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# 5-Arylamino-2-methyl-4,7-dioxobenzothiazoles as Inhibitors of Cyclin-Dependent Kinase 4 and Cytotoxic Agents

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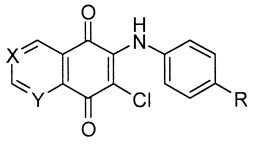
**Abstract**—5-Arylamino-2-methyl-4,7-dioxobenzothiazoles were synthesized as inhibitors of cyclin-dependent kinase 4 (CDK4) and cytotoxic agents. Most of the 4,7-dioxobenzothiazoles exhibited selective inhibitory activities for the CDK4 and cytotoxic potential against human cancer cell lines. 

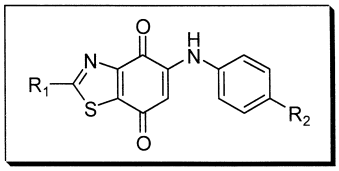
Cyclin-dependent kinases (CDKs) play essential roles in cell cycle regulation.[1] Alternation and deregulation of CDK activity are pathogenic hallmarks of neoplasia.[2,3] Thus, CDK inhibitors have shown antitumor activities in animal models and would be of interest to explore as novel therapeutic agents in cancers as well as other hyperproliferative disorders.[3,4]

Quinones have been frequently studied with their antitumor activities.[5] We also newly synthesized and evaluated the CDK inhibitory activities (CDK2 and CDK4) and cytotoxic potential of various quinone derivatives such as l,4-naphthoquinones **1**, 5,8-quinolinediones **2**, 5,8-isoquinolinediones **3**,[5] 5,8-quinazolinediones **4** and 4,7-dioxobenzothiazoles **5**. Among the quinones tested, 4,7-dioxobenzothiazoles **5** showed potent cytotoxicities against human cancer cell lines and selective inhibitory activities for CDK4 compared to CDK2.

We report herein the synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **5** and their inhibitory activities for CDK4 as well as their cytotoxic potential against human cancer cell lines.




R = H, F, Cl, ..
**1** : X = Y = CH
**2** : X = CH, Y = N
**3** : X = N, Y = CH
**4** : X = Y = N




**5** : $R_1$ = H or $CH_3$
$R_2$ = H, F, Cl, ..

The 4,7-dioxobenzothiazole derivatives[6,7] have been reported as antimalarial agents[6] and inhibitors of mitochondrial cytochrome complex in mammalians.[7] However, the inhibitory activities for CDKs and cytotoxicity of the 4,7-dioxobenzothiazoles against cancer cell lines have not been reported. Many compounds such as olomoucine, roscovitine, flavopiridol and staurosporine are potent and/or relatively selective inhibitors of CDK2, but none are selective inhibitors of CDK4.[3,4] Based on these considerations, we evaluated 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **5a**–**5j** on CDK4 inhibitory activity and cytotoxicities (Scheme 1, Table 1).

## Chemistry

A convenient method for the synthesis of the 4,7-dioxobenzothiazoles **5a**–**5j** is shown in Scheme 1. Experimental details and data for this procedure are cited in

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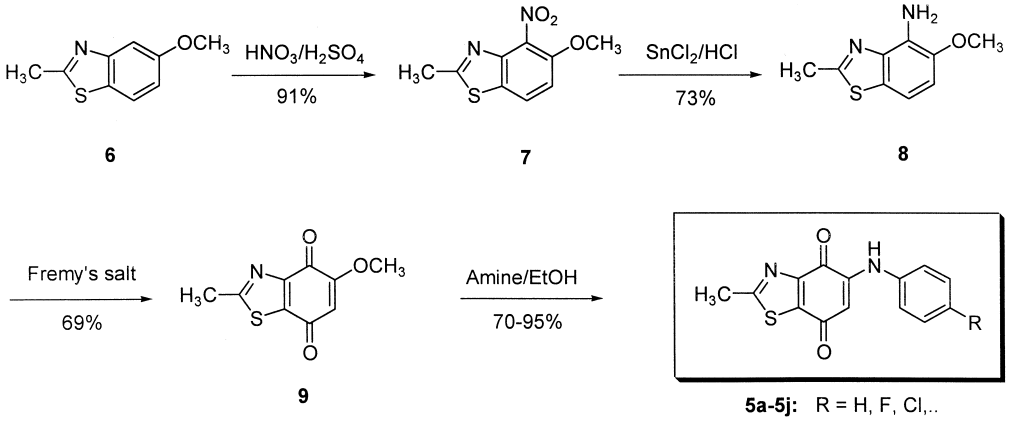
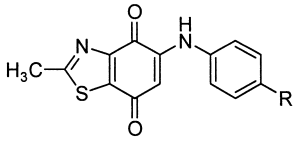



