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DEVELOPMENT OF N^6-(HETEROARYLCARBONYL)ADENINES AS BRD4 INHIBITORS

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Abstract – N^6-(Heteroarylcarbonyl)adenines were synthesized as candidate BRD4 inhibitors, and their structure-activity relationships were investigated. Among the synthesized compounds, N^6-[(3-methoxythiophen-2-yl)carbonyl]adenine (**15**) showed the most potent BRD4-inhibitory activity.

Histone acetylation is an important post-translational modification that is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1-3] It is involved in the regulation of many cellular processes, including the cell cycle, cell differentiation, chromatin remodeling and DNA damage/repair. Acetylated lysine residues in histone tails and in non-histone proteins can be recognized by bromodomains, which are found in many chromatin- and transcription-modifying proteins, including HATs, ATP-dependent chromatin-remodeling complexes, transcriptional coactivators, methyltransferases, and the bromodomain and extra-terminal domain (BET) family proteins.[4-7] The BET family consists of BRD2, BRD3, BRD4, and BRDT in humans, and these proteins regulate transcription and cell growth.[8] Among the bromodomain and BET family proteins, BRD4 has been extensively studied because of its recruitment of positive transcriptional elongation factor b (P-TEFb), associated with phosphorylation of RNA polymerase II, resulting in transcriptional elongation.[1] BRD4 plays a key role in cell-cycle progression,[8] and in transcription of c-*myc*, an oncogenic driver gene.[5] BRD4 also enhances the transcriptional activation of nuclear factor kappa B (NF-κB), which mediates transcription of many inflammatory genes, including those encoding interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α).[9,10] Therefore, BRD4 inhibitors are considered to have potential for the treatment of cancers and inflammatory disorders. There have been many studies on BET bromodomain inhibitors since the first potent inhibitors, (+)-JQ1 and I-BET762, were reported in 2010,[11-13] and some, such as I-BET762 and OTX-015, are already in clinical trials for treatment of cancers (Figure 1).[5] We previously discovered N^6-benzoyladenine derivatives as novel BRD4 inhibitors, and investigated their cell

differentiation-inducing activities.[14,15] As part of our BRD4 project, we replaced the benzoyl group with various heteroarylcarbonyl groups and examined the structure-activity relationships (SAR) of the resulting compounds.

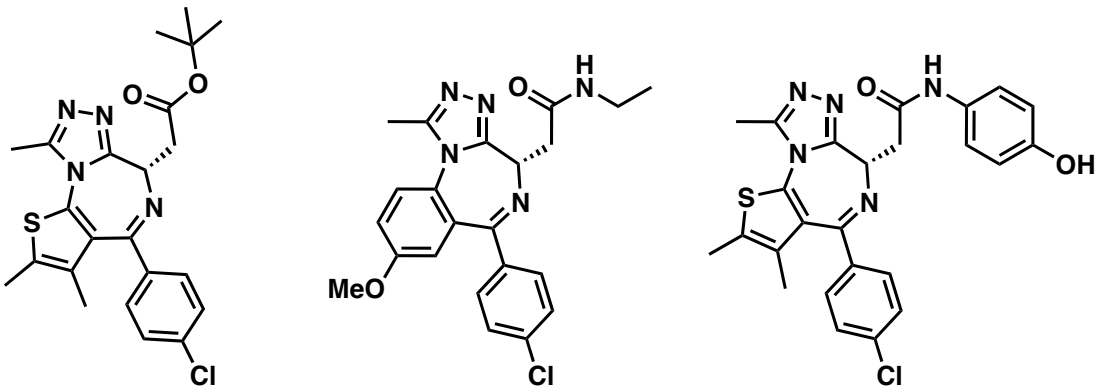
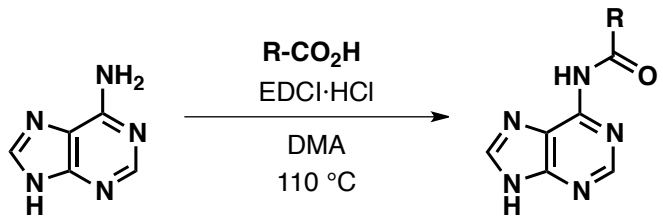


Figure 1. Typical BET inhibitors

To investigate the effect of the benzoyl/heteroarylcarbonyl replacement on BRD4-inhibitory activity, N^6-(heteroarylcarbonyl)adenines were prepared by condensation of adenine with appropriate carboxylic acids as summarized in Scheme 1.

