The Potential of Aspirin in Prodrug Synthesis: A New Potential Delivery System of AZT and FLT

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Summary

Aspirin (*O*-acetylsalicylic acid) has been used to synthesize prodrugs of 3′-azido-3′-deoxythymidine (AZT) and 3′-deoxy-3′-fluorothymidine (FLT). The mixed anhydride between aspirin and trifluoroacetic acid was synthesized and reacted with AZT and FLT to give the blocked nucleosides attached through the 5′-*O* position to the 2-position of 2-methyl-4*H*-1,3-benzodioxin-4-one. The prodrugs showed the same activities against HIV-1 in MT-4 cells as the original drugs. Hydrolysis of the synthesized prodrugs in the growth medium, used for anti-HIV investigations, resulted in formation of 5-*O* acetylated drugs which were subsequently hydrolyzed into the original drugs.

Introduction

A site specific delivery system designed for the central nervous system (CNS) would be of great importance. It is well known that many of the pharmacologically active and important agents, including endogenous neurotransmitters, cannot be transported from the blood into the brain due to the presence of a set of specialized barriers at the blood-brain interface. This barrier system is generally called the blood-brain barrier (BBB), and is composed of a set of anatomical and enzymatic components.[1]

A general method which can be applied to the delivery of drugs to the brain is the prodrug approach.[2] The term prodrug was coined by Albert[3] and refers to the result of a transient chemical modification of a pharmacologically active agent. This change imparts to the compound an improvement in some deficient physicochemical property such as water solubility or membrane permeability. Ideally, a prodrug is biologically inactive, but transformed into the parent compound *in vivo*. This transformation can be mediated by an enzyme or may occur chemically due to some designed instability in the agent. The aim of these manipulations is to increase the concentration of the active agent at its site of action, thereby increasing its efficacy. While potentially there are many different types of pro-derivatives, most of the synthesized ones are simple esters or amides. These compounds are transformed to the parent acid, alcohol or amine by the ubiquitous hydrolase which are present *in vivo*. By temporarily masking the polar groups of a drug, the lipophilicity of a drug is increased and its ability to pass membranes is enhanced.[4] On the other hand, it has become evident that not only 3′-azido-3′-deoxythymidine (AZT), but also 3′-deoxy-3′-fluorothymidine (FLT) are compatible with anti HIV activity.[5] A series of prodrugs of zidovudine (AZT) has been synthesized in an effort to enhance the uptake of the prodrugs by the HIV-1 infected cells.[6]

In our search for a new type of prodrugs of AZT and FLT, we have noticed in the literature that salicyl chloride, when used as a chlorodehydroxylation reagent[7,8] and for 2,2′-anhydro formation[9,10] in nucleosides, could at the same time block at the 5′-*O* with a bond to the 2-position to a 2-methyl-4*H*-1,3-benzodioxin-4-one moiety. In the present paper we want to utilize this finding through the synthesis of more lipid soluble prodrugs using *O*-acetylsalicylic acid (the common aspirin) as masking agent for the anti-HIV drugs AZT and FLT. We thought it advantageous to use a prodrug system where the possible degradation products salicylic acid and/or *O*-acetylsalicylic acid, have well established pharmacologic, pharmacokinetic and biochemical profiles.[11–18]

Chemistry

It was reported by *Brinkman* and *Rüchardt*[19] that *O*-acetylsalicyl chloride exists in an equilibrium mixture with the corresponding cyclic benzodioxinone structure and 2-alkoxy-2-methyl-4H-1,3-benzodioxin-4-one derivatives were isolated as the main products when a solution of *O*-acetylsalicyl chloride and alcohol (or phenol) was heated in tetrahydrofuran (THF) in the presence of a base.[20] Accordingly, *O*-acetylsalicylic acid (**1**) was allowed to react with trifluoroacetic anhydride in carefully dried ethanol-free chloroform to give the mixed anhydride **2** which was allowed to react with AZT[21] (**3a**) and FLT[22] (**3b**) at ambient temperature to give the corresponding orthoester **4a**, and **4b,** respectively, in a moderate yield. The assignment of the structures was established on the basis of its characteristic IR, ^{1}H- and ^{13}C-NMR and mass spectral signals.[21,23] The attempts of reacting 2′,3′-didehydro-3′-deoxythymidine[24] (D4T) and commercially available 2′,3′-dideoxyadenosine (DDA) in the same manner as AZT and FLT failed since both D4T and DDA were insoluble in chloroform. To overcome the solubility problem, it was attempted to use DMSO and DMF as solvents, but only very little conversion was observed in both cases, because these highly hygroscopic solvents, although carefully dried, still may contain water which is detrimental for the mixed anhydride **2**.

