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Synthesis and in vitro antileishmanial activity of 5-substituted-2′-deoxyuridine derivatives

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Abstract

We report herein the synthesis and the in vitro antileishmanial evaluation of 5-substituted-2′-deoxyuridine nucleosides. The most active compound against *Leishmania donovani* promastigotes was Thia-dU (**3a**) with an $IC_{50} = 3\,\mu M$. This compound exhibited the same activity as zidovudine (3′-azido-2′-deoxythymidine) used as nucleoside reference compound. Considering the cytotoxicity of synthetic compounds on peritoneal murine macrophages, the most toxic compound was MeThio-dU (**3d**) with a MTC at $10\,\mu M$. Only Methia-dU (**3b**) was active against intramacrophagic amastigotes with an $IC_{50} = 6.5\,\mu M$. This latter can now be evaluated in vivo, for further investigations through structure-based drug design.

Keywords: Nucleosides; Antileishmanial activity

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1. Introduction

The chemotherapy of visceral leishmaniasis (VL) is still based on pentavalent antimonials and amphotericin B in the case of resistance to antimonials which frequently occurs in India (Bihar) and Souddan [1]. Despite the progress of amphotericin B formulations able to reduce its toxicity and to enhance its antileishmanial effect, and the recent launch of miltefosine as the first orally active antileishmanial drug, there is an urgent need for new molecules active against *Leishmania* sp. because of some toxicological and economical limitations for these drugs [2]. Some nucleoside derivatives have interesting antileishmanial properties. Sinefungin (Fig. 1), a natural nucleoside antibiotic produced by *Streptomyces griseolus*, is structurally related to *S*-adenosyl-L-methionine and exhibits strong antifungal and antiparasitic activities both in vitro and in vivo [3]. Various uracil analogs of sinefungin have been previously synthesized to optimize the structural characteristics of this series [4]. This article describes the synthesis of 5-substituted-2′-deoxyuridine nucleosides and their biological activity on both *Leishmania donovani* promastigote and amastigote stages and their cytotoxicity on peritoneal murine macrophages. The rationale relies on chemical modulations at the 5-position that is critical for biological activity of pyrimidine nucleosides. The choice of aryl substituents such as benzothiazole, thiazole, benzimidazole, and thiophene systems, well known for their antiprotozoal activities was justified in the aim to obtain a synergistic effect in the biological activity by a combination of two active moieties in one molecule. The putative mechanism of action of these compounds will be discussed in this article.

2. Materials and methods

2.1. General methods

All reactions were run under an atmosphere of nitrogen. Tetrahydrofuran (THF) was purchased from commercial sources and dried over 4 Å molecular sieves before use. All other reagents and solvents were purchased from commercial sources and used without purification. Chromatography was made on silica gel purchased from Merck (silica gel 60, 0.063–0.200 mm). Analytical thin-layer chromatography (TLC) was carried out using 0.2 mm commercial silica gel plates (silica gel 60 with fluorescent indicator UV_{254}). Mass spectra were recorded on an Esquire 3000 Plus equipment, in both positive or negative Electron Spray ionization (ESI) mode and are reported as *m*/*z*. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded with a Bruker

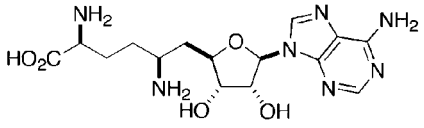


Fig. 1. Structure of sinefungin.

Advance Spectrometer at 200 or 500 MHz. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from used solvents, specified for each product. Coupling constants are reported in Hertz (Hz).

