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Synthesis and *in vitro* anti-HIV activities of didanosine prodrugs

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Abstract

A series of prodrugs of didanosine were synthesized in an effort to enhance the anti-HIV activity. The 5′-OH function of didanosine was esterified with different aryl piperazine acetic acid derivatives and evaluated for anti-HIV-1 activity in MT-4 cell line using the MTT assay method. Among the synthesized compounds, (tetrahydro-5-(1,6-dihydro-6-oxopurin-9-yl)furan-2-yl)methyl 2-(4-(4-chlorophenyl)piperazin-1-yl)acetate (**4b**) was found to be the most potent compound with EC_{50} of 0.64 μM and was not toxic to the MT-4 cells up to 1000 μM with a selectivity index of >1562. Compound **4b** was found to be seven times more potent than the parent drug didanosine (EC_{50} of 4.8 μM) *in vitro*. *In vitro* hydrolysis of the various esters in human plasma indicated that these agents were relatively stable toward plasma esterases with $t_{1/2}$ ranging from 20–60 min.

Keywords: *Didanosine, prodrugs, anti-HIV activity*

Introduction

Acquired immunodeficiency syndrome (AIDS) is a degenerative disease of the immune and central nervous system caused by the human immunodeficiency virus (HIV). Invasion of the central nervous system (CNS) by HIV, the causative agent of AIDS, leads to serious neurological disorders and may be a factor in the development of persistent HIV infections [1]. Many investigations have focused on the development of agents which can more readily penetrate the CNS by crossing the blood-brain barrier. These studies involved esterification of the 5′-hydroxyl group of the parent anti-HIV nucleoside, e.g., zidovudine [2–3], stavudine [4], and 3′-azido- 2′,3′-dideoxyuridine [5], or modification of the nucleoside base with lipophilic functional groups [6] or the phosphate groups of nucleotides [7]. These investigations aimed at increasing lipid solubility, since the correlation between lipophilicity, membrane permeability, and CNS penetration has long been established [8]. From our earlier work, it was found that aryl piperazine derivatives were promising [3,4] and hence in this work, eight aryl piperazine acetic acid ester prodrugs of didanosine were prepared and evaluated for their anti-HIV activity and also *in vitro* hydrolytic stability.

Materials and methods

Chemistry

Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silicagel-G (Merck) coated aluminium plates,

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visualized by iodine vapour. Developing solvents were chloroform-methanol (9:1).

*Synthesis of didanosine chloroacetate (**3**)*. To a stirred suspension of didanosine (**1**) (30 mmol) in methylene chloride (50 mL), dried pyridine (35 mmol) was added followed by the dropwise addition of chloroacetyl chloride (35 mmol) (**2**) over 30 min and then the mixture was refluxed for 4 h. The resulting solution was cooled, washed with water (3 × 100 mL) and dried over anhydrous sodium sulfate. The filtrate was evaporated to dryness under reduced pressure and crystallized from ether to yield 59% didanosine chloroacetate (**3**), mp 260 °C. ^{1}H-NMR (DMSO-d_6): δ ppm: 1.72–1.9 (m, 2H, H-3′), 2.12–2.6 (m, 2H, H-2′), 3.40 (s, 2H, $ClCH_2CO$), 4.09–4.34 (m, 3H, H-4′, and H-5′), 5.9–6.0 (m, 1H, H-1′), 7.9 (s, 1H, H-8), 8.0 (s, 1H, NH, exchangeable with D_2O), 8.4 (s, 1H, H-2).

*General method for synthesis of ester prodrugs (**4a-h**)*. A mixture of the respective aryl piperazine (10 mmol) and didanosine chloroacetate (**3**) (10 mmol) in dry dioxane (50 mL), was stirred at 40 °C for 24 h. The solvent was evaporated under reduced pressure and the residue was dissolved in methylene chloride (100 mL), washed with a saturated solution of sodium chloride (3 × 100 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure and the residue recrystallized from a mixture of ether:ethanol to give the titled compound.

