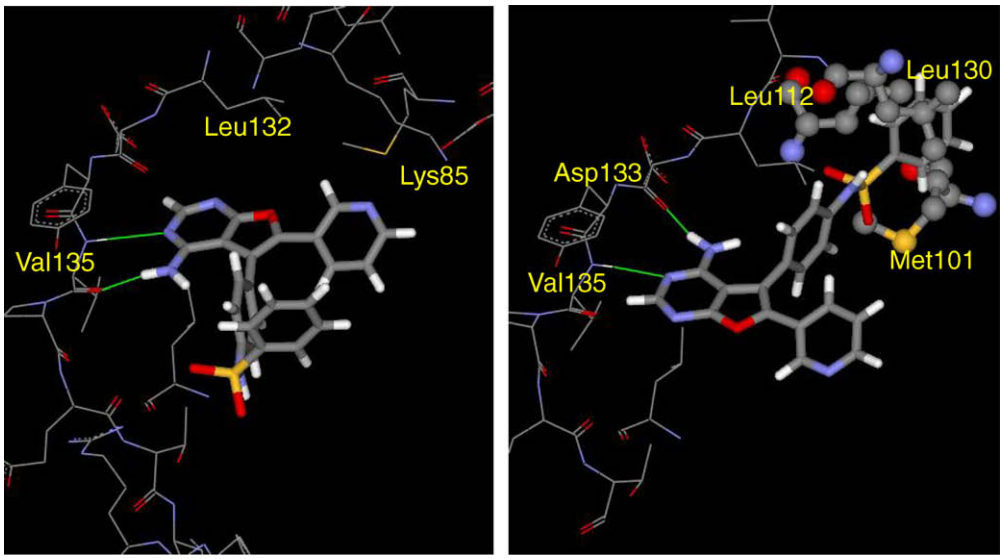


Figure 3. Proposed flipped binding (left) and compliant binding (right) of compound **12** in the GSK-3β protein. In the compliant mode phenyl ring is bumped the residues composed by Met101, Leu112 and Leu130. In the flipped mode compound **12** is likely to be accommodated into the pocket well. Key interactions with hinge region are indicated by green lines.

Table 2. Selectivity profile of compound **12**[a]

AMPK	CHK1	JNK1	MAPK	MEK1	PKA	PKBa	SGK	CDK2	GSK-3
44	3	7	5	0	0	29	3	27	100

[a] Values are % inhibition at 10 μM using 100 μM ATP.

the binding mode observed in the case of VEGFR2. These results indicate that one chemical series can exhibit different potent kinase inhibitory activities by switching its binding mode.

Having identified furopyrimidines with GSK-3 activity, we profiled compound **12**, one of the most potent analogue, by cross screening against a variety of kinases.[11] As shown in Table 2, furopyrimidine **12** showed an excellent overall selectivity profile against those kinases tested. It is noteworthy that compound **12** showed good selectivity against CDK2, where there is a high degree of homology with GSK-3β.[12]

In conclusion, we identified novel potent GSK-3β inhibitors, represented by 4-amino-5-(4-(benzenesulfonylamino)-phenyl)-6-(3-pyridyl)-furo[2,3-*d*]pyrimidine **12**, by modification of an existing VEGFR2 series. The binding mode is likely to be different from that observed in VEGFR2; the core structure being flipped to better occupy the catalytic domain of GSK-3.

Acknowledgments

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References and notes

1. (a) Hunter, T. *Cell* **1995**, *80*, 225; (b) Johnson, L. N.; Lewis, R. J. *J. Chem. Rev.* **2001**, *101*, 2209.
2. (a) Blume-Jensen, P.; Hunter, T. *Nature* **2001**, *411*, 355; (b) Cohen, P. *Nat. Rev. Drug Disc.* **2002**, *1*, 309.
3. Traxler, P.; Furet, P. *Pharmacol. Ther.* **1999**, *82*, 195.
4. (a) Miyazaki, Y.; Matsunaga, S.; Tang, J.; Maeda, Y.; Nakano, M.; Philippe, R. J.; Shibahara, M.; Liu, W.; Sato, H.; Wang, L.; Nolte, R. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2203; (b) Miyazaki, Y.; Tang, J.; Maeda, Y.; Nakano, M.; Wang, L.; Nolte, R. T.; Sato, H.; Sugai, M.; Okamoto, Y.; Truesdale, A. T.; Hassler, D. F.; Nartey, E. N.; Patrick, D. R.; Ho, M. L.; Ozawa, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1773.
5. Witherington, J.; Bordas, V.; Garland, S. L.; Hickey, D. M.; Ife, R. J.; Liddle, J.; Saunders, M.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1577.
6. Related structure series of 4-acylamino-6-arylfuro[2,3-*d*]pyrimidines were reported. (a) Nakano, M; Maeda, Y. PCT Int. Application, WO2005061516A1, July 07, 2005; (b) Maeda, Y.; Nakano, M.; Sato, H.; Miyazaki, Y.; Schweiker, S. L.; Smith, J. L.; Truesdale, A. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3907.
7. Reviews for therapeutic targets of GSK-3 inhibitors: (a) Eldar-Finkelman, H. *Trends Mol. Med.* **2002**, *8*, 126; (b) Kaidanovich, O.; Eldar-Finkelman, H. *Expert Opin. Ther. Targets* **2002**, *6*, 555; (c) Bullock, W. H.; Magnuson, S. R.; Choi, S.; Gunn, D. E.; Rudolph, J. *Curr. Top. Med. Chem.* **2002**, *2*, 915; (d) Dorronsoro, I.; Castro, A.; Martinez, A. *Expert Opin. Ther. Pat.* **2002**, *12*, 1527.
8. Human GSK-3β protein residues 27-393 tagged at the N-terminal with a 10His sequence were expressed from baculovirus and purified to >95% homogeneity. GSK-3β kinase inhibitory activity was determined using a fluorescence anisotropy binding assay. The kinase, a fluorescently