*2.1.1. Synthesis of the free nucleoside **3h**: analytical and spectral data for selected products*

*2.1.1.1. Indolyltin derivative **4**.* To a solution of Boc-protected indole (10 mmol) in THF (30 mL) was slowly added one equivalent of lithium diisopropylamide (2 M solution in hexane/THF, 5 mL) at −78 °C. After 30 min, a solution of Bu_3SnCl (1.1 equiv., 11 mmol) in THF (5 mL) was added dropwise. The reaction was stopped after 3 h by addition of aq. NH_4Cl solution (20 mL) and the mixture was extracted with diethyl ether (3 × 30 mL). The organic layers were dried over $MgSO_4$ filtered and the solvents were evaporated under vacuo. The obtained crude oil was used in the next coupling step without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.88–1.02 (m, 15H, Bu), 1.60–1.73 (m, 12H, Bu), 1.75 (s, 9H, tBu), 6.77 (d, 1H, $J = 0.6$ Hz, H-2 ind.), 7.18–7.33 (m, 3H, H-ind.), 8.01 (d, 1H, $J = 8.8$ Hz, H-4 ind.). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 11.69 ppm (CH_2), 13.70 (CH_3), 27.47 (CH_2), 28.28 (CH_3-Boc), 29.27 (CH_2), 83.82 (C-(CH_3)$_3$), 115.34 (C-4 ind.), 118,63 (C-2 ind.), 120.09 (CH-ind.), 122.25 (CH-ind.), 125.63 (CH-ind.), 132.51 (C-Ar), 137.50 (C-Ar), and 152.26 (CO). MS (ESI^+) $m/z = 508$ (MH^+).

*2.1.1.2. 3′,5′-Di-O-acetyl-5-(N-tertbutoxycarbonylindolyl-2)-2′-deoxyuridine (**1h**).* To a solution of **1** (2 mmol) and indole-tin derivative **4** (4 mmol) in DMF (10 mL) was added $Pd(Ph_3)_2Cl_2$ (0.1 mmol), CuI (0.2 mmol) and Et_3N (2.4 mmol). The mixture was heated at 70 °C for 3 h cooled and filtered over celite. The solvent was removed and the residue purified by flash chromatography (CH_2Cl_2/AcOEt: 100/0 to 85/15) to yield **1h** as a colorless oil (67%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 1.46 (s, 9H, tBu), 1.79 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.19 (m, 1H, H-2′), 2.43 (ddd, 1H, $J = 1.9$, 5.8 and 14.3 Hz, H-2′), 4.16 (m, 1H, H-4′), 4.22 (m, 2H, H-5′), 5.13 (m, 1H, H-3′), 6.28 (dd, 1H, $J = 8.2$ and 5.7 Hz, H-1′), 6.43 (s, 1H, H-3 ind.), 7.10 (t, 1H, $J = 7.4$ Hz, H-5 or H-6 ind.), 7.20 (t, 1H, $J = 7.7$ Hz, H-6 or H-5 ind.), 7.40 (d, 1H, $J = 7.9$ Hz, H-4 or H-7 ind.), 7.54 (s, 1H, H-6), 8.02 (d, 1H, $J = 8.5$ Hz, H-7 or H-4 ind.). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 20.82 (CH_3), 20.93 (CH_3), 27.91 (tBu), 37.76 (C-2′), 62.33 (C-5′), 75.09 (C-3′), 83.92 (C-(CH_3)$_3$), 85.41 (C-4′), 86.03 (C-1′), 110.64 (C-3 ind.), 111.53 (C-5), 115.40 (C-7 ind.), 122.62 (CH-ind.), 124.55 (CH-ind.), 128.42 (CH-ind.), 131.14 (C-ind.), 131.93 (C-ind.), 132.01 (C-6), 149.76 (CO), 150.19 (CO), 161.82 (CO), and 170.56 (CO). MS (ESI^+) $m/z = 450$ [$(MNa)^+$-Boc].

*2.1.1.3. 3′,5′-Di-O-tolouyl-5-(N-tertbutoxycarbonylindolyl-2)-2′-deoxyuridine (**2h**).* **2h** was obtained by using the same experimental conditions as for **1h** (43% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 1.56 (s, 9H, tBu), 2.19 (s, 3H, CH_3), 2.39 (m, 1H, H-2′), 2.44 (s, 3H, CH_3), 2.78 (dd, 1H, $J = 5.4$ and 14.5 Hz, H-2′), 4.56 (m, 1H, H-4′), 4.62 (d, 1H, $J = 11.0$ Hz, H-5′), 4.81 (d, 1H, $J = 12.3$ Hz, H-5′), 5.67 (br d, 1H, $J = 5.9$ Hz, H-3′), 6.23 (s, 1H, H-3 ind.), 6.56 (t, 1H, $J = 5.5$ Hz, H-1′), 6.81 (d, 2H, $J = 7.5$ Hz, H-tol), 7.22 (m, 1H, H-5 or H-6 ind.), 7.28 (m, 2H, H-tol), 7.32 (m, 1H, H-6 or H-5 ind.),