(**4a**) yield 64%, m.p. 201°C, IR(KBr): 3450, 3140, 3050, 1680, 1150 cm^1; ^{1}H-NMR (DMSO-d_6): δ ppm: 1.7–1.92 (m, 2H, H-3′), 2.16–2.4 (m, 2H, H-2′), 2.6–3.34 (m, 8H, piperazine-H), 3.42 (s, 2H, NCH_2CO), 4.09–4.34 (m, 3H, H-4′, and H-5′), 5.9–6.0 (m, 1H, H-1′), 6.59–7.12 (m, 5H, Ar-H), 7.9 (s, 1H, H-8), 8.0 (s, 1H, NH, exchangeable with D_2O), 8.4 (s, 1H, H-2); Calculated for $C_{22}H_{26}N_6O_4$: C,60.26; H,5.98; N,19.17; found: C, 59.98; H, 5.93; N, 19.15%.

(**4c**) yield 73%, m.p. 238°C, IR(KBr): 3450, 3140, 3054, 1680, 1150 cm^1; ^{1}H-NMR (DMSO-d_6): δ ppm: 1.68–1.93 (m, 2H, H-3′), 2.16–2.41 (m, 2H, H-2′), 2.49–3.34 (m, 8H, piperazine-H), 3.46 (s, 2H, NCH_2CO), 3.73 (s, 3H, -OCH_3), 4.09–4.34 (m, 3H, H-4′, and H-5′), 5.95–6.0 (m, 1H, H-1′), 6.48–6.59 (m, 4H, Ar-H), 7.92 (s, 1H, H-8), 8.0 (s, 1H, NH, exchangeable with D_2O), 8.38 (s, 1H, H-2); Calculated for $C_{23}H_{28}N_6O_5$: C,58.96; H,6.02; N,17.94; found: C, 58.97; H, 5.99; N, 17.92%.

(**4e**) yield 69%, m.p. 231 °C, IR(KBr): 3450, 3146, 3050, 1678, 1150 cm^1; ^{1}H-NMR (DMSO-d_6): δ ppm: 1.68–1.93 (m, 2H, H-3′), 2.16–2.41 (m, 2H, H-2′), 2.46–2.6 (m, 8H, piperazine-H), 3.32 (s, 2H, NCH_2CO), 3.62 (s, 2H, -CH_2 benzyl), 4.09–4.34 (m, 3H, H-4′, and H-5′), 5.95–6.0 (m, 1H, H-1′), 7.0–7.16 (m, 5H, Ar-H), 7.97 (s, 1H, H-8), 8.1 (s, 1H, NH, exchangeable with D_2O), 8.32 (s, 1H, H-2); Calculated for $C_{23}H_{28}N_6O_4$: C,61.05; H,6.24; N,18.57; found: C, 60.99; H, 6.28; N, 18.54%.

(**4h**) yield 78%, m.p. 256 °C, IR(KBr): 3450, 3140, 3054, 1680, 1150 cm^1; ^{1}H-NMR (DMSO-d_6): δ ppm: 0.34–0.8 (m, 4H, cyclopropyl-H), 1.10 (d, 3H, CH_3 of pip), 1.68–1.93 (m, 2H, H-3′), 2.16–2.38 (m, 2H, H-2′), 3.32 (s, 2H, NCH_2CO), 3.5 (s, 3H, —OCH_3), 3.64–3.68 (m, 1H, cyclopropyl-H), 3.8–4.0 (m, 7H, -piperazine-H), 4.09–4.34 (m, 3H, H-4′, and H-5′), 5.95–6.0 (m, 1H, H-1′), 6.8–6.9 (m, 1H Ar-H), 7.92 (s, 1H, H-8), 8.0 (s, 1H, NH, exchangeable with D_2O), 8.38 (s, 1H, H-2), 8.6 (s, 1H, C_2-H), 14.22 (bs, 1H, COOH); Calculated for $C_{31}H_{34}FN_7O_8$: C,57.14; H,5.26; N,15.05; found: C, 57.19; H, 5.24; N, 15.99%.

Anti-HIV activity

The compounds were tested for anti-HIV activity against replication of HIV-1 (III B) in MT-4 cells [9]. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine Scotland), supplemented with 10% (v/v) heat-inactivated calf serum and 20-μg/mL gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) was obtained from the culture supernatant of HIV-1 infected MT-4 cell lines and the virus stocks were stored at − 70 °C until used. Anti-HIV assays were carried out in microtitre plates filled with 100 μL of medium and 25 μL volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and control infected cells. Fifty microlitres of HIV at 100 $CCID_{50}$ medium were added to either the HIV infected or control infected part of the microtitre tray. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Five days after infection the viability of control and HIV-infected cells were examined spectrophotometrically by the MTT method.