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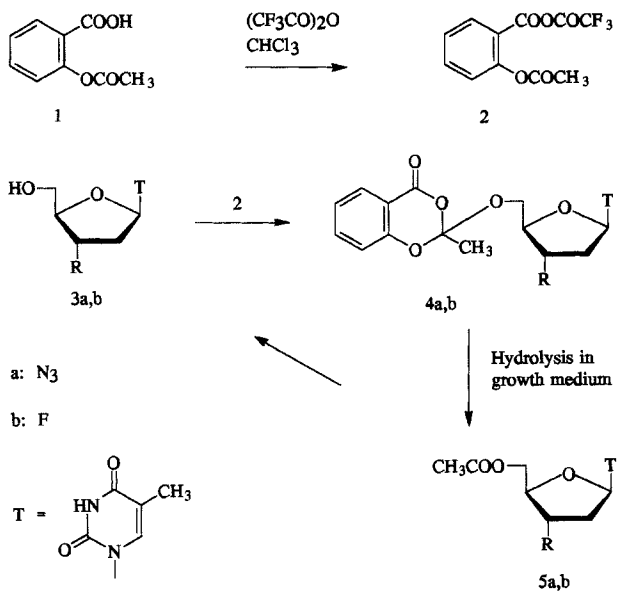

Biological Properties and Discussion

The primary objective of this project was to design and synthesize aspirin prodrugs of AZT and FLT from aspirin. In Table 1 it is shown that the activities against HIV-1 in MT-4 cells are maintained for the new potential prodrugs when compared with the original drugs.

Table 1. Antiviral activity of prodrugs **4a,b**, AZT and FLT against HIV-1 in MT-4 cells.

compd	ED_{50},[a] μM	CD_{50},[b] μM
4a	0.005	36
4b	0.002	100
AZT	0.003	52
FLT	0.003	100

[a] Effective dose of compound, achieving 50% inhibition of HIV-1 antigen production in MT-4 cultures. [b] Cytotoxic dose of compound, required to reduce the proliferation of normal uninfected MT-4 cells by 50 %

One could speculate whether the maintained activity of the prodrugs **4a,b** might be due to an immediate hydrolysis of the 1,3-benzodioxin-4-one ring to give the original drugs. However, hydrolysis of these compounds at 37 °C in the same growth medium, as used for determination of the antiviral activity, showed resistance of the prodrugs to hydrolysis for almost 1 h. The FLT prodrug **4b** was dissolved and hydrolyzed and after 1 h, the HPLC analysis showed the appearance of the hydrolyzed product **5b** which was identified as 5′-*O*-acetyl-FLT by mass spectrometry of the proper fraction from HPLC. An authentic sample prepared by acetylation of FLT with acetic anhydride in dry pyridine showed the same mass spectrum as **5b**. After 3 h, the recorded chart showed the appearance of FLT. The disappearance of the prodrug **4b** and the formation of the intermediate **5b** as well as the formation of the end product FLT is shown in Fig 1. The formation of FLT was completed after 44 h. The prodrug **4a** was submitted to the same conditions as mentioned for **4b**. After 1 h, HPLC analysis showed the appearance of an intermediate which is believed to be **5a**. After 16 h, AZT appeared and within 30 h the prodrug **4a** was consumed with formation of **5a** and AZT. After 46 h, only AZT could be observed by HPLC analysis.