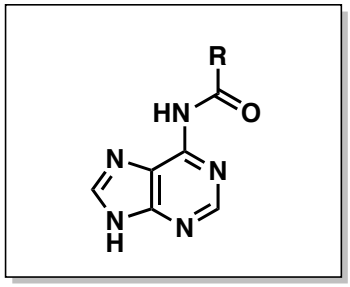


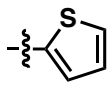
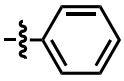
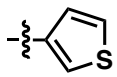
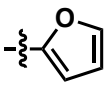
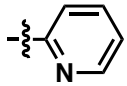
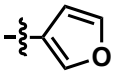
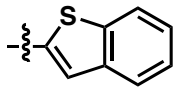
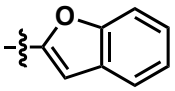
Scheme 1. General synthesis of N^6-(heteroarylcarbonyl)adenines

The BRD4-inhibitory activities of the obtained compounds were evaluated by means of europium-based LANCE TR-FRET (time-resolved fluorescence resonance energy transfer) assay using a BRD4 bromodomain 1 TR-FRET assay kit (No. 600520, Cayman). The BRD4-inhibitory activities of the test compounds are expressed as IC_{50} values, which were determined from the sigmoidal dose–response curves using R software (Tables 1 and 2; for compounds that did not reach 50% inhibition at 100 or 300 μM, the % inhibitions at 100 or 300 μM are shown; N.A. means no activity at 300 μM). Although the values differed from experiment to experiment, the results, including the order of the activities between the test compounds, were basically reproducible. The assay was performed in triplicate, and repeated at lease three times. All data are presented in Table 1, and typical sets of data are presented in Table 2. Basically, as shown in Table 1, the BRD4-inhibitory activities decreased in the following order: 2-thienyl

(**5**) > 3-thienyl (**6**) > 2-furyl (**2**) > 3-furyl (**3**) ≃ phenyl (**1**) ≃ benzothiophen-2-yl (**8**) ≃ benzofuran-2-yl (**4**) > 2-pyridyl (**7**). Compounds **2**, **3**, **5** and **6** which possess electron-donating heteroaryl groups, seem to show slightly higher inhibitory activities than N^6-benzoyladenine (**1**). On the other hand, **7** bearing an electron-withdrawing heteroaryl group, showed weaker activity than **1**. These observations imply the importance of enhanced basicity (or hydrogen bond acceptor ability) of the carbonyl oxygen for the inhibitory activity. Interestingly, compounds **4** and **8**, possessing relatively large heteroaromatic rings, retained the activity. Based on these results, we selected **5** as a lead compound.

Table 1. BRD4-inhibitory activity of N^6-(heteroarylcarbonyl)adenines



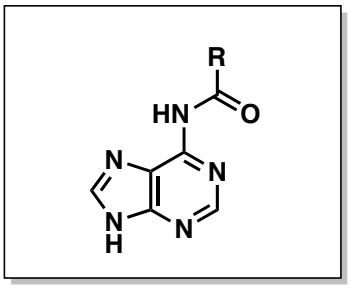
compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
(+)-JQ1	-	0.34-0.82	**5**		84-110 (45-54%)[a]
1		170-190 (26-33%)[a]	**6**		(46%)[a]
2		(24-40%)[a]	**7**		(25%)[a]
3		(30%)[a]	**8**		(38%)[a]
4		(31%)[a]			

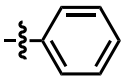
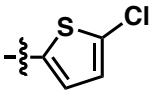
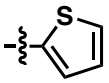
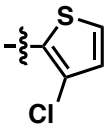
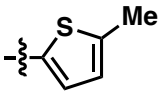
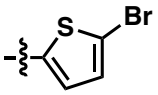
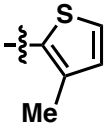
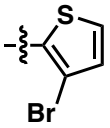
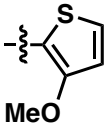
[a] Inhibitory rate at 100 μM is shown in parenthesis.

