Synthesis of new 3-acetyl-1,3,4-oxadiazolines combined with pyrimidines as antileishmanial and antiviral agents

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Abstract
A new series of 3-acetyl-1,3,4-oxadiazoline hybrid molecules was designed and synthesized using a condensation between acyclonucleosides and substituted phenylhydrazone. All intermediates and final products were screened against Leishmania donovani, a Protozoan parasite and against three viruses SARS-CoV-2, HCMV and VZV. While no significant activity was observed against the viruses, the intermediate with 6-azatymine as thymine and 5-azathymine-3-acetyl-1,3,4-oxadiazoline hybrid exhibited a significant antileishmanial activity. The later compound was the most promising, exhibiting an IC50 value at 8.98 µM on L. donovani intramacrophage amastigotes and a moderate selectivity index value at 2.4.

Graphical Abstract

Keywords 3-Acetyl-1,3,4-oxadiazoline · Pyrimidines analogs · Antileishmanial activity · Antiviral activity
**Introduction**

Neglected tropical diseases (NTDs) are widespread in several countries in Africa, Europe, America, and Asia. Every year, NTDs contaminate millions of people in 149 countries, which cause wasting billions of dollars and claiming thousands of lives [1]. According to the World Health Organization (WHO), leishmaniases are a class of illnesses prompted by the Protozoan parasite Leishmania, showing four crucial clinical syndromes: post kala azar dermal leishmaniasis (PKDL), cutaneous leishmaniasis (CL), visceral leishmaniasis/kala azar (VL), and mucocutaneous leishmaniasis (MCL) [2].

On the other hand, the immune system response to the illnesses is altered by co-infection with the human immunodeficiency virus, HIV [3]. Furthermore, leishmaniases have an increased medical value in infected people with HIV-1 (human immunodeficiency virus type 1) because of the spread of both pathogens in several regions of the world (e.g., South America, India, the Mediterranean Basin, and many African countries). There is absolutely no doubt that the exact number of reported cases of co-infection is undercounted due to various issues with HIV-1 infection, leishmaniasis, or both in the setting of developing countries [4]. Ellen Heirwegh et al., reported that *Leishmania major* with phleboviral infection was more infectious than *L. major* alone. A better understanding of the possible role of viral co-infection might lead to more effective treatment regimens [5]. One of the key challenges in the management of leishmaniasis and viral disease co-infection is the invention of a clinically effective treatment that not only treats leishmaniasis but also prevents viral disease. However, there is a lack of knowledge on the correlation between leishmaniasis and viral disease co-infection. Moreover, Antonis Pikoulas et al., presented a case of co-infection with COVID-19 and visceral leishmaniasis, and discuss recent reports on co-existence of leishmania and SARS-CoV2 spp. to date [6].

Current treatments for visceral leishmaniasis including antimonials, liposomal amphotericin B, miltefosine, and paromomycin, have some issues related to toxicity, emerging resistance, high cost and relatively long treatment regimens. Therefore, there is an urgent need for the development of new and better medicines [7, 8].

1,3,4-Oxadiazoles are a very important series in chemistry as they have several biological activities. The 1,3,4-oxadiazole scaffold is present in numerous drugs: Nesapidil® (anti-hypertensive) [9], Furamizol® (antibiotic) [10], Raltegravir® (anti-retroviral) [11], and Zibotentan® (anticancer) [12, 13]. We also notice that the *N*-acetyl-1,3,4-oxadiazoline is among the derivatives of 1,3,4-oxadiazoles that showed different antileishmanial activities. For instance, Taha et al. have synthesized phenyl-linked oxadiazoline–phenylhydrazone hybrids I as the most potential antileishmanial candidate, with an IC₅₀ value of 0.95 ± 0.01 µM [14]. Moreover, another series of quinolinyl–oxadiazole hybrids had promising antileishmanial activities, and compound II emerged as the most potent agent with an IC₅₀ value of 0.10 ± 0.001 µM [15]. Meanwhile, some *N*-acetyl-1,3,4-oxadiazoline derivatives have been reported as antiviral agents. For instance, Ali et al. prepared a series of monosaccharide 1-acetyl-hydrazinouracils that was evaluated for antiviral activity against hepatitis B virus and showed moderate activities III [16]. Moreover, they showed activities as antibacterial [17, 18], antimicrobial [19, 20], antifungal [21], and anti-inflammatory [22] (Fig. 1).