7.39 (d, 1H, $J = 7.8$ Hz, H-4 or H-7 ind.), 7.62 (s, 1H, H-6), 7.64 (d, 2H, $J = 7.2$ Hz, H-tol), 7.97 (d, 2H, $J = 7.2$ Hz, H-tol), and 8.10 (d, 1H, $J = 8.5$ Hz, H-7 or H-4 ind.). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 21.45 (CH_3), 21.60 (CH_3), 27.83 (tBu), 38.28 (C-2′), 64.05 (C-5′), 74.90 (C-3′), 82.91 (C-4′), 83.64 (C-(CH$_3$)$_3$), 84.98 (C-1′), 110.62 (C-3 ind.), 112.20 (C-5), 115.32 (C-7 ind.), 120.54 (CH-ind.), 122.49 (CH-ind.), 124.45 (CH-ind.), 125.86 (C-Ar), 126.11 (C-Ar), 128.42 (C-Ar), 128.86 (CH-tol), 128.97 (CH-tol), 129.17 (CH-tol), 129.70 (CH-tol), 130.26 (C-Ar), 134.78 (C-6), 136.86 (C-Ar), 144.05 (C-Ar), 144.47 (C-Ar), 149.47 (CO), 149.90 (CO), 161.48 (CO), 165.80 (CO), and 165.96 (CO). MS (ESI$^+$) $m/z = 702$ (MNa$^+$).

2.1.1.4. 3′,5′-di-O-tolouyl-5-(indolyl-2)-2′-deoxyuridine (indole-deprotected side product [5]). This compound was obtained as a side product in the palladium coupling step. It is also an advanced intermediate in the synthesis of nucleoside **3h**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 2.16 (m, 1H, H-2′), 2.27 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.75 (dd, 1H, $J = 5.2$ and 14.3 Hz, H-2′), 4.51 (m, 1H, H-4′), 4.72 (m, 2H, H-5′), 5.54 (br d, 1H, $J = 5.9$ Hz, H-3′), 6.29 (br m, 1H, H-1′), 6.45 (s, 1H, H-3 ind.), 7.02 (t, 1H, $J = 7.4$ Hz, H-6 ind. or H-5 ind.), 7.09 (t, 1H, $J = 7.8$ Hz, H-5 ind. or H-6 ind.), 7.11 (d, 2H, $J = 8.1$ Hz, H-tol), 7.27 (d, 2H, $J = 8.1$ Hz, H-tol), 7.34 (d, 1H, $J = 7.9$ Hz, H-4 ind. or H-7 ind.), 7.41 (d, 1H, $J = 8.2$ Hz, H-7 ind. or H-4 ind.), 7.92 (d, 2H, $J = 7.9$ Hz, H-tol), 7.97 (d, 2H, $J = 8.2$ Hz, H-tol), 8.05 (s, 1H, H-6), and 10.46 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 21.55 (CH_3), 21.70 (CH_3), 38.36 (C-2′), 64.00 (C-5′), 74.65 (C-3′), 83.29 (C-4′), 85.90 (C-1′), 98.02 (C-3 ind.), 107.55 (C-5), 111.35 (CH-ind.), 119.75 (CH-ind.), 120.06 (CH-ind.), 121.81 (CH-ind.), 126.33 (2× C-Ar), 127.75 (2× C-Ar), 129.28 (CH-tol), 129.39 (CH-tol), 129.56 (CH-tol), 129.80 (CH-tol), 134.28 (C-6), 136.14 (C-Ar), 144.51 (C-Ar), 144.56 (C-Ar), 149.13 (CO), 162.34 (CO), 166.02 (CO), and 166.39 (CO). MS (ESI$^+$) $m/z = 580$ (MH)$^+$, 603 (MNa)$^+$.