We next examined substituent effects in the 2-thienyl moiety of **5**. Compounds **9**, **11** and **13** substituted at the 5-position of thiophene showed more potent inhibitory activities than did the corresponding 3-substituted compounds (**10**, **12** and **14**) (Table 2). These results suggest that 5-substitution is preferable to 3-substitution for potent BRD4-inhibitory activity. Moreover, compounds **9** and **10**, which possess electron-donating substituents, showed stronger activities than **11**-**14**, which have electron-withdrawing substituents. This tendency is consistent with the results in Table 1 and the reported SAR of N^6-benzoyladenine derivatives,[14,15] in which introduction of electron-donating substituents (alkoxy and

dimethylamino groups) into the benzoyl group greatly enhances BRD4-inhibitory activity. Overall, our results indicate that electron-donating aromatic or heteroaromatic rings are important for the activity. Among the compounds we synthesized here, N^6-[(3-methoxythiophen-2-yl)carbonyl]adenine (**15**) was the most potent BRD4 inhibitor.

Table 2. BRD4-inhibitory activity of N^6-[(thiophen-2-yl)carbonyl]adenine derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
1		190	**11**		(45%)[a]
5		110	**12**		(25%)[a]
9		67	**13**		(24%)[a]
10		170	**14**		N.A.[b]
			15		28

[a] Inhibitory rate at 300 μM is shown in parenthesis.
[b] N.A. = no activity at 300 μM

In summary, we synthesized N^6-(heteroarylcarbonyl)adenines as candidate BRD4 inhibitors. Among the prepared compounds, N^6-[(3-methoxythiophen-2-yl)carbonyl]adenine (**15**) exhibited the most potent BRD4-inhibitor activity with an IC_{50} value of 28 μM. The SAR data obtained here should be helpful in the further development of N^6-benzoyladenine-based BRD4 inhibitors.

EXPERIMENTAL

General synthetic procedure for N^6-(heteroarylcarbonyl)adenines Heteroaryl carboxylic acid (1.0 eq) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.2 eq) were added to a

suspension of adenine (1.0 eq) in anhydrous DMA, and the mixture was stirred at 110 °C. After completion of the reaction, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$) and by washing with CH_2Cl_2/hexane (2:1 or 1:1) to afford the product. Several by-products arising from EDCI and adenine (but not from carboxylic acid) existed in the crude materials. Therefore, we set up column chromatography several times for each products.

N^6-(2-Furanoyl)adenine (2)

White solid (11%): mp 202.0-203.0 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.60-6.61 (m, 1H), 7.43 (d, *J*=3.5 Hz, 1H), 7.63 (d, *J*=1.7 Hz, 1H), 8.46 (s, 1H), 8.72 (s, 1H); ^{13}C-NMR (500 MHz, $CDCl_3$) δ 113.3, 113.6, 118.4, 143.8, 144.4, 145.9, 146.6, 152.1, 156.7, 160.8; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{10}H_7N_5O_2Na$ 252.0492; found 252.0499.

N^6-(3-Furanoyl)adenine (3)

Pale yellow solid (15%): mp 260.5-261.5 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.09 (s, 1H), 7.84 (s, 1H), 8.61 (s, 1H), 8.65 (s, 1H), 8.73 (s, 1H), 11.45 (brs, 1H); ^{13}C-NMR (500 MHz, DMSO-d_6) δ 110.1, 115.3, 121.9, 145.2, 145.4, 146.1, 148.4, 151.8, 160.6, 161.9; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{10}H_7N_5O_2Na$ 252.0492; found 252.0503.