Substituted pyrimidines are widely available in living organisms and are one of the most important compounds studied by chemists. Pyrimidines, including thymine, uracil, and derivatives, are among the most abundant heterocyclic diazines. According to the literature, pyrimidine derivatives exhibit a wide range of pharmacological properties, including antifungal activity [23, 24], antibacterial [25, 26], antileishmanial [27], antiviral [28, 29], and anticancer [30, 31]. A rational discovery of novel antiparasitic drugs should be based on parasite-specific metabolisms [32]. The biosynthesis of pyrimidine is a biologically important process that occurs via both de novo production and pyrimidine salvage routes. The enzymes of this pathway are considered potential drug targets. Meanwhile, several studies have shown that pyrimidines have widely antileishmanial activities. For instance, the synthesis of pyrimidine derivatives led to the discovery of the potent compound P1 (Fig. 2) with an EC₅₀ value of 0.65 µM against intracellular *Leishmania donovani* amastigotes [33]. Moreover, pyrimidines P2 have been demonstrated to be efficacious in vivo, with each compound leading to 78% parasite inhibition when dosed in *L. donovani* infected hamsters for five days (50 mg kg⁻¹, i.p.) [34]. Finally, Chauhan and coworkers have described that aza-pyrimidine–pentamidine hybrids P3 have promising activity on intracellular *L. donovani* amastigotes (EC₅₀ values of < 1 µM) [35].

Prompted with these literature reports, we designed some novel hybrid molecules which combined the pyrimidine and 3-acetyl-1,3,4-oxadiazoline scaffolds in order to investigate firstly their in vitro antileishmanial activity against the axenic amastigotes and intramacrophage amastigotes forms of *L. donovani*, and second, their antiviral activity against SARS-CoV-2, HCMV, and VZV (Fig. 3).
Results and discussion

Chemistry

The synthetic strategy to achieve compounds (14–23) is shown in Schemes 1, 2, and 3. The crucial step for synthesizing acyclonucleosides derivatives (7–10) (Scheme 1) known as modified Hilbert-Johnson reaction [36] corresponded to the condensation between silylated nucleobases (3′–6′) and alkylating agent 2 [37]. The desired products were obtained in synthetically good yields between 53 and 84%. Thereafter, the methyl benzoate derivatives (11″–13″) were subjected to a hydrazinolysis reaction after treatment with 80% aqueous hydrazine hydrate in ethanol under reflux to afford the corresponding hydrazides (11–13) in good yield (Scheme 2). The latter were reacted with the aldehyde derivatives (7–10) to give the imine derivatives (14′–23′) which were treated with acetic anhydride under heating (155 °C) to promote cyclization and furnished the N3-acetyl-1,3,4-oxadiazoline derivatives (14–23) in yield ranging from 40 to 71%. All the oxadiazoles were completely characterized using NMR (13C and 1H), IR, and high-resolution mass spectrometry.
In the $^1$H NMR spectra, the signal for the hydrogen present in the oxadiazole ring (H-2) was observed within the 7.20–7.67 ppm range. The hydrogen atoms of methylene group attached to nitrogen (nucleobases) were noticed as singlet in the $^1$H NMR spectra. The carbon chemical shifts are compatible with the structures of the compounds. In the IR spectra, expected bands for functional groups were noticed. The exact mass of the hybrid molecules (14–23) was confirmed by high-resolution mass spectrometry analyses.

**Biological evaluation**

**Antileishmanial activity**

The novel series of nucleosides analogs of 3-acetyl-1,3,4-oxadiazoline 14–23 and alkylated pyrimidines (uracil, thymine, 5-fluorouracil, and 5-azathymine) 7–10 were tested for their biological activity. This series was not highly cytotoxic on the RAW 264.7 macrophage model since eleven compounds exhibited CC$_{50}$ values superior to 100 µM. However, the most cytotoxic (compounds 10, 20, 23) with CC$_{50}$ values in a range from 19 to 56 µM, were the single ones having a significant antileishmanial activity against the axenic amastigotes and intramacrophage amastigotes forms of *L. donovani*.
The IC\textsubscript{50} and CC\textsubscript{50} values of the compounds were compared to those of miltefosine as the reference drug. The results, presented in Table 1, clearly showed that the starting material compound 10 presented a moderate activity (CC\textsubscript{50} = 56.15 µM, IC\textsubscript{50} = 29.02 µM (Axenic amastigotes), IC\textsubscript{50} = 29.96 µM (intramacrophage amastigotes), and SI = 1.87). It is worth to note that when thymine as a nucleobase 8 was changed to 6-azathymine 10, the IC\textsubscript{50} decrease from 100 to 29.02 µM. Furthermore, when the oxadiazole group is introduced in the hybrid molecule 20, the IC\textsubscript{50} decrease from 29.02 to 6.05 µM. The most active derivatives 20 showed a significant activity: IC\textsubscript{50} = 6.05 µM (Axenic amastigotes) and IC\textsubscript{50} = 8.98 µM (Intramacrophage amastigotes) CC\textsubscript{50} = 21.66 µM and very moderate selectivity index value of up to 2.41 comparatively to miltefosine (Table 1).

The structure–activity relationship showed that oxadiazole derivatives 14–23 had better activity than the acyclonucleosides 7–10. As a result, it was concluded that the creation of a stereoisomer center C2 on the oxadiazole ring may influence the antileishmanial activity, this was supported by the fact that the oxadiazole derivative 20 was 5 times more potent than the acyclonucleoside derivative 10. From the biological data collected with derivatives 19 and 23 having 5-fluorouracil as a nucleobase, SARs showed that, at the para position of the phenyl moiety, the chlorine substituent was favorable while the meta-chlorine was not suitable for providing antileishmanial activity.

