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MODELISATION, SYNTHESIS AND ANTIVIRAL EVALUATION OF NEW 2,3-DISUBSTITUTED THIAZOLIDINONE NUCLEOSIDE ANALOGUES

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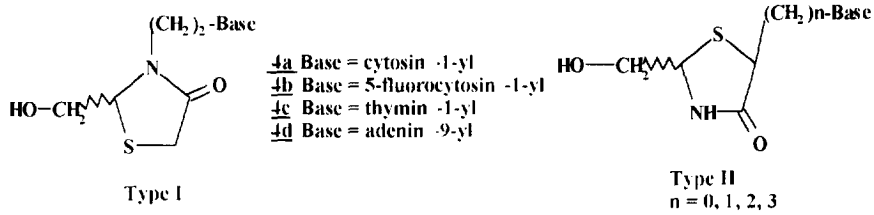
Abstract. The syntheses, anti-HIV and anti-HBV *in vitro* evaluations of new thiazolidinone nucleoside analogues are described. Preliminary molecular modeling studies demonstrated that these new analogues conserved the essential elements of the hypothesized biological active synthon.[1]

Introduction

Structural modifications in the sugar part of nucleosides have led to the development of several nucleoside analogues with antiviral properties. Wellknown examples are acyclic nucleoside analogues (e.g. ACV),[2] fluorinated nucleosides,[3a,b] carbocyclic nucleosides (e.g. carbovir),[4] nucleosides with a four-membered ring (e.g. oxetanocin)[5] and more recently the unatural L-enantiomer of 2',3'-dideoxy-3'-thiacytidine (e.g. Lamivudine, 3TC)[6,7] which is so far the first approved drug for treatment of HIV infections. The success encountered with 3TC, encouraged us to search for other heterocycles mimicking the ribose ring cycle which can act as competitive inhibitors and/or alternate substrates of the HIV reverse transcriptase. Predictions derived from molecular modeling studies led us to propose some inhibitors prior to their synthesis. Thiazolidinone N-substituted by a two carbons alkyl chain linked to a nucleic base (type I model, Figure 1), appeared to conserve the essential elements of the hypothesized biological active synthon deduced from the following modeling studies.

Figure 1



Type I

Type II
n = 0, 1, 2, 3

Molecular modeling studies

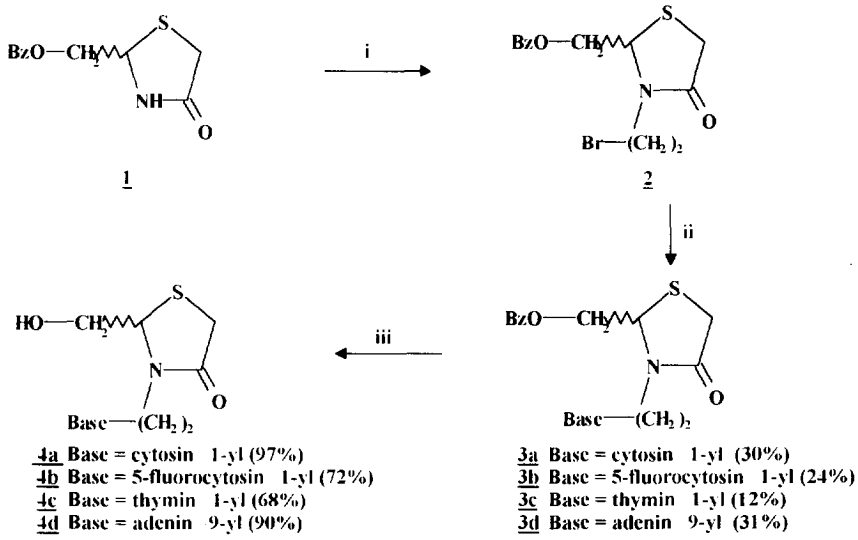
A specific molecular model defining the required geometrical, electrical and energetical parameters for a given molecule to elicit anti-HIV activity was established. As already reported by Van Roey et al.[8] C3' exo nucleoside conformation is active on both kinase and reverse transcriptase enzyme active sites. In its C3' exo conformation, the nucleoside drug must have an envelope in terms of both geometry and electrostatic potential, similar to that of the natural substrate (e.g. 2'-deoxycytidine) for kinase and reverse transcriptase. The required energy to put the type I thiazolidinone nucleoside (Figure 1) into cytidine-like geometry (C3' exo conformation) was calculated. All the calculations were performed using GenMol software.[9,10] The difference ΔE (Kcal.Mol^{-1}) between the strain energy of the thiazolidinone nucleoside in C3' exo conformation and the minimum strain energy after relaxation, corresponds to the increase of the transition state energy with cytidine. ΔE is the value calculated for the cytosine thiazolidinone derivatives (n=2). For compounds **4a** and **4b** ΔE was found to be equal to 0.9 ± 0.1 Kcal **Mol**$^{-1}$. From the thermodynamic point of view, this low ΔE value means that the cytosinyl thiazolidinone analogue presents an envelope similar to that of cytidine in terms of both geometry and electrostatic potential. Consequently, these new analogues should present the chemical structures allowing them to fit after their triphosphorylation into HIV reverse transcriptase active sites. In contrast, it should be mentioned that ΔE values (Kcal **Mol**$^{-1}$) calculated for type II thiazolidinone cytosine nucleosides (Figure 1) are 8.0 for n=0, 2.6 for n=1, 3.8 for n=2 and 5.0 for n=3. These rather large ΔE values indicate that these heteronucleosides present an envelope significantly different from the natural cytidine substrate. Therefore, they should be predicted inactive as anti-HIV inhibitors. The synthesis and the antiviral evaluation of these type II thiazolidinone nucleosides already reported[11] have confirmed these *a posteriori* predictive calculations. Following molecular modeling studies, the syntheses and the anti-HIV evaluation of type I thiazolidinone nucleosides, predicted as potent anti-HIV inhibitors, were investigated.