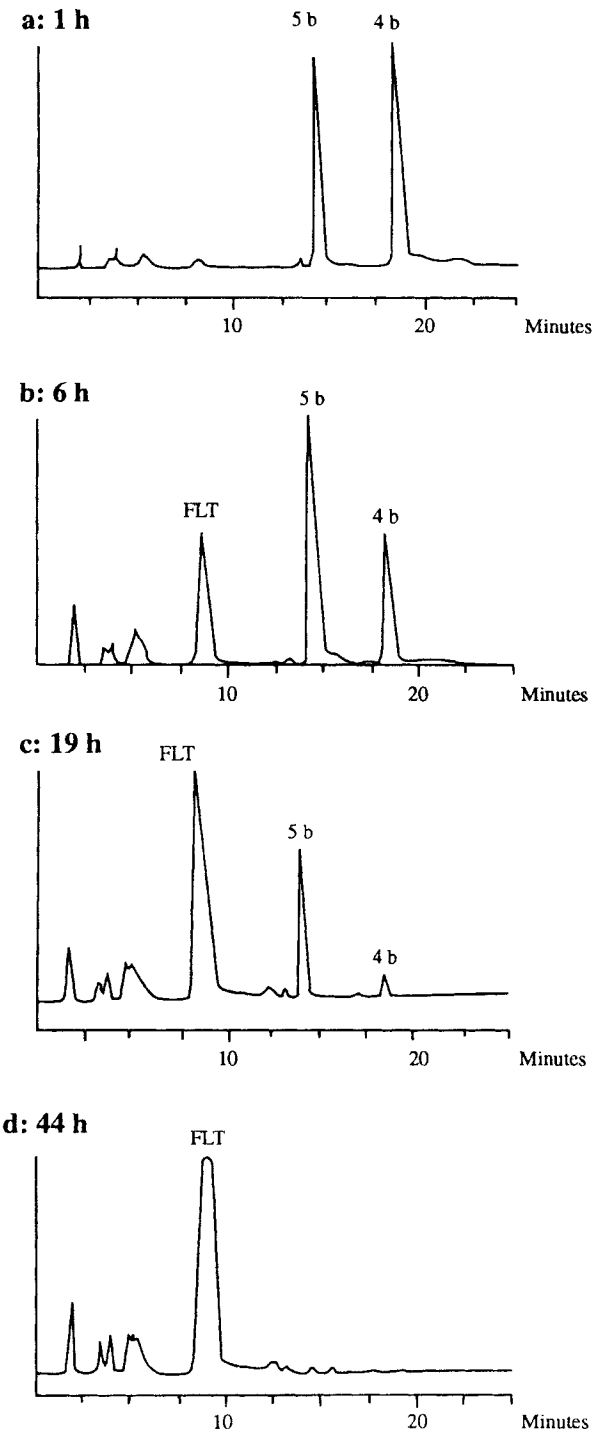

Figure 1. GLC diagrams showing hydrolysis of the FLT prodrug **4b** into FLT (**3b**) *via* its corresponding 5-*O*-acetyl derivative **5b** in the growth medium RPMI 1640.

2-Alkoxy(aryloxy)-2-methyl-4*H*-1,3-benzodioxin-4-ones (aspirin prodrugs) have been prepared by a number of authors for different purposes. *Rüchardt* and *Rochlitz*[20] prepared a number of these compounds, but they did not investigate their hydrolysis. *Paris et al.*[25] synthesized a series of cyclic aspirin triglycerides and found that the plasma salicylate level obtained after 5 h was 70 % that of aspirin at the same molar dose. *Senning* and coworkers,[23,26–31] however, found in enzymatic and non-enzymatic hydrolysis experiments that 2-alkoxy(aryloxy)-2-methyl-4*H*-1,3-benzodioxin-4-ones in most cases produced salicylic acid instead of aspirin which was only produced when the substituents were properly selected. The alkoxy part was not identified in their experiments. Our observation of **5** (the acetylated derivatives of AZT and FLT), as the primary hydrolysis product, confirm a direct formation of salicylic acid from the so called aspirin prodrug without intermediate formation of aspirin.