**Scheme 1.** Synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles.

**Table 1.** CDK inhibitory and cytotoxic activities of 5-arylamino-2-methyl-4,7-dioxobenzothiaoles



| Compound | R | $IC_{50}$ (μM)[a] | | Cytotoxicity[b] $IC_{50}$ (μg/mL) | | | |
|---|---|---|---|---|---|---|---|
| | | CDK4 | CDK2 | A 549[c] | Col 1 | HL-60 | HepG2 |
| **5a** | H | 6.0 | >200 | 0.64 | 1.93 | 0.28 | 1.30 |
| **5b** | F | 6.3 | >200 | 1.69 | 1.94 | 0.23 | 1.51 |
| **5c** | Cl | 7.0 | >200 | 3.60 | 20.00 | 0.33 | 3.23 |
| **5d** | Br | 6.7 | >200 | 3.60 | 6.57 | 0.28 | 2.78 |
| **5e** | I | 3.0 | >200 | 2.08 | 2.08 | 0.24 | 1.95 |
| **5f** | OH | 6.7 | >200 | 0.70 | 1.85 | 0.26 | 1.36 |
| **5g** | $CH_3$ | 6.0 | >200 | 0.61 | 1.08 | 0.30 | 1.21 |
| **5h** | $OCH_3$ | 5.6 | >200 | 1.65 | 2.01 | 0.27 | 1.39 |
| **5j** | $OCH_2CH_3$ | 20.0 | >200 | 1.76 | 2.09 | 0.29 | 1.53 |
| Olomoucine | | >200 | 7.0 | 57.00 | 87.00 | 50.0 | NT[d] |
| Roscovitine | | 100 | 0.6 | NT | NT | NT | NT |
| Cisplatin | | NT | NT | 1.80 | 1.38 | 0.70 | 0.33 |

[a]CDK4/cyclin Dl and CDK2/cyclin A inhibition assay according to ref 2.
[b]Cytotoxicity evaluation: SRB assay according to the NCI (National Cancer Institute) protocols.[11,12]
[c]Human cancer cell lines: A 549 (non-small cell lung from NCI), HL-60 (myeloid leukemia from ATCC), HepG2 (hepatocarcinoma from ATCC) and Col 1 cells (human colon carcinoma from Department of Surgical Oncology, University of Illinois at Chicago, USA).
[d]NT: not tested.

References and Notes.[8–10] Nitration of 5-methoxy-2-methylbenzothiazole (**6**) followed by reduction afforded 4-amino-5-methoxy-2-methyl-benzothiazole (**8**) in about 73% yield. 5-Methoxy-2-methyl-4,7-dioxobenzothiazole (**9**) was synthesized by oxidizing the compound **8** with Fremy's salt (potassium nitrosodisulfonate) in 69% yield. 5-Arylamino-2-methyl-4,7-dioxobenzothiazoles **5a**–**5j** were formed by regioselective nucleophilic substitution of the 4,7-dioxobenzothiazole (**9**) with the appropriate arylamines.