Moreover, a comparison of the activity profiles of compounds 10 and 20 draws speculation that the oxadiazole group may be serving as a chromophore group. Thus, additional modifications will be pursued in subsequent studies, as this approach has been shown to greatly enhance antileishmanial activity of oxadiazole analogs.

**Determination of compound lipophilicity**

There are physical differences between the test systems on promastigote and amastigote forms of \textit{L. donovani}, which
could explain the obtained results. The promastigote test employs an extracellular axenic parasite, which is more accessible than the intracellular amastigotes. Indeed, the parasite starts the creation of a membrane (parasitophorous vacuole membrane, which surrounds the intracellular parasite) during the invasion process. Permeation of the two membranes (plasmatic and vacuole) is a critical criterion for activity and varies depending on the hydrophobicity of the compound [38, 39].

The octanol/water partition coefficients (ClogP) reflect the hydrophobicity, and products that have higher ClogP are more lipophilic. Chemdraw was used to predict partition coefficients in this study. As shown in Table 1, we notice that the incorporation of 1,3,4-oxadiazoline increases the hydrophobic character. For instance, when we compare compound 10 and 20, ClogP is enhanced by about two times, which explain that oxadiazoline 20 is more active than 10. Similarly, by comparing all nucleobases, 6-azathymine is the most hydrophobic, which follows the results of biological activities.

### Antiviral activity

All the synthesized compounds (7–10, 14–23) were evaluated for their antiviral activities against human cytomegalovirus (HCMV) and human varicella-zoster virus (VZV), both thymidine kinase-deficient TK and wild type. The pyrimidine–1,3,4-oxadiazole hybrids were unable to substantially inhibit the replication of these two DNA viruses at non-toxic concentrations, in contrast to the compounds used as reference (Ganciclovir, and Cidofovir against for HCMV, and Acyclovir for VZV) (Tables 2, 3).

Only three compounds 10, 17, and 19 showed moderate activity against VZV with EC₅₀ = 46.95 µM, 43.02 µM, and 34.38 µM, respectively. We notice that when the fluorine atom is present in the acyclonucleoside, the value of EC₅₀ decreases from 43.02 µM for uracil acyclonucleoside 17 to 34.38 µM for 5-fluorouracil acyclonucleoside 19 (Table 2). Whereas only compound 10 is moderately active against HCMV with EC₅₀ = 63.14 µM (AD-169 strain) and 44.72 µM (Davis strain) (Table 3).

The rapidly developing pandemic, known as coronavirus disease 2019 (COVID-19), is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The rapid global emergence of SARS-CoV-2 has advanced research efforts toward the development of therapeutic intervention and finding viral drug to control the pandemic. The functional importance of RNA-dependent RNA polymerase (RdRp) in the viral life cycle, makes it an attractive target for designing antiviral drugs. Moreover, SARS-CoV-2 is an RNA virus like HCV, HIV, and other flaviviruses that share a similar replication mechanism requiring an RNA-dependent RNA polymerase (RdRp). In addition, the most promising broad-spectrum class of viral RdRp inhibitors are nucleos(t) ide analogs [40, 41]. Elfiky used a molecular docking study to predict that Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir may have inhibitory activity against SARS-CoV-2 RdRp [42]. Significant efforts have been made to discover novel or repurposed therapeutics to select the best candidates for the management of COVID-19 disease. To date, the US Food and Drug Administration (US FDA) has proved eleven agents emergency for the treatment of COVID patients, including two nucleosides analogs: remdesivir and Beta-d-N4-hydroxycytidine (NHC, molnupiravir) [43]. In this context, all synthesized hybrid molecule analogs
Table 2  Anti-VZV activities of compounds 7–10 and 14–23

| Product  | Antiviral activity EC\textsubscript{50} (µM)\textsuperscript{a} | Cytotoxicity (µM) |  |
|----------|---------------------------------------------------------------|------------------|---|
|          | TK\textsuperscript{+} VZV strain | TK\textsuperscript{−} VZV strain | Cell morphology | Cell growth |
| OKA 07-1 | (MCC)\textsuperscript{b} | (CC\textsubscript{50})\textsuperscript{c} |  |
| 7        | > 100 | > 100 | > 100 | ND |
| 8        | > 100 | > 100 | > 100 | ND |
| 9        | > 100 | > 100 | > 100 | ND |
| 10       | 46.95 | > 20 | ≥ 20 | ND |
| 14       | > 100 | > 100 | ≥ 100 | ND |
| 15       | > 100 | > 100 | ≥ 20 | ND |
| 16       | > 100 | > 100 | ≥ 100 | ND |
| 17       | 43.02 | > 20 | 100 | ND |
| 18       | > 20 | > 20 | 100 | ND |
| 19       | 34.38 | > 20 | ≥ 100 | ND |
| 20       | > 20 | > 20 | 100 | ND |
| 21       | > 20 | > 20 | 100 | ND |
| 22       | > 20 | > 20 | 100 | ND |
| 23       | > 20 | > 20 | 100 | ND |
| Acyclovir (ACV) | 6.26 | 46.80 | > 444 | > 444 |