Experimental Section

^{1}H- and ^{13}C-NMR spectra were recorded at 250 MHz on Bruker AC 250 FT using tetramethylsilane as the internal reference. EI-mass spectra were recorded on Varian MAT 311 A. IR-spectra were recorded on Perkin-Elmer Model 1720 FTIR. Silica gel plates (Merck F 254) were used for thin-layer chromatography. The compounds were detected by visual examination under short- and long-wavelength UV light. The HPLC were recorded on Waters Delta Prep 3000 Preparative Chromatography Systems using reversed phase Delta Pak C18, 15 µ.

General Procedure for the Synthesis of 2-Substituted 2-Methyl-4H-1,3-benzodioxin-4-ones **4a,b**

O-Acetylsalicylic acid (0.54 g, 3 mmol) was suspended in dry ethanol-free chloroform (chloroform was filtered through neutral Al_2O_3, activity grade I). The flask was closed with a septum, flushed with nitrogen, the trifluoroacetic anhydride (0.42 ml, 0.3 mmol) added and the suspension was heated at 45 °C for 2 min until a clear solution was obtained. The reaction mixture was allowed to cool with stirring to room temperature (30 min). The appropriate nucleoside (2 mmol), dissolved in chloroform, was added via a syringe to the mixture containing the mixed anhydride **3**. The mixture was stirred at room temperature for 2 h in the case of **4a** and overnight in the case of **4b**. The mixture was cooled to 0 °C and sat. aq. $NaHCO_3$ was added. After extraction with (3 × 30 ml) chloroform, drying over Na_2SO_4 and evaporation of the chloroform, **4** was obtained as an oil.

4a: Oil, 801 mg, purified by chromatography (Merck silica, 230–400 mesh, pet. ether: ethylacetate *v:v* = 1:1) to give **4a** as amorphous foam. Yield 480 mg; 56 %. ^{1}H-NMR ($CDCl_3$) two diastereomers: δ (ppm) = 1.89/1.90 (2 × s, 3H, 5-CH_3), 1.98 (s, 3H, 2″-CH_3), 2.02–2.38 (m, 2H, H-2′), 3.79–3.99 (m, 2H, H-3′, H-4′), 4.02–4.15 (m, 2H, H-5′), 6.16 (m, 1H, H-1′), 7.05 (t, 1H, H-6″), 7.23 (m, 1H, H-7″), 7.35 (2 × s, 1H, H-6), 7,65 (m, 1H, H-8″), 7.98 (m, 1H, H-5″), 9.40 (bs, 1H, NH).– ^{13}C-NMR ($CDCl_3$) two stereoisomers: δ (ppm) = 12.34/12.39 (5-CH_3), 22.60/22.76 (2″-CH_3), 37.39/37.49 (C-2′), 60.37/60.55 (C-3′), 63.41/63.46 (C-5′), 82.00/82.10 (C-4′), 84.63/84.92 (C-1′), 111.03/111.41 (C-2″), 112.71/113.00 (C-5), 116.67/116.73 (C-8″), 116.88/117.00 (C-4″a), 123.57/123.64 (C-6″), 129.52 (C-5″), 124.89/135.01 (C-7″), 136.77/136.80 (C-6), 150.13/150.22 (C-2), 154.07/154.53 (C-8″a), 159.80/159.82 (C-4″), 163.65/163.70 (C-4).– FAB MS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 430 ($M+H^+$).