## Biological Activities

The 4,7-dioxobenzothiazoles **5a**–**5j** were tested for their CDKs/cyclins (CDK2 and CDK4) inhibitory activities according to ref 2. The $IC_{50}$ values determined are compared with those of olomoucine and roscovitine. As indicated in Table 1, the 4,7-dioxobenzothiazoles **5a**–**5j** showed inhibitory activities for CDK4/cyclin D1 in the range of 3.0∼20.0 μM as an $IC_{50}$ value without inhibitory activity for CDK2/cyclin A at the test concentration up to 200 μM. In contrast, the olomoucine and roscovitine showed more selective inhibitory activities for CDK2 compared to CDK4. The result indicates that the 4,7-dioxobenzothiazoles **5a**–**5j** could be considered as selective CDK4 inhibitors.

The cytotoxic potential of compounds **5a**–**5j** against human cancer cells was determined by the SRB (sulforhodamine B) assay according to the National Cancer Institute (NCI) protocols as described previously.[11,12] The following human tumor cell lines were used: A 549, Col 1, HL-60 and HepG2. The $IC_{50}$ values of **5a**–**5j** were compared with those of cisplatin as a reference agent. As indicated in Table 1, the 4,7-dioxobenzothiazoles

**5a**–**5j** showed generally potent cytotoxic activities against all cancer cell lines tested, and especially potent activity was observed in HL-60 cells with the $IC_{50}$ values of 0.23–0.33 μg/mL. Actually, activities of the compounds **5a**, **5b**, **5f**, **5g**, **5h** and **5j** were superior or comparable to those of cisplatin against HL-60 and A 549 cell lines. In addition, the compounds **5a**, **5f**, **5g** and **5h** also exhibited approximately 3 times more potent cytotoxicity than cisplatin against A 549. These potent cytotoxic congeners **5a**, **5f**, **5g** and **5h** also exhibited inhibitory activities for CDK4.

## Conclusion

5-Arylamino-2-methyl-4,7-dioxobenzothiazoles **5a**–**5j** are selective CDK4 inhibitors and potent cytototoxic agents against HL-60 cancer cell line.