\textsuperscript{a}Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU)

\textsuperscript{b}Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology

\textsuperscript{c}Cytostatic concentration required to reduce cell growth by 50%

Table 3  Anti-HCMV activities of compounds 7–10 and 14–23

| Product  | Antiviral activity EC\textsubscript{50} (µM)\textsuperscript{a} | Cytotoxicity (µM) |  |
|----------|---------------------------------------------------------------|------------------|---|
|          | AD-169 strain | Davis strain | Cell morphology | Cell growth |
| AD-169 strain | (MCC)\textsuperscript{b} | (CC\textsubscript{50})\textsuperscript{c} |  |
| 7        | > 100 | > 100 | ≥ 100 | ND |
| 8        | > 20 | > 100 | ≥ 100 | ND |
| 9        | > 100 | > 100 | 100 | ND |
| 10       | 63.14 | 44.72 | ≥ 100 | ND |
| 14       | > 100 | > 100 | 100 | ND |
| 15       | > 100 | > 100 | ≥ 100 | ND |
| 16       | > 100 | > 100 | 100 | ND |
| 17       | > 100 | > 100 | 100 | ND |
| 18       | > 20 | > 20 | 100 | ND |
| 19       | > 20 | > 20 | 100 | ND |
| 20       | > 20 | > 20 | ≥ 20 | ND |
| 21       | > 20 | > 20 | 100 | ND |
| 22       | > 20 | > 20 | ≥ 20 | ND |
| 23       | > 20 | > 20 | 100 | ND |
| Ganciclovir (GCV) | 6.5 ± 1.77 | 1.64 ± 0.22 | > 394 | > 394 |
| Cidofovir (CDV) | 0.84 ± 0.21 | 0.12 ± 0.03 | 317 | > 317 |

\textsuperscript{a}Effective concentration required to reduce virus-induced cytopathic effect by 50%. Virus input was 100 plaque forming units (PFU)

\textsuperscript{b}Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology

\textsuperscript{c}Cytostatic concentration required to reduce cell growth by 50%
Experimental section

Chemistry

General

Melting points have been measured without correction using a Büchi B-545 electronic capillary melting temperature apparatus. TLC was employed to check reactions utilizing aluminum sheets coated with Merck’s silica gel 60 F254. FTIR spectra were taken on a Perkin-Elmer VERTEX 70 FTIR spectroscopy covering the frequency 400–4000 cm⁻¹. Melting points have been measured without correction using a Büchi B-545 electronic capillary melting temperature apparatus.

HPLC/MS conditions

HPLC/MS was performed on a Thermo Scientific Dionex Ultimate 3000 consisting of a quaternary (HPG-3400RS) pump, a WPS-3000 (TSL) ultraviolet detector (DAD), an autosampler, a TCC-300 column oven, and a quaternary (HPG-3400RS) pump, a WPS-3000 (TSL) ultraviolet detector (DAD). The HPLC/MS system flow rate was set to 1.0 mL min⁻¹, and the solvent used was acetonitrile (A) and water/0.1% formic acid (B) with an elution program as follows: 20% of (A) for 8.6 min and 85% of (A) for 5.4 min. The solvent system flow rate was set to 1.0 mL min⁻¹, and the sample injection volume was 20 L. UV–Vis detection was monitored at 260 nm, while DAD acquisition was done between 200 and 600 nm.

General procedure for the preparation of the 2-hydroxybenzaldehyde homonucleosides

The mixture of the pyrimidine base (1 mmol) (3–6) and ammonium sulfate (0.10 mmol, 10 mg) in hexamethyldisilazane (1 mL) was refluxed (3 h) at 120 °C to give a clear solution (silylation step). Then the benzyl derivative (2.5 mmol, 425 mg), KI (0.50 mmol, 84 mg) and the solvent (acetonitrile) (5 mL) were added. The mixture was heated at 85 °C for 12 h, diluted with dichloromethane/methanol, and evaporated to dryness. The residue was purified by flash chromatography (solvent CH₂Cl₂/MeOH 99/1).

5-((2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl)-2-hydroxybenzaldehyde (7) 

Rf = 0.26 [CH₂Cl₂/MeOH (97/3)]. Yield = 84%, m = 207 mg. Mp = (218–220) °C. UV (methanol) λₘₐₓ = 259 nm. IR ν (cm⁻¹): 3026 (=CH); 1696 (C=O aldehyde); 1364 (CH₂). ¹H NMR (300 MHz, DMSO-d-6) δ (ppm): 4.76 (s, 2H, CH₂); 10.78 (s, 1H, OH); 11.30 (s, 1H, H-3). ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 11.88 (CH₃); 48.90 (CH₂); 109.00 (C₆H₅); 135.92 (C₆H₅); 141.07 (C₆H₅); 150.95 (C₆H₅); 160.40 (C₆H₅); 163.59 (C₆H₅); 190.82 (C-aldehyde). HPLC/MS (m/z) 247.07 (M+H)⁺, HRMS for C₁₂H₁₁N₂O₄: Calc 247.0713, Found 247.0716.