4b: 0.861 g oil was purified by chromatography (Merck silica, 230–400 mesh, methanol: chloroform *v:v* = 1:99) to give **4b** as a foam. Yield 440 mg (54 %). ^{1}H-NMR ($CDCl_3$) two diasteromers: δ (ppm) = 1.88/1.89 (2 × s, 3H, 5-CH_3), 1.99 (s, 3H, 2″-CH_3), 2.43 (m, 2H, H-2′), 3.80–4.10 (m, 2H, H-5′), 4.33/4.39 (4 × m, $J_{F,4'-H}$ = 27 Hz, 1H, H-4′), 4.68/5.05 (4 × m, $J_{F,3'-H}$ = 53.5 Hz, 1H, H-3′), 6.39 (m, 1H, H-1′), 7.18 (m, 2H, H-6″and H-7″), 7.44 (s, 1H, H-6), 7.61 (m, 1H, H-8″), 7.99 (m, 1H, H-5″), 9.03 (bs, 1H, NH).– ^{13}C-NMR ($CDCl_3$) two diastereomers: δ (ppm) = 12.39/12.44 (5-CH_3), 22.57/22.70 (C-9″), 38.16 (d, $J_{F,C-2'}$ = 21.2 Hz, C-2′), 63.75/63.93, 63.86/64.04 (d, $J_{F,C-5'}$ = 11.3 Hz, C-5′), 82.44/82.86, 82.61/83.03 (d, $J_{F,C-4'}$ = 26.4 Hz, C-4′), 84.70/85.01 (C-1′), 92.57/95.41, 92.67/95.51 (d, $J_{F,C-3'}$ = 178.6 Hz, C-3′), 111.30/111.71 (C-2″), 112.79/112.99 (C-5), 116.59/116.74 (C-8″), 116.85/116.96 (C-4″a), 123.73/123.76 (C-6″), 129.59 (C-5″), 134.73/134.77 (C-7″), 136.89/136.94 (C-6), 150.15/150.28 (C-2), 154.06/154.54 (C-8″a), 159.31/159.38 (C-4″), 163.47/163.53 (C-4).– FAB MS (DMSO + 3-nitrobenzylalcohol): *m/z* = 407 ($M + H^+$).

Hydrolysis of 4a,b. 1 mg of **4a** or **4b** was dissolved in 1 ml of growth medium [RPMI 1640 cat. No. 61870-010 Life Technologies Inc.] with stirring at 37 °C. Samples from the obtained clear solution were analyzed with reversed phase HPLC with 10 % ethanol in water.

Virus and Cells. The HIV-1 strain HTLV-IIIB[32] was propagated in H9 cells[33] at 37 °C, 5 % CO_2 using RPMI 1640 with 10 % heat-inactivated Fetal Calf Serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquotted, and stored at –80 °C until use.

Inhibition of HIV-1 Replication. Compounds were examined for possible antiviral activity against HIV-1 using MT-4 cells as target cells. For screening studies MT-4 cells were incubated with virus (0.005 MOI) for 2 h, washed, and thereafter added in a proportion of 1:10 to uninfected cells, which had been preincubated in growth medium containing the test compound for 2 h. Cultures were maintained with the test compound for 6 days in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantitated by HIV-1 antigen detection ELISA.[34] Compounds mediating less than 30 % reduction of antigen expression were considered without biological activity. Compounds mediating a reduction of 30 % or more were examined for cytotoxic effect using concentration-dependent inhibition of MT-4 cell proliferation as measure of cytotoxicity using the MTT assay as previously described.[35] A 30 % inhibition of cell growth relative to control cultures was considered significant.

References

[1] N. Bodor, M.E. Brewster, *Pharmac. Ther.* **1983**, *19*, 337–386.

[2] A.A. Sinkula, S.H. Yalkowsky, *J. Pharm. Sci.* **1975**, *64*, 181–210; N. Bodor, K.B. Sloan, R.J. Little, S.H. Selk, L. Caldwell, *Int. J. Pharm.* **1982**, *10*, 307–321.

[3] E.N. Albert, J.M. Kerns, *Brain Res.* **1981**, *230*, 153–164.

[4] R.D. Smyth, M. Pfeffer, D.R. Van Harker, A. Cohen, G.H. Hottendorf, *Antimicrob. Ag. Chemother.* **1981**, *19*, 1004–1012.

[5] H. Hartmann, M.W. Vogt, A.G. Durno, M.S. Hirch, G. Hunsmann, F. Eckstein, *AIDS Res. Hum. Retroviruses* **1988**, *4*, 457–466.

[6] S.K. Aggarwal, S.R. Gogu, S.R.S. Rangan, K.C. Agrawal, *J. Med. Chem.* **1990**, *33*, 1505–1510.

[7] A.A. Akhrem, G.V. Zaitseva, E.N. Kalinichenko, I.A. Mikhailopulo, *Bioorg. Khim.* **1976**, *2*, 1325–1337; *CA* **1977**, *86*, 73047f.