## Acknowledgements

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8. Experimental: All melting points (mp) were measured in open capillary tubes with Thomas Hoover Capillary Apparatus model and were uncorrected. The thin layer chromatography (TLC) was performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ for solvent. The compounds were detected under UV light (254 nm) or by heating at 110 °C after spraying 30% $H_2SO_4$–vanillin solution. Column chromatography was performed on silica gel 560 (70–230 mesh, ASTM, Merck). The purity of 4,7-dioxobenzothiazoles **5a**–**5j** was also verified by GC (Hewlett Packard 5890A, HP-S capillary column at 260 °C, $N_2$ gas, 17 mL/min as carrier gas, FID). The IR spectra were taken from Perkin–Elmer 1420r IR spectrometer with KBr pellets. $^1$H NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDC1_3$ or DMSO-$d_6$ as solvents, and chemical shifts are given in ppm with TMS as a standard. Mass spectra were obtained on JMS AX 505 WA spectrometer (electronic impact at 70 eV). Elemental analyses were performed by CE instruments EA1110 with sulfanilamide as a standard material. 5-Methoxy-2-methylbenzothiazole (**6**) was obtained from TCI Co. $CDC1_3$, DMSO-$d_6$ and other reagents were purchased from Aldrich Chemical Co.
9. General procedure for synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **5**. 5-Methoxy-2-methylbenzothiazole (**6**, 3.2 g, 17.85 mmol) in 6 mL of c-$H_2SO_4$ and 6 mL of c-$HNO_3$ was stirred at rt for 2 h. The precipitate was filtered and crystallized from $CHCl_3$. 5-Methoxy-2-methyl-4-nitrobenzothiazole (**7**) was obtained (3.64 g, 91%): mp 144–145 °C. To 6 g (32 mmol) of $SnCl_2$ in 12 mL of c-HCl was added 1 g (9.42 mmol) of compound **7**. The mixture was stirred at 60 °C for 2 h and was extracted twice with $CH_2Cl_2$. The extract was evaporated and crystallized from $CH_2Cl_2$. 4-Amino-5-methoxy-2-methylbenzothiazole (**8**) was obtained (1.26 g, 73%): mp 118–119 °C. To a solution of compound **8** (1.94 g, 10 mmol) in 400 mL of acetone was added a solution of potassium nitrosodisulfonate (5 g, 18.65 mmol) in sodium dihydrogen phosphate buffer (0.3 M, 800 mL). The mixture was stirred at rt for 1 h and was extracted twice with $CH_2Cl_2$. The extract was evaporated and crystallized from $CH_2Cl_2$. 5-Methoxy-2-methyl-4,7-dioxobenzothiazole (**9**) was obtained (1.44 g, 69%). A solution of compound **9** (0.209 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4–5 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH (Scheme 1, Table 1). Crystallization from aq EtOH afforded 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **5a**–**5j**.
10. Characterization data for 4,7-dioxobenzothiazoles. 5-Methoxy-2-methyl-4,7-dioxobenzothiazole (**9**): yellow powder (69%). Mp: 248–249 °C. IR (KBr): ν 3030 (w), 2940, 1690 (s, C=O), 1595–1470 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 6.0 (s, 1H, H6), 3.57 (s, 3H, $OCH_3$), 2.7 (s, 3H, $CH_3$). MS (*m*/*z*): 209 ($M^+$), 194 ($M^+-CH_3$). Anal. calcd for $C_9H_7NO_3S$ (209.22): C, 51.67; H, 3.37; N, 6.69; S, 15.33. Found: C, 51.66; H, 3.38; N, 6.69; S, 15.32. 5-Phenylamino-2-methyl-4,7-dioxobenzothiazole (**5a**): dark purple powder (70%). Mp: 219–220 °C. IR (KBr): ν 3255 (NH), 3000 (w, aromatic ring), 1694 (s, C=O), 1590–1470 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.4–7.5 (m, 5H, Ph–H), 5.9 (s, 1H, H6), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 270 ($M^+$), 255 ($M-CH_3$), 241, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{14}H_{10}N_2O_2S$ (270.31): C, 62.21; H, 3.73; N, 10.36; S, 11.86. Found: C, 62.20; H, 3.74; N, 10.35; S, 11.84. 5-[*N*-(4-Fluorophenyl)lamino-2-methyl-4,7-dioxobenzothiazole (**5b**): dark green powder (95%). Mp: 231–233 °C. IR (KBr): ν 3200 (NH), 3055 (w, aromatic ring), 1692 (s, C=O), 1600–1480 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.2–7.5 (m, 4H, Ph–H), 5.8 (s, 1H, H6), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 288 ($M^+$), 260, 232, 126, 95, 76. Anal. calcd for $C_{14}H_9FN_2O_2S$ (288.30): C, 58.33; H, 3.15; N, 9.72; S, 11.12. Found: C, 58.29; H, 3.16; N, 9.71; S, 11.11. 5-[*N*-(4-Chlorophenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5c**): dark purple powder. Mp: 271–273 °C. IR (KBr): ν 3255 (NH), 3050 (w, aromatic ring), 1694 (s, C=O), 1590–1470 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.4–7.5 (m, 4H, Ph–H), 5.9 (s, 1H, H6), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 304 ($M^+$), 269 (M–Cl), 241, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{14}H_9ClN_2O_2S$ (304.75): C, 55.18; H, 2.98; N, 9.19; S, 10.52. Found: C, 55.19; H, 2.99; N, 9.18; S, 10.49. 5-(*N*-(4-Bromophenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5d**): dark purple powder (80%). Mp: 318–320 °C. IR (KBr): ν 3280 (NH), 3060 (w, aromatic ring), 1687 (s, C=O), 1600–1480 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.3–7.7 (m, 4H, Ph–H), 5.9 (s, 1H, H6), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 348 ($M^+$), 269 ($M-Br$), 253, 224, 126, 76. Anal. calcd for $C_{14}H_9BrN_2O_2S$ (349.20): C, 48.15; H, 2.60; N, 8.02; S,