Chemistry

Our synthetic strategy starts from 2-benzoyloxymethyl-1,3-thiazolidin-4-one **1** which has already been described.[11] N-alkylation of this thiazolidinone intermediate **1** was achieved according to a procedure described by Nishimoto et al.[12] by reacting one equivalent of powdered KOH in anhydrous DMF with one equivalent of **1** at room temperature, followed by the addition of one equivalent of 1,2-dibromoethane. The corresponding bromoalkylated thiazolidinone **2** was isolated, purified and fully characterized. Various nucleic bases were then condensed on the intermediate **2** using cesium carbonate as a coupling reagent. The reaction

was conducted in DMF at 100°C for 2 hours. Low yields of the desired purified compounds were obtained as indicated on scheme 1. The final deprotection step was achieved using saturated methanolic ammonia solution at room temperature for 12 hours, and the resulting compounds (**4a-d**) were characterized by ^{1}H, ^{13}C NMR and FAB mass spectroscopy. The overall synthesis is summarized on scheme 1.

Scheme 1.



4a Base = cytosin 1-yl (97%)
4b Base = 5-fluorocytosin 1-yl (72%)
4c Base = thymin 1-yl (68%)
4d Base = adenin 9-yl (90%)

3a Base = cytosin 1-yl (30%)
3b Base = 5-fluorocytosin 1-yl (24%)
3c Base = thymin 1-yl (12%)
3d Base = adenin 9-yl (31%)

i: KOH/DMF/25°C then $Br(CH_2)_2Br$/25°C/12h, ii: nucleic base/Cs_2CO_3/DMF/100°C/2h; iii: NH_3/MeOH/25°C/12h

Antiviral Activity

Compounds **3a**, **3b**, **3c**, **3d** and **4a**, **4b**, **4c**, **4d** were tested for their ability to inhibit HIV-1 replication in cell cultures. The fusogenic effect of HIV-1 in the MT4 cell line[13] was determined as described by Rey et al.[14,15] The anti-HBV (anti-Hepatitis B Virus) assays for compounds **4a**, **4b**, **4c** and **4d** were conducted according to procedures previously described[16] for duck hepatocytes culture, and according to Fourel et al.[17, 18] to estimate DHBV (Duck Hepatitis B Virus) production. Anti-HIV and anti-DHBV evaluations for the tested thiazolidinone N-nucleosides are reported in table 1. The reference compound 3TC (Lamivudine) known for its antiviral activity against both HIV and DHBV was tested in the same experimental conditions.