In-vitro *stability studies*

To 990 μl of human plasma was added 10 μl of a solution of compound (10 mg/mL in dimethylsulfoxide) and the mixture was incubated at 37 °C in a water bath. At various time intervals (0-4 h), 100 μL of the samples were withdrawn and added immediately to ice-cold methanol (400 μL). The samples were centrifuged and the supernatants were filtered through nylon 66 filters (0.45 μm) and analyzed according to the method previously reported [3].

Result and discussion

Synthesis and characterisation

Prodrugs are pharmacologically inactive derivatives of active agents, which undergo chemical or

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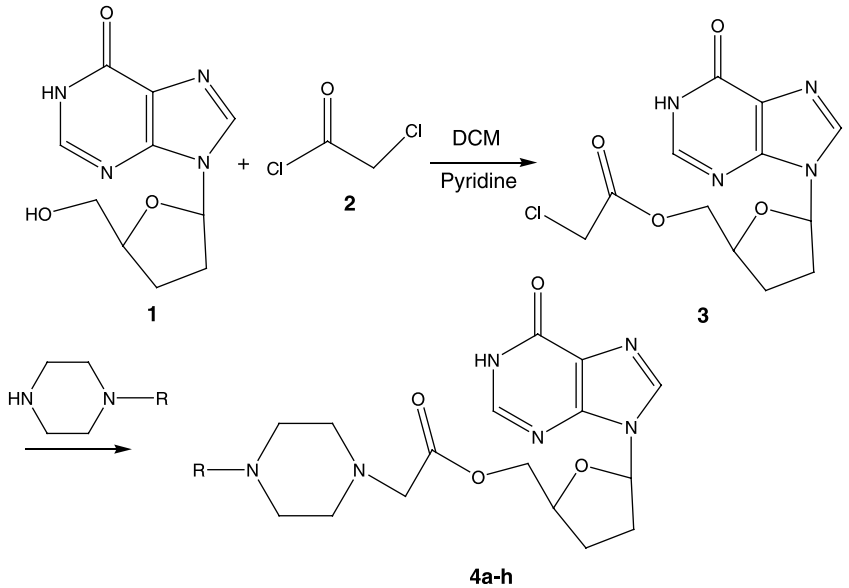
enzymatic biotransformation resulting in the release of the active drug after administration. The metabolic product (e.g. parent drug) subsequently elicits the desired pharmacological response. Esters have dominated prodrug research because they have ideal characteristics, exhibiting reasonable chemical stability *in vitro* which allows them to be formulated with adequate shelf-lives. In addition, by virtue of their ability to function as esterase substrates, esters are suitably labile *in vivo*. Esterification of the 5′-hydroxyl group was a common approach to enhance brain uptake and *in vivo* efficacy of anti-HIV nucleoside derivatives [10].

The reaction sequence used for the preparation of didanosine prodrugs (**4a-h**) was achieved in two steps (Scheme I). The precursor didanosine chloroacetate (**3**) was obtained by refluxing didanosine (**1**) and chloroacetyl chloride (**2**) in dichloro methane using pyridine as an acid scavenger. Subsequent condensation of the intermediate (**3**) with the respective aryl piperazines, at room temperature gave the title compounds **4a-h** in 64–81% yield (Table I).

The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) and elemental analyses and the structures were identified by spectral data. In general, Infra red spectra (IR) showed a carbonyl stretching peak for the ester at 1680 cm^{-1} and CH_2 (methylene) peak at 3050 cm^{-1}. In the ^1H NMR spectra the signals for the respective protons of the prepared didanosine derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 3.4 ppm corresponding to the $-NCH_2-CO-$ group; multiplet at δ 2.4–3.3 ppm for piperazine protons; singlet at δ 8.0 ppm for the NH proton; singlet at δ 7.9 ppm for C_8-H. The elemental analysis results were within ± 0.4% of the theoretical values.