N^6-[(Benzofuran-2-yl)carbonyl]adenine (4)

White solid (14%): mp 262.5-263.5 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.31 (dd, *J*=6.9, 8.0 Hz, 1H), 7.47 (dd, *J*=7.5, 8.0 Hz, 1H), 7.56 (d, *J*=8.6 Hz, 1H), 7.69 (d, *J*=8.0 Hz, 1H), 7.78 (s, 1H), 8.57 (s, 1H), 8.73 (s, 1H); ^{13}C-NMR (500 MHz, $CDCl_3$) δ 112.1, 113.1, 113.7, 114.7, 123.2, 124.3, 127.0, 128.6, 144.2, 146.0, 146.5, 152.0, 155.5, 157.5; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{14}H_9N_5ONa$ 302.0648; found 302.0667.

N^6-[(Thiophen-2-yl)carbonyl]adenine (5)

White solid (15%): mp 240.0-241.0 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.24-7.26 (m, 1H), 7.98 (d, *J*=5.2 Hz, 1H), 8.31 (d, *J*=4.0 Hz, 1H), 8.57 (s, 1H), 8.73 (s, 1H); ^{13}C-NMR (500 MHz, DMSO-d_6) δ 115.6, 129.2, 132.5, 134.6, 138.4, 145.5, 146.2, 151.7, 160.7, 161.4; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{10}H_7N_5OSNa$ 268.0264; found 268.0258.

N^6-[(Thiophen-3-yl)carbonyl]adenine (6)

White solid (16%): mp 239.0-240.0 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.67-7.68 (m, 1H), 7.73-7.74 (m, 1H), 8.45 (s, 1H), 8.65 (s, 1H), 8.69 (s, 1H), 11.37 (brs, 1H), 12.32 (brs, 1H); ^{13}C-NMR (500 MHz, DMSO-d_6) δ 114.3, 127.3, 127.6, 132.4, 135.7, 144.4, 146.0, 151.1, 161.4, 162.1; ESI-TOF-HRMS $(M-H)^-$ calculated for $C_{10}H_6N_5OS$ 244.0288; found 244.0278.

N^6-(2-Pyridinoyl)adenine (7)

Yellow solid (7%): mp 273.5-274.5 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.54-7.57 (m, 1H), 7.92-7.95 (m, 1H), 8.26 (d, *J*=8.0 Hz, 1H), 8.65 (d, *J*=4.6 Hz, 1H), 8.70 (s, 1H), 8.76 (s, 1H); ^{13}C-NMR (500 MHz, $CDCl_3$) δ 113.4, 123.3, 128.2, 138.2, 144.1, 144.3, 147.3, 148.8, 152.8, 157.7, 163.6; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{11}H_8N_6ONa$ 263.0652; found 263.0641.

N^6-[(Benzothiophen-2-yl)carbonyl]adenine (8)

Pale yellow solid (3%): mp 292.0-293.0 ºC; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.43 (dd, *J*=6.9, 7.5 Hz, 1H), 7.48 (dd, *J*=7.4, 7.5 Hz, 1H), 7.87 (d, *J*=8.0 Hz, 1H), 7.94 (d, *J*=8.1 Hz, 1H), 8.38 (s, 1H), 8.76 (s, 1H), 8.80 (s, 1H); ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{14}H_9N_5OSNa$ 318.0420; found 318.0411.

N^6-[(5-Methylthiophen-2-yl)carbonyl]adenine (9)

White solid (3%): mp 258.0-259.0 ºC; ^{1}H-NMR (500 MHz, CD_3OD) δ 2.45 (s, 3H), 6.85 (d, *J*=4.0 Hz, 1H), 7.86 (d, *J*=4.0 Hz, 1H), 8.52 (s, 1H), 8.71 (s, 1H); ESI-TOF-HRMS $(M-H)^-$ calculated for $C_{11}H_8N_5OS$ 258.0444; found 258.0435.