Compounds **4b**, **4c** and **4d** demonstrated a marked activity against HIV-1 and DHBV. However, these compounds were one order of magnitude less active than the reference drug

3TC.**Surprisingly,** the cytosinyl analogue **4a** was found inactive against HIV-1 but as active as the other congeners against DHBV. Moreover, the 5'-O protected compound **3b** exhibited activity against HIV-1.

Table I : Anti-HIV and anti-HBV activities of N-substituted thiazolidinone derivatives.

Compound N°	anti-HIV activity		anti-DHBV activity	
	IC50 (μM)[a]	CC50 (μM)[b]	IC50 (μM)[a]	CC50 (μM)[b]
3TC	0.1±0.05	100	0.1-0.5	≥ 400
3a	≥100	≈100	nd.	nd.
3b	1-10	100	nd.	nd.
4a	≥300	≈300	0.5-2	100
4b	1-10	100	0.5-2	150
4c	5-15	100	0.5-2	≥200
4d	1-10	100	2	200

a. IC_{50} : concentration required to inhibit 50% of the viral replication (HIV or DHBV).
b. CC_{50} : concentration required to reduce the cell viability 50%; nd. not determined.

We had already reported that type II compounds (Figure 1) were inactive against HIV-1.[19] The present results obtained with type I thiazolidinone nucleosides corroborate at least partially the conclusions derived from the reported molecular modeling studies. The relative low antiviral activity of this new class of compounds compared to 3TC could be due to their poor delivery properties.[20] However, **4c** was shown to be a selective inhibitor of DHBV replication and deserves further assessment in the human HBV study models in comparison with 3TC. Because of their more polar character, type I thiazolidinone nucleosides may enter the cells with difficulty. In the case of AZT, similar observations have shown that prodrugs incorporating polar species like glutamic acid were found to be less permeable to the cells in comparison to those carrying less polar moieties such as isoleucine.[21]

Since the developed modeling software did not integrate important biophysical parameters such as lipophilicity and pharmacokinetic parameters (bioavailability, cell membrane permeation) the hypothesized biological active synthon deduced from modeling studies should be only taken as a useful tool.

In conclusion, using molecular computational modeling, we have designed and achieved the synthesis of a new class of thiazolidinone derivatives, which exhibited moderate anti-HIV and anti-HBV activity. This work, originally initiated with the discovery of Lamivudine (3TC), strengthens the need to develop the synthesis of other anti-HIV heteronucleoside containing modified sugar ring.

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Analytical data of final compounds.

Compound **4a**: ^{1}H NMR (DMSO d_6) δ 3.2-3.8 (m,8H,4CH_2), 4.6 (t,1H,H-2), 5.6 (d,1H,H-5' J=7.0Hz), 6.9 (brs,2H,NH_2), 7.45 (d,1H,H-6' J=7.0Hz); MS (FAB$^+$): 271 (M+H$^+$).

Compound **4b**: ^{1}H NMR (DMSO d_6) δ 3.2-3.8 (m,8H,4CH_2), 4.75 (t,1H,H-2), 5.3 (brs,1H,OH), 7.4 (brs,2H,NH_2), 7.85 (d,1H,H-6' J=7.0Hz); MS (FAB$^+$): 289 (M+H$^+$).

Compound **4c**: ^{1}H NMR (DMSO d_6) δ 1.75 (s,3H,CH_3), 3.2-3.9 (m,8H,4CH_2), 4.8 (t,1H,H-2), 5.2 (d,1H,OH), 7.40 (d,1H,H-6'); MS (FAB+): 286 (M+H$^+$).

Compound **4d**: ^{1}H NMR (DMSO d_6) δ 3.2-3.9 (m,6H,3CH_2), 4.3 (m,2H,CH_2), 4.6 (s,1H,H-2), 7.1 (brs,2H,NH_2), 8.1 (s,1H,H-adenine), 8.2 (s,1H,H-adenine); MS (FAB$^+$): 295 (M+H$^+$).

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