2-Hydroxy-5-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl) benzaldehyde (8) 

Rf = 0.38 [CH₂Cl₂/MeOH (97/3)]. Yield = 76%, m = 198 mg. Mp = (218–220) °C. UV (methanol) λₘₐₓ = 259 nm. IR ν (cm⁻¹): 3026 (=CH); 1696 (C=O aldehyde); 1364 (CH₂). ¹H NMR (300 MHz, DMSO-d-6) δ (ppm): 1.74 (s, 3H, CH₃); 4.76 (s, 2H, CH₂); 4.80 (s, 2H, CH₂); 6.97 (d, J=8.4 Hz, 1H, H₅); 7.47 (d, J=8.4 Hz, 1H, H₆); 7.59 (s, 1H, H-6); 7.63 (s, 1H, H₅p); 10.24 (s, 1H, H-aldehyde); 10.76 (s, 1H, OH); 11.29 (s, 1H, H-3). ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 49.63 (CH₃); 101.22 (C₆H₅); 117.63 (C₆H₅); 122.20 (C₆H₅); 127.84 (C₆H₅); 127.93 (C₆H₅); 135.94 (C₆H₅); 145.40 (C₆H₅); 150.95 (C₆H₅); 160.40 (C₆H₅); 163.59 (C₆H₅); 190.80 (C-aldehyde). HPLC/MS (m/z) 265.06 (M+H)⁺, HRMS for C₁₂H₁₀FN₂O₄: Calc 265.0619, Found 265.0623.

2-Hydroxy-5-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl)-2-hydroxybenzaldehyde (9) 

Rf = 0.37 [CH₂Cl₂/MeOH (97/3)]. Yield = 72%, m = 190 mg. Mp = (236–238) °C. UV (methanol) λₘₐₓ = 258 nm. IR ν (cm⁻¹): 3026 (=CH); 1691 (C=O aldehyde); 1348 (CH₂). ¹H NMR (300 MHz, DMSO-d-6) δ (ppm): 4.76 (s, 2H, CH₂); 6.97 (d, J=8.4 Hz, 1H, H₅); 7.49 (d, J=8.4 Hz, 1H, H₆); 7.63 (s, 1H, H₅p); 8.21 (d, J=6.9 Hz, 1H, H-6); 10.24 (s, 1H, H-aldehyde); 10.78 (s, 1H, OH); 11.82 (s, 1H, H-3). ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 48.61 (CH₃); 117.59 (C₆H₅); 122.10 (C₆H₅); 127.37 (C₆H₅); 128.27 (C₆H₅); 129.60 (d, J=30.8 Hz, C-5); 149.58 (C-2); 160.30 (C₆H₅); 157.16 (d, J=25.75 Hz, C-4); 190.82 (C-aldehyde). HPLC/MS (m/z) 265.06 (M+H)⁺, HRMS for C₁₂H₁₀FN₂O₄: Calc 265.0619, Found 265.0623.
2-Hydroxy-5-[(6-methyl-3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl) methyl] benzaldehyde (10) \( R_1 = 0.47 \) \( \text{CH}_2\text{Cl}_2/\text{MeOH} \ (97/3) \). Yield = 53\%, \( m = 138 \) mg. Mp = (198–200 °C). UV (methanol) \( \lambda_{\text{max}} = 278 \) nm. IR \( \nu \) (cm\(^{-1}\)):

\( 3018(=\text{CH}) \); 1664 (C=O aldehyde); 1379 (C-H aromatic); 11.35 (s, 1H, H-3). \(^{13}C\) NMR (75 MHz, DMSO d-6) \( \delta \) (ppm):

123.81 (Cph); 124.35 (Cph); 126.42 (Cph); 128.19 (Cph); 135.94 (Cph); 143.44 (C-5); 148.69 (C-2); 157.02 (C=O aldehyde); 167.02 (CO acetyl). HPLC/MS \( (m/z) \) 262.08 (M+H)

HRMS for C\(_{12}\)H\(_{12}\)N\(_3\)O\(_4\): Calc 262.0822, Found 262.0823.

General procedure for the preparation of the \( N \)-acetyl 1,3,4 oxadiazol homonucleosides

A mixture of 2-hydroxy benzaldehyde homonucleosides (0.3 mmol) \((7–10)\) and benzoic hydrazide acid (0.36 mmol) \((11–13)\) was refluxed in ethanol (4 mL) for 3 h in the presence of catalytic amount of acetic anhydride, added, and agitated at 155 °C for 90 min. The reaction mixture was evaporated to dryness, 3 mL of acetic acid added, and agitated at 155 °C for 90 min. The solution was poured into ice and neutralized with NaHCO\(_3\). The obtained solid was filtered and purified by flash chromatography \( \text{CH}_2\text{Cl}_2/\text{MeOH} \ 98/2 \).

