Synthesis and Biological Activity of 9-(2,6-Difluorobenzyl)-9H-purines Bearing Chlorine

Shigetada KOZAI and Tokumi MARUYAMA*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University Yamashiro-cho, Tokushima 770–8514, Japan.
Received November 13, 1998; accepted January 13, 1999

Mitsunobu reaction of 2,6-dichloropurine with 2,6-difluorobenzyl alcohol gave 2,6-dichloro-9-(2,6-difluorobenzyl)-9H-purine and 7-benzylated congener in 73% and 10% yield, respectively. Treatment of the former compound with ammonia gave 2-chloro-9-(2,6-difluorobenzyl)adenine (**1**) in good yield. Also, 2-amino-6-chloropurine was condensed with 2,6-difluorobenzyl alcohol in a similar manner to afford 2-amino-6-chloro-9-(2,6-difluorobenzyl)-9H-purine (**2**) as a major product. Inhibition of phosphodiesterase (PDE) isozymes by 9-(2,6-difluorobenzyl)-9H-purines was investigated and both **1** and **2** were found to be good inhibitors of PDE2 in addition to PDE4.

Key words Mitsunobu reaction; 9-benzylpurine; phosphodiesterase inhibitor; phosphodiesterase isozyme; nucleobase

Phosphodiesterases (PDEs) are enzymes which catalyze the hydrolysis of 3',5'-cyclic nucleotides cAMP and cGMP to their 5'-monophosphates. PDEs are classified into seven families, five of which, PDE1–PDE5, are characterized.¹⁾ For instance, PDE4 specifically hydrolyzes cAMP and exerts antiinflammatory and tracheal relaxant activity. Recently, Bourguignon *et al.*²⁾ reported 9-benzyladenines as potent and selective PDE inhibitors, in which 9-(2-fluorobenzyl)-N⁶-methyladenine (BWA 78U)³⁾ and its 2-trifluoromethyl congener (NCS 613)²⁾ were found to be selective inhibitors of PDE4. However, purines bearing halogen at nucleobase have not been examined for their inhibition of PDEs in the literature. Because biological activities of 6-chloropurine and 2-chloropurine nucleosides^{4,5)} have been reported, we are interested in the activity of purine analogs bearing chlorine at the base moiety. 2,6-Difluorobenzyl group was introduced in the anticonvulsant agent (534U87),⁶⁾ so this group was chosen as a substituent at N9 of purine. In this paper, synthesis of 9-(2,6-difluorobenzyl)-9H-purine analogs bearing chlorine at the base moiety and their inhibition of PDEs are described.

Synthesis

Toyota *et al.*⁷⁾ reported alkylation of 6-chloropurine with alcohols by Mitsunobu reaction⁸⁾ and described the preparation of 9-alkylated adenines. We adopted this method for the synthesis of 2-substituted 9-(2,6-difluorobenzyl)-9H-purines. Thus, 2,6-dichloropurine was treated with 2,6-difluorobenzyl alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate in dry THF at 50 °C for two days. After work-up of the mixture, two products were obtained following separation by silica gel chromatography. Compound **1a** was obtained from the first fraction as white crystals in 73% yield and showed similar UV spectrum to that of 2,6-dichloropurine, indicating alkylation at N9. Therefore,

the structure was determined to be 2,6-dichloro-9-(2,6-difluorobenzyl)-9H-purine together with elemental analysis and other spectroscopic data. 2,6-Dichloro-7-(2,6-difluorobenzyl)-7H-purine (**1b**) was obtained from the second fraction in 10% yield, showing absorption maximum at 282 nm in the UV spectrum.⁹⁾ 2-Amino-6-chloropurine was also treated with 2,6-difluorobenzyl alcohol in a manner similar to that of **1a** to give two products **2a,b** in 54% and 13.4% yield, respectively. Treatment of **1a** with ammonia in methanol (saturated at 0 °C) gave 2-chloro-9-(2,6-difluorobenzyl)adenine (**3**) in good yield.¹⁰⁾ 6-Chloro-9-(2,6-difluorobenzyl)-9H-purine (**4**) was prepared according to the previously published method.¹¹⁾

Biological Results and Discussion

Inhibition of PDE1–PDE5 by 9-(2,6-difluorobenzyl)-purines bearing chlorine was evaluated according to the methods in the indicated references.^{12–14)} As shown in the Table, 6-chloro-9-(2,6-difluorobenzyl)-9H-purine (**4**) exhibited weak *in vitro* activity for PDE2 (IC₅₀ = 10.2 μM). An indication of inhibition was also observed for PDE1, but no activity was noted for PDE3, PDE4 or PDE5. This is the first case to inhibit PDEs by purines without 6-amino or amidine group. In the case of 2-amino-6-chloro-9-(2,6-difluoroben-

Chart 1

Chart 2

* To whom correspondence should be addressed.