*2.1.1.5. 5-(N-tertbutoxycarbonylindolyl-2)-2′-deoxyuridine (**5**).* To a solution of the protected nucleoside **1h** or **2h** obtained above (0.5 mmol) in MeOH (5 mL) was added K_2CO_3 (3 equiv.) and the reaction mixture was stirred over night at room temperature. The mixture was filtered and concentrated in vacuo. The residual oil was purified on silica gel chromatography (CH_2Cl_2/MeOH: 95/5 to 90/10) to afford quantitative yield of pure product **5** as a foam. ^{1}H NMR (CD_3OD, 500 MHz) δ (ppm) 1.53 (s, 9H, tBu), 2.28 (m, 1H, H-2′), 2.31 (m, 1H, H-2′), 3.70 (dd, 1H, $J = 3.3$ and 11.9 Hz, H-5′), 3.78 (dd, 1H, $J = 3.0$ and 11.9 Hz, H-5′), 3.94 (m, 1H, H-4′), 4.41 (m, 1H, H-3′), 6.33 (t, 1H, $J = 6.6$ Hz, H-1′), 6.59 (s, 1H, H-3 ind.), 7.19 (t, 1H, $J = 7.5$ Hz, H-5 or H-6 ind.), 7.26 (t, 1H, $J = 7.5$ Hz, H-6 or H-5 ind.), 7.52 (d, 1H, $J = 7.6$ Hz, H-4 or H-7 ind.), 8.13 (d, 1H, $J = 8.3$ Hz, H-7 or H-4 ind.), 8.19 (s, 1H, H-6). ^{13}C NMR (CD_3OD, 125 MHz) δ (ppm) 28.43 (tBu), 42.05 (C-2′), 62.84 (C-5′), 72.39 (C-3′), 85.28 (C-(CH$_3$)$_3$), 87.07 (C-4′), 89.31 (C-1′), 111.86 (C-3 ind.), 112.60 (C-5), 116.44 (C-7 or C-4 ind.), 121.91 (C-4 or C-7 ind.), 124.05 (C-5 or C-6 ind.), 125.79 (C-6 or C-5 ind.), 130.43 (C-ind.), 133.20 (C-ind.), 138.81 (C-ind.), 139.17 (C-6), 151.50 (CO), 152.34 (CO), and 164.97 (CO). MS (ESI$^+$) $m/z = 466$ (MNa)$^+$, 482 (MK)$^+$.

*2.1.1.6. 5-(indolyl-2)-2′-deoxyuridine (**3h**).* To a solution of **5** (133 mg, 0.3 mmol) in dioxane (2 mL) was added TFA (4 mL) and the reaction mixture was stirred over night at room temperature. The mixture was evaporated and the residual oil was loaded on silica gel column and eluted with CH_2Cl_2/MeOH (95/5 to 85/15) to afford 81 mg (79%) of **3h** as a foam. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 2.21 (m, 1H, H-2′), 2.30 (m, 1H, H-2′), 3.65 (m, 1H, H-5′), 3.71 (m, 1H, H-5′), 3.85 (m, 1H, H-4′), 4.34 (m, 1H, H-3′), 6.26 (t, 1H, $J = 6.6$ Hz, H-1′), 6.81 (s, 1H, H-3 ind.), 6.97 (t, 1H, $J = 7.4$ Hz, H-5 or H-6 ind.), 7.05 (t, 1H, $J = 7.1$ Hz, H-6 or H-5 ind.), 7.46 (m, 2H, H-4 and H-7 ind.), 8.45 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm) 38.86 (C-2′), 60.48 (C-5′), 69.54 (C-3′), 84.10 (C-4′), 86.83 (C-1′), 98.46 (C-3 ind.), 105.82 (C-5), 110.81 (CH-ind.), 118.61 (CH-ind.), 118.96 (CH-ind.), 120.51 (CH-ind.), 127.22 (C-ind.), 127.26 (C-ind.), 130.53 (C-ind.), 136.01 (C-6), 148.88 (CO), and 161.18 (CO). MS (ESI$^+$) $m/z = 344$ (MH^+). UV (MeOH) λ_{max} 213 nm (ε 54,800), 288 nm (ε 31,400), 331 nm (ε 29,700).