labelled inhibitor, and a variable concentration of test compound were incubated together to reach thermodynamic equilibrium under conditions such that in the absence of test compound, the fluorescent inhibitor is significantly (>50%) enzyme bound and in the presence of a sufficient concentration (>10 K_i, where K_i = dissociation constant for inhibitor binding) of a potent inhibitor the anisotropy of the unbound fluorescent ligand is measurably different from the bound value. Inhibitor binding to GSK-3β was assessed by a fluorescence anisotropy competitive binding assay. All components were dissolved in buffer of composition 50 mM Hepes, pH 7.4, 1 mM CHAPS, 1 mM DTT, 10 mM $MgCl_2$ with final concentrations of 65 nM GSK-3β and 5 nM fluorescently labelled inhibitor. This reaction mixture is added to wells containing various concentrations of test compound (typically 24 pM–25 μM final) or DMSO vehicle (<3% final) in black 384-well microtitre plates and equilibrated for 60–180 min at ambient temperature. Fluorescence anisotropy was measured in a Molecular Devices Acquest spectrofluorimeter (excitation wavelength 485 nm: emission wavelength 535 nm). The error of the assay was estimated to be within ±0.2 log units, based on the median standard deviation of compounds which have been tested more than eight times.

9. IC_{50} value of 4-amino-5,6-bis(4-methoxylphenyl)-furo[2,3-*d*]pyrimidine in GSK-3 enzyme assay was over 15 μM. Replacement of pyridine of compound **12** with 4-methoxylphenyl reduced the GSK-3 inhibitory activity (IC_{50} > 15 μM). These results indicated interaction of pyridine plays one of the key roles to exhibit activity.
10. EC_{50} value of compound **12** in the glycogen accumulation assay in L6 cells was 3.2 μM. The method was described by: Peat, A. J.; Garrido, D.; Boucheron, J. A.; Schweiker, S. L.; Dickerson, S. H.; Wilson, J. R.; Wang, T. Y.; Thomson, S. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2127.
11. Davis, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95.
12. Compound **12** showed IC_{50} value of 1120 nM against VEGFR2.
13. Experimentals for some intermediates are as follows.
(a) 4′-Acetamido-2-hydroxyacetophenone (**5**). To a suspension of 4′-acetamido-2-bromoacetophenone (10.0 g, 39 mmol) in EtOH (800 ml), aqueous potassium formate (35.4 g in 200 ml) and subsequently sodium bicarbonate (3.68 g) were added. The mixture was stirred at 38 °C (inner temp) for 22 h. The mixture was concentrated in vacuo until the total volume reached to ca. 300 ml, diluted with ethyl acetate (1200 ml), and washed with water (200 ml) and brine (2× 400 ml). Aqueous layers were re-extracted with ethyl acetate (800 ml then 400 ml). Combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo to give crude **5**: ^{1}H NMR (DMSO-d_6) ppm 10.36 (s, 1H), 7.88 (m, 2H), 7.71 (m, 2H), 4.99 (m, 1H), 4.73 (m, 2H), 2.09 (s, 3H) as a solid (7.17 g, 95%), which was used for the next step without purification.
(b) 5-(4-Acetamidophenyl)-4-amino-furo[2,3-*d*]pyrimidine (**7**). 4-(4-Acetamidophenyl)-3-cyano-2-[(ethoxymethylidene)amino]furane (10.26 g, 34.5 mmol), which was obtained from compound **6** by ethoxymethylidenation of the amine, was suspended in a mixture of EtOH (410 ml) and THF (410 ml). Into the mixture cooled in an ice-water bath and under vigorous stirring, NH_3 gas was bubbled for 40 min. The suspension became a clear solution at once, and subsequently it began to make a precipitation. The mixture in the sealed reaction vessel was stirred at room temperature for 3 h, concentrated in vacuo, and dried under reduced pressure. The residual solid was triturated with small amount of methanol, filtrated, and dried under reduced pressure to give 4-(4-acetamidophenyl)-2-[(aminomethylidene)amino]-3-cyano-furan (6.16 g). ^{1}H NMR (DMSO-d_6): ppm 10.06 (s, 1H), 8.21 (br, 2H), 7.87 (br, 1H), 7.64 (d, 2H, J = 8 Hz), 7.63 (s, 1H), 7.52 (d, 2H, J = 8 Hz), 2.06 (s, 3H); MS(ESI) m/z 269 $(M+1)^+$. Thus, obtained solid (6.00 g, 22.4 mmol) was suspended in a mixture of EtOH (180 ml) and THF (180 ml). To the mixture was added dropwise 27.3 ml (0.9 N, 24.6 mmol) of sodium ethoxide (freshly prepared from sodium in ethanol). The suspension gradually dissolved and gave a clear solution during stirring at room temperature. After stirring for 4 h, the mixture was concentrated in vacuo to give a solid, which was triturated with water, collected by filtration, washed with water, and dried under reduced pressure to give **7** (6.01 g) as a brown solid. ^{1}H NMR (DMSO-d_6): ppm 10.12 (s, 1H), 8.25 (s, 1H), 7.93 (s, 1H), 7.73 (d, 2H, J = 8 Hz), 7.45 (d, 2H, J = 8 Hz), 6.55 (br, 2H), 2.09 (s, 3H); MS(ESI) m/z 269 $(M+1)^+$.