Table 1. Inhibition of PDEs by 9-(2,6-Difluorobenzyl)-9H-purines

Name	IC ₅₀ (μM)				
	PDE1	PDE2	PDE3	PDE4	PDE5
2a	80.4	5.9	ns	1.4	ns
3	49.6	0.72	93.5	1.7	ns
4	82	10.2	ns	ns	ns

ns: IC₅₀ > 100 μM.

zyl)-9H-purine (**2a**), inhibition of PDE2 (IC₅₀ = 5.9 μM) was similar to **4**. However, compound **2a** also showed strong inhibition of PDE4 (IC₅₀ = 1.4 μM). It appeared that the introduction of amino group to 2-carbon of the purine caused marked inhibition of PDE4 in addition to PDE2. We are interested in the tendency that substitution at both 2- and 6-carbon causes inhibition of two PDE isozymes, PDE2 and PDE4. Thus, a regioisomer of **2a**, 2-chloro-9-(2,6-difluorobenzyl)adenine (**3**) was prepared and evaluated for PDEs. Compound **3** showed more potent activity (IC₅₀ = 0.72 μM) than **2a** for PDE2 and similar activity for PDE4 (IC₅₀ = 1.7 μM). The biological profile of **2a** and **3** suggest that amino function at 6-carbon is not essential to suppress the PDE isozymes that hydrolyze cAMP. NCS 613 was found to be highly selective in inhibiting the PDE4 since IC₅₀ values for PDE1, PDE3, PDE4 and PDE5 were 40, 380, 0.04, and 5, respectively.²⁾ It should be noted that both **2a** and **3** strongly inhibited PDE2 as well as PDE4, so that inhibition of PDE2 should be evaluated in choosing the selective inhibitor for PDE4.

Experimental

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low resolution mass spectra were obtained on a Shimadzu-LKB 9000B mass spectrometer in the direct-inlet mode. High resolution mass spectra were obtained on a JMS AX-500 spectrometer in the direct-inlet mode. ¹H-NMR spectra were recorded on either Varian UNITY 200 (200 MHz) or Varian UNITY 600 (600 MHz) in CDCl₃ (or dimethyl sulfoxide (DMSO)-*d*₆) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with Silica gel 60 containing fluorescent indicator F₂₅₄ were used for thin-layer chromatography and Silica gel 60 (Merck 7734, 60–200 mesh) was employed for column chromatography.

Condensation of 2,6-Dichloropurine with 2,6-Difluorobenzyl Alcohol by Mitsunobu Reaction To a mixture of 2,6-difluorobenzyl alcohol (2.24 ml, 20 mmol) and 2,6-dichloropurine (1.89 g, 10 mmol) in dry tetrahydrofuran (THF) (150 ml) was added triphenylphosphine (5.3 g, 20 mmol) and diisopropyl azodicarboxylate (4.3 ml, 20 mmol) and the solution was stirred at 50 °C for 2 d, then concentrated to a small volume. The residual solution was chromatographed over a column of Silica gel G (3×50 cm) using a 0–67% AcOEt in benzene (4 l). The first fraction was evaporated to give a solid, which was recrystallized from EtOH to afford 2,6-dichloro-9-(2,6-difluorobenzyl)-9H-purine (**1a**) as white crystals (2.30 g, 73%), mp 123 °C. ¹H-NMR (CDCl₃) δ: 8.11 (1H, s, H8), 7.36–7.41 (1H, m, H4'), 6.96–7.04 (2H, m, H3', H5'), 5.51 (2H, s, –CH₂–). UV λ_{max} MeOH nm: 274. MS *m/z*: 314, 316, 318 (M⁺). Anal. Calcd for C₁₂H₆Cl₂F₂N₄: C, 45.74; H, 1.92; N, 17.78. Found: C, 45.43; H, 1.91; N, 17.65. From the second fraction 2,6-dichloro-7-(2,6-difluorobenzyl)-7H-purine (**1b**) was obtained as white crystals (0.32 g, 10%), mp 167–169 °C. ¹H-NMR (CDCl₃) δ: 8.22 (1H, s, H8), 7.37–7.52 (1H, m, H4'), 6.97–7.09 (2H, m, H3', H5'), 5.78 (2H, s, –CH₂–). UV λ_{max} MeOH nm: 282. MS *m/z*: 314, 316, 318 (M⁺).

Anal. Calcd for C₁₂H₆Cl₂F₂N₄: C, 45.74; H, 1.92; N, 17.78. Found: C, 45.88; H, 1.71; N, 17.79.