Biological investigation and discussion

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in MT-4 cell lines and their EC_{50} (effective concentration of compound (μM) achieving 50% protection in MT-4 cell lines against the cytopathic effect of HIV-1), and CC_{50} (cytotoxic concentration of compound (μM) required to reduce the viability of control infected CEM cells by 50%), are reported in the Table I with didanosine as standard drug for comparison. A rapid glance of the obtained results reveal that compounds **4a-h** exhibited excellent anti-HIV activity. Among the synthesized compounds (tetrahydro-5-(1,6-dihydro-6-oxopurin-9-yl)furan-2-yl)methyl 2-(4-(4-chlorophenyl)piperazin-1-yl)acetate (**4b**) was found to be the most potent compound with EC_{50} of 0.64 μM and was not toxic to the MT-4 cells up to 1000 μM with a selectivity index (CC_{50}/EC_{50}) of >1562. The lipophilicity (logP) of the synthesized compounds increased considerably compared with the parent drug, didanosine (Table I). This may render them more capable of penetrating various biomembranes [11], consequently improving their permeation properties through viral cell membranes. The results showed that there was an improvement in anti-HIV activity compared to the parent drug. Though these drugs have been found to have improved lipophilicity, further work in improving the log P to a value of 2 ± 0.7 (Hansch CNS optimum) for targeting the CNS would be beneficial.



Scheme 1. Synthesis of didanosine prodrugs.

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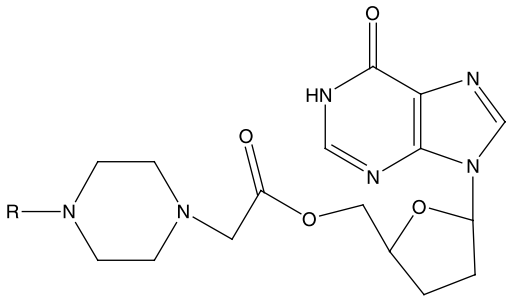
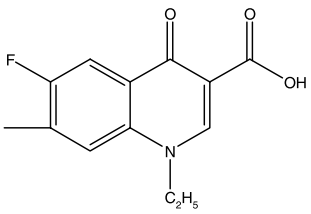
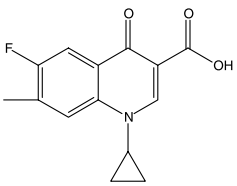
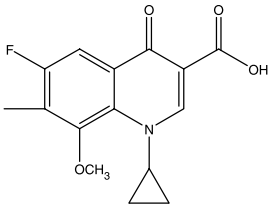
Table I. Physical constants and anti-HIV activity of **4a-h**.



4a-h

Comp	R	Yield %	m.p °C	logP[a]	EC_{50}[b]	CC_{50}[c]	$t_{1/2}$[d]
4a	Phenyl	64	201	0.78	1.28	>1000	20
4b	4-Chlorophenyl	69	185	1.34	0.64	>1000	60
4c	4-Methoxyphenyl	73	238	0.65	1.87	828	60
4d	4-Nitro phenyl	81	194	–	4.78	>1000	ND
4e	Benzyl	69	231	0.44	2.2	>1000	ND
4f		76	247	1.04	1.16	560	ND
4g		70	>260	0.87	3.12	380	ND
4h		78	256	1.06	0.82	770	ND
DDI	–	–	–	−1.1	4.8	>1000	–

[a] logP values calculated with Chem office 2004 software; [b] effective concentration of compound (μM) achieving 50% protection in MT-4 cell lines against the cytopathic effect of HIV-1; [c] CC_{50} cytotoxic concentration of compound (μM) required to reduce the viability of control infected CEM cells by 50%; [d] *In vitro* hydrolysis $t_{1/2}$ (min), **4h** contains methyl group at 3-position of piperazine moiety.

The usefulness of the prodrugs of didanosine should depend not only on the stability of the prodrug for its transport across the cell membrane but also upon its reversion to the parent compound intracellularly, especially in the virally infected cells. The half-lives ($t_{1/2}$) of the prodrugs were therefore determined in human plasma. The data showed that the *in vitro* hydrolysis of the various esters in human plasma (Table I) indicated that these agents were relatively stable toward plasma esterases with $t_{1/2}$ ranging from 20–60 minutes.

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