2.2. Toxicity on macrophages [6]

The toxicity of the nucleosides was evaluated on mouse peritoneal macrophages which were then used for the intramacrophagic amastigotes in vitro model. Peritoneal macrophages were harvested from female CD1 mice (Charles River, Cléon, France) 3 days after an intraperitoneal injection of 1.5 ml of sodium thioglycolate (Biomérieux) and were dispensed into eight-well chamber slides (LabTek Ltd.) at a concentration of 5×10^4/well (400 μl/well) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 25 mM Hepes, and 2 mM L-glutamine (Life Technologies, Cergy-Pontoise, France). Four hours after the macrophages were plated, they were washed to eliminate fibroblasts. The culture medium was renewed 48 h later and a new culture medium containing the drug was added. The experiment was stopped at day 5, and the viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, expressed in μM ± SD and the maximum tolerated concentration (MTC) expressed in μM was evaluated for each compound.

2.3. Antileishmanial activity [6]

Leishmania donovani (MHOM/IN/80/DD8) promastigotes used in this study were cultured in Hepes (25 mM)-buffered RPMI 1640 medium enriched with 10% heat-inactivated fetal calf serum (hi-FCS) and 50 μg/ml gentamycin at 27 °C in a dark environment.

2.3.1. In vitro evaluation on promastigote forms

The antileishmanial screening was performed in flat-bottomed 96-well plastic tissue-culture trays maintained at 27 °C in an atmosphere of 95% air/5% CO_2. Promastigote forms from a logarithmic phase culture were suspended to yield 10^6 cells/ml after hemocytometer counting. Each well was filled with 100 μl of the parasite suspension, and plates were incubated at 27 °C for 1 h before drug addition. The compounds to be tested were dissolved in DMSO and then added to each well to obtain

the final concentration of 100 μM and further concentrations were twice diluted. At up to 2% (v/v), DMSO had no effect on parasite growth. Each concentration was screened in triplicate. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The MTT cell proliferation assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution was added to lyse the cells and solubilize the coloured crystals. The samples were read using an ELISA plate reader at a wavelength of 570 nm. The amount of colour produced was directly proportional to the number of viable cells. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) after a 3-day incubation period. Pentamidine and amphotericin B were used as antileishmanial reference compounds.

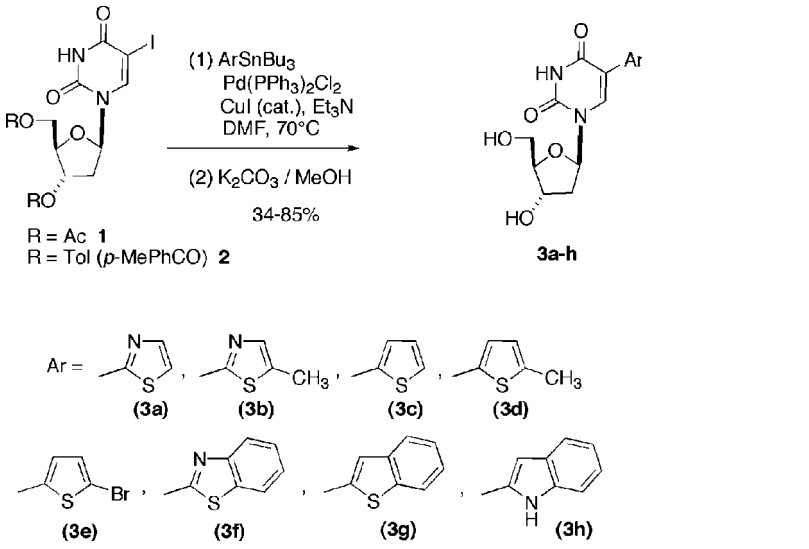
2.3.2. In vitro evaluation on intramacrophage amastigotes

Concerning the amastigote in vitro model, the macrophages were infected after a 24 h incubation period with promastigote forms of *L. donovani* DD8 wild type (WT) in a stationary phase at a ratio of 10 parasites per macrophage, to obtain 87% of infected macrophages and 10 ± 3 amastigotes per macrophage. At 18 h after the promastigotes had entered macrophages, the free promastigotes were eliminated and intramacrophagic amastigotes were treated at various concentrations of the compounds. Pentamidine and amphotericin B were used as reference compounds. Each experiment was performed in triplicate. The culture medium was renewed 48 h later and a new culture medium containing the drug was added. The experiment was stopped at day 5, and the percentage of infected macrophages was evaluated microscopically after Giemsa staining. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, and expressed in $\mu M \pm SD$.