Condensation of 2-Amino-6-chloropurine with 2,6-Difluorobenzyl Alcohol by Mitsunobu Reaction To a mixture of 2,6-difluorobenzyl alcohol (2.24 ml, 20 mmol) and 2-amino-6-chloropurine (1.70 g, 10 mmol) in dry THF (150 ml) was added triphenylphosphine (5.3 g, 20 mmol) and diisopropyl azodicarboxylate (4.3 ml, 20 mmol) and the solution was stirred at 50 °C for 2 d, then concentrated to a small volume. The residual solution was chromatographed over a column of Silica gel G (3×50 cm) using 0–67% AcOEt in benzene (4 l) and 10% EtOH in CHCl₃ (1 l). The first fraction was evaporated to dryness and the residue was crystallized from EtOH to afford 2-amino-6-chloro-9-(2,6-difluorobenzyl)-9H-purine (**2a**) as white crystals (1.60 g, 54%), mp 207–208 °C. ¹H-NMR (DMSO-*d*₆) δ: 8.10 (1H, s, H8), 7.45–7.53 (1H, m, H4'), 7.12–7.20 (2H, m, H3', H5'), 6.92 (2H, br s, NH₂), 5.36 (2H, s, –CH₂–). UV λ_{max} MeOH nm: 310. MS *m/z*: 295, 297 (M⁺). Anal. Calcd for C₁₂H₈ClF₂N₅: C, 48.75; H, 2.73; N, 23.69. Found: C, 49.0; H, 2.66; N, 23.44. From the second fraction 2-amino-6-chloro-7-(2,6-difluorobenzyl)-7H-purine (**2b**) was obtained as pale yellowish crystals (0.397 g, 13.4%), 230 °C (dec.). ¹H-NMR (DMSO-*d*₆) δ: 8.43 (1H, s, H8), 7.41–7.56 (1H, m, H4'), 7.10–7.20 (2H, m, H3', H5'), 6.66 (2H, br s, NH₂), 5.68 (2H, s, –CH₂–). UV λ_{max} MeOH nm: 323. MS *m/z*: 295, 297 (M⁺). Anal. Calcd for C₁₂H₈ClF₂N₅: C, 48.75; H, 2.73; N, 23.69. Found: C, 48.73; H, 2.73; N, 23.65.

2-Chloro-9-(2,6-difluorobenzyl)adenine (3) 2,6-Dichloro-9-(2,6-difluorobenzyl)-9H-purine (200 mg, 0.63 mmol) was suspended in methanol (10 ml) saturated with ammonia at 0 °C and the solution was kept at 80 °C for 1 d in a sealed tube. Concentration of the solution gave white crystals (0.17 g, 91%), mp 250–252 °C. ¹H-NMR (DMSO-*d*₆) δ: 8.12 (1H, s, H8), 7.75 (2H, br s, NH₂), 7.42–7.50 (1H, m, H4'), 7.09–7.17 (2H, m, H3', H5'), 5.38 (2H, s, –CH₂–). UV λ_{max} MeOH nm: 266. MS *m/z*: 295, 297 (M⁺). Anal. Calcd for C₁₂H₈ClF₂N₅: C, 48.75; H, 2.73; N, 23.69. Found: C, 48.66; H, 2.77; N, 23.73.

References and Notes

- Beavo J. A., *Physiol. Rev.*, **75**, 725–748 (1995).
- Bourguignon J.-J., Desaubry L., Raboisson P., Wermuth C.-G., Lugnier C., *J. Med. Chem.*, **40**, 1768–1770 (1997).
- Kelley J. L., Sokoro F. E., *J. Med. Chem.*, **29**, 1133–1134 (1986).
- a) Honjo M., Maruyama T., Horikawa M., Balzarini J., De Clercq E., *Chem. Pharm. Bull.*, **35**, 3227–3234 (1987); b) Maruyama T., Sato Y., Oto Y., Takahashi Y., Snoeck R., Andrei G., Witvrouw M., De Clercq E., *Chem. Pharm. Bull.*, **44**, 2331–2334 (1996).
- a) Carson D. A., Wasson D. B., Esparza L. M., Carrera C. J., Kipps T. J., Cottam H. B., *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 2970–2974 (1992); b) Secrist J. A. III, Shortnacy A. T., Montgomery J. A., *J. Med. Chem.*, **31**, 405–410 (1988); c) Christensen L. F., Broom A. D., *J. Med. Chem.*, **15**, 735–739 (1972); d) Angus J. A., Cobbin L. B., Einstein R., Maguire M. H., *Br. J. Pharmacol.*, **41**, 592–599 (1971).
- Kelley J. L., Koble C. S., Davis R. G., McLean E. W., Soroko F. E., Cooper B. R., *J. Med. Chem.*, **38**, 4131–4134 (1995).
- Toyota A., Katagiri N., Kaneko C., *Chem. Pharm. Bull.*, **40**, 1039–1041 (1992).
- Mitsunobu O., Yamada M., *Bull. Chem. Soc. Jpn.*, **40**, 2380–2382 (1967).
- a) Montgomery J. A., Temple, Jr. C., *J. Am. Chem. Soc.*, **83**, 630–635 (1961); b) Townsend L. B., Robins R. K., Loeppky R. N., Leonard N. J., *J. Am. Chem. Soc.*, **86**, 5320–5325 (1964).
- Schaeffer H. J., Thomas H. J., *J. Am. Chem. Soc.*, **80**, 3738–3742 (1958).
- Maruyama T., Kozai S., Uchida M., *Nucleosides & Nucleotides*, in press.
- Beavo J. A., Reifsnnyder D. H., *Trends Pharmacol. Sci.*, **11**, 150–155 (1990).
- Hidaka H., Asano T., *Biochim. Biophys. Acta*, **429**, 485–497 (1976).
- Nicholson C. D., Chaliss R. A., Shalid M., *Trends Pharmacol. Sci.*, **12**, 19–27 (1991).