2-(3-Acetyl-5-phenyl-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl phenyl acetate (14) \( R_1 = 0.26 \) \( \text{CH}_2\text{Cl}_2/\text{MeOH} \ (97/3) \). Yield = 50\%, \( m = 67 \) mg. Mp = (214–216 °C). UV (methanol) \( \lambda_{\text{max}} = 271 \) nm. IR \( \nu \) (cm\(^{-1}\)):

3459 (NH); 1765 (C=O aldehyde); 1379 (C-H aromatic); 11.35 (s, 1H, H-3). \(^{13}C\) NMR (75 MHz, DMSO d-6) \( \delta \) (ppm):

123.81 (Cph); 124.35 (Cph); 126.42 (Cph); 128.19 (Cph); 135.94 (Cph); 143.44 (C-5); 148.69 (C-2); 157.02 (C-4); 160.21 (Cph); 191.12 (C-aldehyde). HPLC/MS \( (m/z) \) 449.15 (M+H)

HRMS for C\(_{23}\)H\(_{21}\)N\(_4\)O\(_6\): Calc 449.1456, Found 449.1453.

2-(3-Acetyl-5-phenyl-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl phenyl acetate (15) \( R_1 = 0.31 \) \( \text{CH}_2\text{Cl}_2/\text{MeOH} \ (97/3) \). Yield = 47\%, \( m = 66 \) mg. Mp = (153–155 °C). UV (methanol) \( \lambda_{\text{max}} = 278 \) nm. IR \( \nu \) (cm\(^{-1}\)):

3480 (NH); 1760 (C=O aldehyde); 1379 (C-H aromatic); 11.35 (s, 1H, H-3). \(^{13}C\) NMR (75 MHz, DMSO d-6) \( \delta \) (ppm):

123.81 (Cph); 124.35 (Cph); 126.42 (Cph); 128.19 (Cph); 135.94 (Cph); 143.44 (C-5); 148.69 (C-2); 157.02 (C-4); 160.21 (Cph); 191.12 (C-aldehyde). HPLC/MS \( (m/z) \) 467.14 (M+H)

HRMS for C\(_{24}\)H\(_{23}\)N\(_4\)O\(_6\): Calc 467.1361, Found 467.1364.

2-(3-Acetyl-5-phenyl-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl phenyl acetate (16) \( R_1 = 0.31 \) \( \text{CH}_2\text{Cl}_2/\text{MeOH} \ (97/3) \). Yield = 50\%, \( m = 89 \) mg. Mp = (229–231 °C). UV (methanol) \( \lambda_{\text{max}} = 278 \) nm. IR \( \nu \) (cm\(^{-1}\)):

3436 (NH); 1763 (C=O); 1689 (C=O); 689 (OH phenol). \(^{1}H\) NMR (300 MHz, DMSO d-6) \( \delta \) (ppm):

7.48 (s, 1H, H-6); 7.39–7.80 (m, 8H, H-aromatic, H-6); 7.49–7.80 (m, 8H, H-aromatic); 11.36 (s, 1H, H-3). \(^{13}C\) NMR (75 MHz, DMSO d-6) \( \delta \) (ppm):

123.81 (Cph); 124.35 (Cph); 126.42 (Cph); 128.19 (Cph); 135.94 (Cph); 143.44 (C-5); 148.69 (C-2); 157.02 (C-4); 160.21 (Cph); 191.12 (C-aldehyde). HPLC/MS \( (m/z) \) 483.11 (M+H)

HRMS for C\(_{24}\)H\(_{23}\)F\(_3\)N\(_4\)O\(_6\): Calc 483.1164, Found 483.1164.
2-(3-Acetyl-5-(3-chlorophenyl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl) phenyl acetate (18) 


gave δmax = 277 nm. IR ν (cm⁻¹): 3486 (NH); 1769 (C=O); 1678 (C=C); 1204 (C–N); 694 (OH phenol). ¹H NMR (300 MHz, DMSO d-6) δ (ppm): 1.74 (s, 3H, CH₃); 2.12 (s, 3H, CH₃); 2.19 (s, 3H, CH₃); 4.87 (s, 2H, CH₂); 7.19 (s, 1H, H-oxadiazoline); 7.66 (s, 1H, H-6); 7.39–7.76 (m, 7H, H-aromatic); 11.34 (s, 1H, H-3). ¹³C NMR (75 MHz, DMSO d-6) δ (ppm): 119.93 (Cph); 125.06 (Cph); 125.74 (Cph); 125.85 (Cph); 127.99 (Cph); 128.60 (Cph); 130.03 (Cph); 131.22 (Cph); 131.75 (Cph); 133.80 (Cph); 134.95 (Cph); 141.17 (C-6); 148.23 (Cph); 150.97 (C-2); 153.42 (C-oxadiazoline aromatic); 164.18 (C-4); 166.41 (CO acetyl); 168.51 (CO acetyl). HPLC/MS (m/z) 501.0972 (M+H)⁺, HRMS for C₂₅H₂₂ClN₅O₆: Calc 501.0975, Found 501.0976.