[8] A.A. Akhrem, E.N. Kalinichenko, I.A. Mikhailopulo, *Zh. Obshch. Khim.* **1977**, *47*, 968–968; *CA* **1977**, *87*, 53515z.

[9] A.A. Akhrem, G.V Zaitseva, E.N. Kalinichenko, U. Makstais, I.A. Mikhailopulo, V. P. Reshchikov, N. M. Fertukova, A.A. Shkarenkov, I. Sternberga, *USSR Pat.* 960189 **1982**; *CA* **1983**, *98*, 89819t.

[10] A.A. Akhrem, E.N. Kalinichenko, E.I. Kvasyuk, I.A. Mikhailopulo, *Bioorg. Khim.* **1977**, *3*, 845-847; *CA* **1977**, *87*, 201973q.

[11] R. Arcas, J. Herreros, E. Rocha, J. Honorato, J. Aranza Perea, *Rev. Med. Univ. Navarra* **1981**, *25*, 119.

[12] M.J. Dominquez, M. Vacas, Y. Saez, I. Olabarria, A. Velasco, J.A. Iriarte, J. Forn, *Clinm. Ther.* **1985**, *7*, 357.

[13] E. Francia, A. Marin, *Drugs Future* **1978**, *3*, 225.

[14] J. Garcia-Rafanell, J.M. Planas, P. Puig-Parellada, *Arch. Int. Pharmacodyn. Ther.* **1978**, *237*, 343–350.

[15] V. Rimbau, J. Forn, A. Torralba, *Arch. Farmacol. Toxicol.* **1982**, *8*,197–200.

[16] V. Rimbau, R. Lopez, A. Fernandez, J. Forn, *Arch. Farmacol. Toxicol.* **1981**, *7*, 11–12.

[17] M.S. Sanchez, J. Garcia-Rafanell, J. Forn, *Arch. Farmacol. Toxicol.* **1981**, *7*, 89–92.

[18] M. S. Sanchez, J. Ramis, J. Garcia-Rafanell, J. Forn, *Arch. Farmacol. Toxicol.* **1982**, *8*, 193–196.

[19] H. Brinkmann, C. Rüchardt, *Tetrahedron Lett.* **1972**, 5221–5224.

[20] C. Rüchardt, S. Rochlitz, *Justus Liebigs Ann. Chem.* **1974**, 15–23.

[21] S. Czernecki, M.J.-M. Valéry, *Synthesis* **1991**, 239–240.

[22] P. Herdewijn, J. Balzarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder, H. Vanderhaeghe, *J. Med. Chem.* **1987**, *30*, 1270–1278.

[23] M. Ankersen, A. Senning, *Acta Chem. Scand.* **1989**, *43*, 793–798.

[24] N.D.P. Cosford, R.F. Schinazi, *Nucleosides Nucleotides* **1993**, *12*, 149–155.

[25] G.Y. Paris, D.L. Garmaise, D.G. Cimon, *J. Med. Chem.* **1980**, *23*, 79–82.

[26] A.B. Hansen, A. Senning, *Acta Chem. Scand. B* **1983**, *37*, 351–359.

[27] M. Ankersen, K.K. Nielsen, A. Senning, *Acta Chem. Scand.* **1989**, *43*, 213–221.

[28] M. Hundewadt, A. Senning, *Acta Chem. Scand.* **1990**, *44*, 746–751.

[29] K. K. Nielsen, A. Senning, *Acta Chem. Scand.* **1990**, *44*, 952–956.

[30] M. Hundewadt, A. Senning, *J. Pharm. Sci.* **1991**, *80*, 545–547.

[31] L. Falborg, A. Senning, *Acta Chem. Scand.* **1993**, *47*, 514–520.

[32] M. Popovic, M.G. Sarnagdharan, E. Read, R. C. Gallo, *Science* **1984**, *224*, 497–500.

[33] S. Harada, Y. Koyanagi, N. Yamamoto, *Science* **1985**, *229*, 563–566.

[34] C.M. Nielsen, I.C. Bygbjerg, B.F. Vestergaard, *Lancet (No. 1)* **1987**, 566–567.

[35] T. Mosmann, *J. Imm. Meth.* **1983**, *65*, 55–63.

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