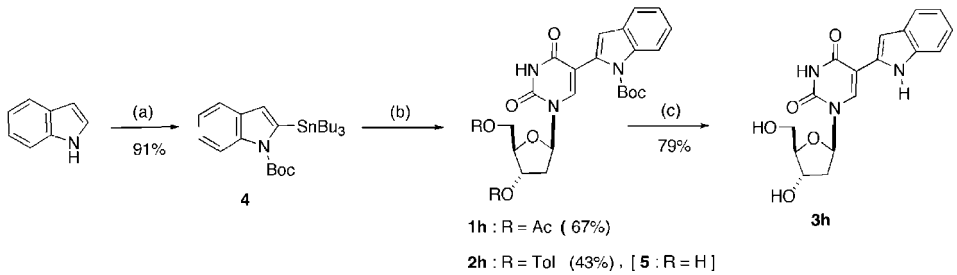
3. Results and discussion

The free nucleosides **3a–h** were synthesized in two to three steps and in good overall yields (34–85%). The key step is based on palladium catalyzed Stille cross coupling [7] of protected 5-iodo-2′-deoxyuridine **1** or **2** [8] and aryltin derivatives (Scheme 1). A typical example is shown in Scheme 2 for the synthesis of indole nucleoside **3h**. Thus, the indole-tin derivative **4** was first obtained in 91% overall yield from indole by amine protection (Boc_2O, DMAP) followed by metalation–transmetalation of the resulting protected indole (LDA/THF then Bu_3SnCl). Palladium coupling of **4** and 5-iodo-2′-deoxyuridine **1** or **2** afforded fluorescent products **1h** and **2h** in 67 and 43% yield, respectively. When the coupling step was carried out without CuI /Et_3N, products **1h** and **2h** were isolated in low yields together with their Boc-cleaved compounds [5]. **1h** and **2h** were then cleanly deprotected by using sequential treatment with K_2CO_3/MeOH and TFA to give the free nucleoside **3h**.

All compounds were evaluated for their in vitro antileishmanial activity (promastigotes and intramacrophagic amastigotes) and cytotoxicity (MTC). Zidovudine, a nucleoside compound, and the currently used drugs pentamidine and amphotericin



Scheme 1. Synthesis of 5-heteroaryl-2′-deoxyuridine.



Scheme 2. Typical synthesis: reagents and conditions: (a) (i) Boc_2O (1.2 equiv.), DMAP (10% molar), CH_3CN, (ii) LDA (1.2 equiv.), THF, −78 °C then Bu_3SnCl (1.2 equiv.); (b) **1** or **2**, $Pd(PPh_3)_2Cl_2$ (0.05 equiv.), DMF, CuI (0.1 equiv.); Et_3N (1.2 equiv.); (c) (i) K_2CO_3/MeOH (3 equiv.) to give **5** then (ii) aq. TFA, dioxane.

B were used as references. All results are listed in Table 1. The most active compound against promastigotes was Thia-dU (**3a**) ($IC_{50} = 3.1\ \mu M$) and all other compounds were at least 5–10-fold less active. Thia-dU **3a** exhibited the same activity as zidovudine used as nucleoside reference compound. The substituent on position 5 of the uridine ring had a minor effect on the activity against promastigotes. Thus, whatever the heterocycle was thiazole (**3a**), thiophene (**3c**) or their substituted (**3b**, **3d**, and **3e**) or benzannulated analogs (**3f**, **3g**, and **3h**), the activity was maintained at the same level. In the same manner, the replacement of the methyl group on the thiophene ring by a bromine atom (**3d** vs. **3e**) did not significantly change the activity against promastigotes. When a methyl group is added on the thiazole ring at position 5 (**3a** vs. **3b**), the IC_{50} decreased 10-fold.