N^6-[(3-Methylthiophen-2-yl)carbonyl]adenine (10)

White solid (24%): mp 225.5-226.5 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2.50 (s, 3H), 7.04 (d, *J*=5.2 Hz, 1H), 7.75 (s, 1H), 8.45 (s, 1H), 8.65 (s, 1H), 11.12 (s, 1H), 12.35 (s, 1H); ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{11}H_9N_5OSNa$ 282.0420; found 282.0416.

N^6-[(5-Chlorothiophen-2-yl)carbonyl]adenine (11)

Pale yellow solid (6%): mp 272.0-273.0 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.30 (d, *J*=4.0 Hz, 1H), 8.18 (d, *J*=4.0 Hz, 1H), 8.61 (s, 1H), 8.73 (s, 1H); ^{13}C-NMR (500 MHz, DMSO-d_6) δ 115.8, 129.4, 132.5, 136.5, 137.6, 145.4, 146.2, 151.6, 160.3, 160.5; ESI-TOF-HRMS $(M-H)^-$ calculated for $C_{10}H_5ClN_5OS$ 277.9898; found 277.9896.

N^6-[(3-Chlorothiophen-2-yl)carbonyl]adenine (12)

White solid (12%): mp 277.5-278.5 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.08 (d, *J*=5.2 Hz, 1H), 7.68 (d, *J*=5.2 Hz, 1H), 8.75 (s, 1H), 8.90 (s, 1H); ESI-TOF-HRMS $(M-H)^-$ calculated for $C_{10}H_5ClN_5OS$ 277.9898; found 277.9894.

N^6-[(5-Bromothiophen-2-yl)carbonyl]adenine (13)

Pale yellow solid (4%): mp 275.5-276.5 ºC; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.16 (d, *J*=4.6 Hz, 1H), 7.84 (d, *J*=4.1 Hz, 1H), 8.57 (s, 1H), 8.73 (s, 1H); ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{10}H_6BrN_5OSNa$ 347.9348; found 347.9351.

N^6-[(3-Bromothiophen-2-yl)carbonyl]adenine (14)

Pale yellow solid (9%): mp 253.0-254.0 ºC; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.18 (d, *J*=5.8 Hz, 1H), 7.72 (d, *J*=5.2 Hz, 1H), 8.68 (s, 1H), 8.76 (s, 1H); ^{13}C-NMR (500 MHz, CD_3OD) δ 113.6, 113.9, 131.6, 133.5, 133.9, 144.3, 145.3, 152.7, 156.2, 160.1; ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{10}H_6BrN_5OSNa$ 347.9348; found 347.9145.

***N*^{6}-[(3-Methoxythiophen-2-yl)carbonyl]adenine (15)**

Pale yellow solid (7%): mp 209.0-210.0 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 4.13 (s, 3H), 7.27 (d, *J*=5.8 Hz, 1H), 8.00 (d, *J*=5.7 Hz, 1H), 8.42 (s, 1H), 8.62 (s, 1H), 9.92 (brs, 1H), 12.40 (brs, 1H); ESI-TOF-HRMS $(M+Na)^+$ calculated for $C_{11}H_9N_5O_2SNa$ 298.0369; found 298.0359.

BRD4 bromodomain 1 TR-FRET assay BRD4-inhibitory activity was evaluated by means of europium-based LANCE TR-FRET (time-resolved fluorescence resonance energy transfer) assay using a BRD4 bromodomain 1 TR-FRET assay kit (No. 600520, Cayman). (+)-JQ1 (No. 11187, Cayman) was used as a positive control. Plates were read with an Envision (PerkinElmer) in the time-resolved format (excitation at 320 nm, emissions at 615 and 665 nm), using a 100 μs delay and a 400 μs reading window. Data analysis was performed using the TR-FRET ratio (665 nm emission/615 nm emission).

ACKNOWLEDGEMENTS

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