9.18. Found: C, 48.13; H, 2.61; N, 8.02; S, 9.17. 5-[*N*-(4-Iodophenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5e**): dark violet powder (80%). Mp: 227–228 °C. IR (KBr): ν 3250 (NH), 3045 (w, aromatic ring), 1690 (s, C=O), 1590–1475 (benzene ring) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.2–7.8 (m, 4H, Ph–H), 6.2 (s, 1H, H6), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 396 ($M^+$), 269, 241, 126, 76. Anal. calcd for $C_{14}H_9N_2O_2IS$ (396.20): C, 42.44; H, 2.29; N, 7.07; S, 8.09. Found: C, 42.43; H, 2.29; N, 7.06; S, 8.08. 5-[*N*-(4-Hydroxyphenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5f**): black powder (70%). Mp: 319–320 °C. IR (KBr): ν 3500–3400 (s, OH), 3250 (NH), 3050 (w, aromatic ring), 1690 (s, C=O), 1600–1480 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 9.6 (s, 1H, NH), 6.8–7.2 (m, 4H, Ph–H), 6.2 (s, 1H, H6), 5.7 (s, 1H, Ph-OH). MS (*m*/*z*): 286 ($M^+$), 269 (M−OH), 241, 160, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{14}H_{10}N_2O_3S$ (286.31): C, 58.73; H, 3.52; N, 9.78; S, 11.20. Found: C, 58.71; H, 3.53; N, 9.77; S, 11.21. 5-(*N*-(4-Methylphenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5g**): black–purple powder (85%). Mp: 231–232 °C. IR (KBr): ν 3250 (NH), 3050 (w, aromatic ring), 2368, 1690 (s, C=O), 1600–1480 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 9.4 (s, 1H, NH), 7.3 (m, 4H, benzene ring), 5.8 (s, 1H, H6), 2.8 (s, 3H, $CH_3$), 2.3 (s, 3H, Ph–$CH_3$). MS (*m*/*z*): 284 ($M^+$), 269 (M−$CH_3$), 241, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{15}H_{12}N_2O_2S$ (284.33): C, 63.36; H, 4.25; N, 9.85; S, 11.28. Found: C, 63.33; H, 4.25; N, 9.84; S, 11.27. 5-[*N*-(4-Methoxyphenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5h**): black powder (80%). Mp; 228–230 °C. IR (KBr): ν 3250 (NH), 3000 (w, aromatic ring), 1694 (s, C=O), 1590–1470, 1270 (s, C–O–C) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 9.3 (s, 1H, NH), 7.0–7.3 (m, 4H, Ph–H), 6.2 (s, 1H, H6), 3.9 (s, 3H, $OCH_3$), 2.8 (s, 3H, $CH_3$). MS (*m*/*z*): 300 ($M^+$), 285 (M−$CH_3$), 269 (M−$OCH_3$), 241, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{15}H_{12}N_2O_3S$ (300.33): C, 59.99; H, 4.03; N, 9.33; S, 10.68. Found: C, 59.97; H, 4.04; N, 9.32; S, 10.69. 5-[*N*-(4-Ethoxyphenyl)amino-2-methyl-4,7-dioxobenzothiazole (**5j**): dark violet powder (85%). Mp: 201–202 °C. IR (KBr): ν 3290 (NH), 3050 (w, aromatic ring), 1685 (s, C=O), 1600–1470 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 9.3 (s, 1H, NH), 7.0–7.3 (m, 4H, Ph–H), 5.7 (s, 1H, H5), 5.7 (s, 1H, H6), 4.0 (d, 2H, $OC_2H_5$, $J$= 7.2 Hz), 2.8 (s, 3H, $CH_3$), 1.3 (d, 3H, $OC_2H_5$, $J$= 7.2 Hz). MS (*m*/*z*): 314 ($M^+$), 285 (M−$C_2H_5$), 269, 160, 126, 77 ($C_6H_5^+$). Anal. calcd for $C_{16}H,_4N_2O_3S$ (314.36): C, 61.13; H, 4.49, N, 8.91; S, 10.20. Found: C, 61.10; H, 4.50; N, 8.91; S, 10.19.

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