Table 1
Antileishmanial activity of compounds **3a**–**h** (IC_{50} defined as inhibitory concentration 50%) and their toxicity on murine macrophage (MTC defined as a maximum tolerated concentration)

Compound (abbreviation)	*L. donovani* DD8		Cytotoxicity on peritoneal murine macrophages MTC (μM)
	Promastigotes IC_{50} (μM) ± SD	Intramacrophagic amastigotes IC_{50} (μM) ± SD	
3a (Thia-dU)	3.1 ± 0.2	>100	100
3b (Methia-dU)	26.2 ± 2.8	6.5 ± 0.7	50
3c (Thio-dU)	30.3 ± 3.2	>50	50
3d (MeThio-dU)	25.2 ± 1.9	>10	10
3e (BrThio-dU)	18.3 ± 1.5	>100	100
3f (BenzoThia-dU)	32.4 ± 3.3	>50	50
3g (BenzoThio-dU)	20.2 ± 1.8	>50	50
3h (Ind-dU)	19.1 ± 2.1	>50	50
Zidovudine	3.1 ± 0.4	>100	>100
Pentamidine	7.2 ± 0.6	14.6 ± 1.6	15
Amphotericin B	0.1 ± 0.1	0.4 ± 0.1	5

The antileishmanial evaluations were performed as previously described [6].

The activity against intramacrophagic amastigotes did not reflect the activity on promastigotes since only Methia-dU (**3b**) was active, with an IC_{50} at 6.5 μM. Therefore, some data have to be taken into consideration to explain these results. Zidovudine like Thia-dU (**3a**), although active on promastigotes was inactive on intramacrophagic amastigotes. Considering the low toxicity of these compounds on uninfected macrophages, they probably cannot enter the phagolysosome containing the amastigotes within the macrophage. It is assumed that nucleosides do not passively enter the parasite and *L. donovani*, like all other Kinetoplastida, is a purine auxotroph [9]. This parasite is incapable of purine biosynthesis and must therefore salvage purine nucleobases or nucleosides from its host. This process is initiated by purine transporters on the parasite cell surface [10]. Furthermore, it has been demonstrated that pyrimidine nucleosides are recognized by these transporters [11].

The difference of activities of compounds **3a**–**h** against the promastigote forms could be partially ascribed to the transporter recognition. Concerning the parasite stage, a recent study revealed that, unlike the promastigote stage, the amastigote possesses two distinct adenosine transporters T1 and T2 both with high affinities [12]. One of these transporters (T1) is identical to the adenosine/pyrimidine nucleoside transporter of the promastigote previously described. The second transporter (T2) is specific to the amastigote stage and only transports purine nucleosides. Therefore, we can consider that synthetic compounds **3a**–**h** could use the same transporter both in promastigote and amastigote forms and, the differences of activity observed could be related to the difficulty for the compound to cross over the phagolysosome membrane. Whereas the substituent on the heterocycle at the C-5 position was of high importance, the nature of the heterocycle itself (thiazole or a thiophene rings) did not induce great changes of the biological activities. Thia-dU (**3a**), the most active compound on promastigotes, seemed incapable to cross over the phagolysosome membrane whereas Methia-dU (**3b**) had significant activity on intramacrophagic amastigotes.

Furthermore, no compound from this series was active against sinefungin-resistant *L. donovani* LRC (data not shown) indicating a cross resistance between these nucleosides and sinefungin. A synergy was searched between Thia-dU (**3a**) or Methia-dU (**3b**) and pentamidine or amphotericin B. Surprisingly, no synergistic effect was observed despite different mode of action of the molecules in association.

4. Conclusion

In summary, we reported a short and efficient synthesis of nucleoside analogs together with their antileishmanial activity. All these compounds were active in vitro in micromolar range. Moreover, compound **3b** was active on both amastigotes and promastigotes and has now to be studied in vivo to assess its relevance in visceral leishmaniasis chemotherapy.

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