2-(3-Acetyl-5-(3-chlorophenyl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl) phenyl acetate (19) 


gave δmax = 277 nm. IR ν (cm⁻¹): 3486 (NH); 1769 (C=O); 1678 (C=C); 1204 (C–N); 694 (OH phenol). ¹H NMR (300 MHz, DMSO d-6) δ (ppm): 1.74 (s, 3H, CH₃); 2.12 (s, 3H, CH₃); 2.19 (s, 3H, CH₃); 4.87 (s, 2H, CH₂); 7.19 (s, 1H, H-oxadiazoline); 7.43–7.76 (m, 7H, H-aromatic); 8.24 (d, 1H, J_H-F = 6 Hz, H-6); 11.88 (s, 1H, H-3). NMR ¹³C (75 MHz, DMSO d-6) δ (ppm): 20.48 (CH₃); 20.95 (CH₃); 49.89 (CH₂); 90.49 (C-oxadiazoline); 124.33 (Cph); 125.06 (Cph); 125.86 (Cph); 128.00 (Cph); 128.76 (Cph); 129.70 (Cph); 130.13 (d, J_C-C=1.2 Hz, C-6); 131.23 (Cph); 131.75 (Cph); 133.80 (Cph); 134.37 (Cph); 148.33 (C-2); 149.63 (Cph); 153.38 (C-oxadiazoline); 154.07 (d, J_C-C=1.2 Hz, C-5); 167.70 (CO acetyl). HPLC/MS (m/z) 501.0972 (M+H)⁺, HRMS for C₂₅H₂₂ClN₅O₆: Calc 501.0972, Found 501.0972.

2-(3-Acetyl-5-(3-chlorophenyl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl) phenyl acetate (20) 


gave δmax = 278 nm. IR ν (cm⁻¹): 3449 (NH); 1762 (C=O); 1702 (C=C); 1203 (C–N); 698 (OH phenol). ¹H NMR (300 MHz, DMSO d-6) δ (ppm): 1.74 (s, 3H, CH₃); 2.12 (s, 3H, CH₃); 2.18 (s, 3H, CH₃); 2.19 (s, 3H, CH₃); 4.87 (s, 2H, CH₂); 7.19 (s, 1H, H-oxadiazoline); 7.38–7.81 (m, 7H, H-aromatic); 11.34 (s, 1H, H-3). NMR ¹³C (75 MHz, DMSO d-6) δ (ppm): 20.48 (CH₃); 20.95 (CH₃); 49.89 (CH₂); 90.49 (C-oxadiazoline); 124.33 (Cph); 125.06 (Cph); 125.86 (Cph); 128.00 (Cph); 128.76 (Cph); 129.70 (Cph); 130.13 (d, J_C-C=1.2 Hz, C-6); 131.23 (Cph); 131.75 (Cph); 133.80 (Cph); 134.37 (Cph); 148.33 (C-2); 149.63 (Cph); 153.38 (C-oxadiazoline); 154.07 (d, J_C-C=1.2 Hz, C-5); 167.70 (CO acetyl). HPLC/MS (m/z) 501.0972 (M+H)⁺, HRMS for C₂₅H₂₂ClN₅O₆: Calc 501.0972, Found 501.0972.

2-(3-Acetyl-5-(4-chlorophenyl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)-4-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl) phenyl acetate (21) 


gave δmax = 278 nm. IR ν (cm⁻¹): 3446 (NH); 1762 (C=O); 1702 (C=C); 1203 (C–N); 698 (OH phenol). ¹H NMR (300 MHz, DMSO d-6) δ (ppm): 1.74 (s, 3H, CH₃); 2.12 (s, 3H, CH₃); 2.18 (s, 3H, CH₃); 2.19 (s, 3H, CH₃); 4.87 (s, 2H, CH₂); 7.19 (s, 1H, H-oxadiazoline); 7.38–7.81 (m, 7H, H-aromatic); 11.34 (s, 1H, H-3). NMR ¹³C (75 MHz, DMSO d-6) δ (ppm): 20.48 (CH₃); 20.95 (CH₃); 49.89 (CH₂); 90.49 (C-oxadiazoline); 124.33 (Cph); 125.06 (Cph); 125.86 (Cph); 128.00 (Cph); 128.76 (Cph); 129.70 (Cph); 130.13 (d, J_C-C=1.2 Hz, C-6); 131.23 (Cph); 131.75 (Cph); 133.80 (Cph); 134.37 (Cph); 148.33 (C-2); 149.63 (Cph); 153.38 (C-oxadiazoline); 154.07 (d, J_C-C=1.2 Hz, C-5); 167.70 (CO acetyl). HPLC/MS (m/z) 501.0972 (M+H)⁺, HRMS for C₂₅H₂₂ClN₅O₆: Calc 501.0972, Found 501.0972.
1H, H-oxadiazoline); 7.42–7.81 (m, 7H, H-aromatic); 8.23
d (1H, JH–H = 6 Hz, H-6); 11.88 (s, 1H, H-3). 13C NMR
(75 MHz, DMSO d-6) δ (ppm): 20.51  (CH3); 20.94  (CH3);
49.96  (CH2); 90.38 (C-oxadiazoline); 122.70  (Cph); 124.32
(Cph); 128.06  (Cph); 128.18  (Cph); 128.75 (Cph); 129.33
(Cph); 129.71 (Cph); 134.35 (Cph); 136.58 (Cph); 130.15 (d,
J = 4.57 Hz, C-6); 145.51 (Cph); 148.32 (C-2); 153.85
(C-oxadiazoline); 141.23 (d, J = 222.07 Hz, C-5); 157.58 (d, J = 22.8 Hz, C-4); 166.31 (CO acetyl); 168.46
(CO acetyl). HPLC/MS (m/z) 501.10 (M+H) +, HRMS for
C23H19ClFN4O6: Calc 501.0972, Found 501.0970.

Materials and methods in biology

SARS-CoV-2

The used SARS-CoV-2 was derived from the Beta-Cov/
Belgium/GHB-03021/2020 (EPI ISL407976|2020-02-03),
which has been isolated in February 2020 from a Belgian
patient having returned from Wuhan. The isolate was passed
through VeroE6 cells seven times, producing two series of
amino acid deletions in the spike protein [44]. Titration on
Vero E6 cells was used to determine the infectious material
of the viral stock.

The antiviral assay for SARS-CoV-2 is established on the
previous used SARS-CoV assay [45]. After infection, the
fluorescence of VeroE6-GFP cell cultures declines because
of the cytopathogenic influence of the replicating virus. The
cytopathogenicity is inhibited, and the fluorescent signal is
maintained in the presence of an antiviral agent. To this end,
VeroE6-GFP cells (kindly provided by Marnix Van Loock,
Janssen Pharmaceutica, Beerse, Belgium) have been used as
described previously [46, 47]. Because VeroE6 cells exhibit
high chemotype efflux, the antiviral assays were carried out
in the presence of the P-glycoprotein (Pgp) efflux inhibitor
CP-100356 (0.5 M) [48].

VZV and HCMV

The synthetized compounds were tested against two human
herpesviruses {cytomegalovirus (HCMV) strains AD-169
and Davis varicella-zoster virus strains [Oka (wild type) and
07-1 (thymidine kinase-deficient strain)]} in human embry-
onic lung (HEL) fibroblasts as reported previously [49].

Antileishmanial evaluation

Cell lines

The mouse monocyte/macrophage cell lines RAW 264.7 and
L. donovani (MHOM/ET/67/HU3, also called LV9) promas-
tigotes and axenic amastigotes were kept in accordance with
the procedures presented by Pomel et al. [50]

Cytotoxicity evaluation of the compounds on RAW 264.7
macrophages

The resazurin technique, described in Pomel et al., was
used to assess cytotoxicity in RAW 264.7 macrophages
[50].

In vitro antileishmanial evaluation on L. donovani axenic
amastigotes

This evaluation was performed using the SYBR Green
method as previously described [50]. IC50 values were cal-
culated using the IC Estimator version 1.2 software [50].
Miltefosine was used as the reference drug.

Evaluation of in vitro antileishmanial on intramacrophage
amastigotes

Cytotoxicity determination, as described above, was
employed to choose the highest concentrations of drug that
could be investigated on the L. donovani intramacrophage
amastigote model using RAW 264.7 cells. Macrophages
were contaminated with L. donovani axenic amastigotes at
a 10 parasites per macrophage ratio. The rate of infected
macrophages was about 80% in these conditions, as well
as the mean number of amastigotes for each infected mac-
rophage was 4 to 5 in the untreated controls. The in vitro
treatment was applied 24 h post-infection during 48 h. The
results of the product’s effect are given as rate reduction of
parasite growth, measured using the SYBR Green incorpora-
tion method. The activity of the compounds is expressed as
IC50, calculated using the IC Estimator version 1.2 software
[50]. Miltefosine was used as the reference drug.

Conclusion

In conclusion, a novel series of homonucleosides analogs of
N-acetyl-1,3,4-oxadiazoline was synthesized and evalu-
ated for their antileishmanial and antiviral activities against
HCM, VZV, and SARS-CoV-2. Furthermore, the synthetic
strategy to synthetize hybrid compounds was proved to be
simple and efficient. Compound 20 was the most active with
IC50 value less than 10 µM against the axenic and intramac-
rophage amastigotes forms of L. donovani. The absence of
significant antiviral activity of this series prompts us to focus
further studies on its antileishmanial potential but trying to
enhance its antiviral effect. Consequently, additional chem-
istry work is under progress to synthesize new derivatives,
to strengthen their development on a rational base, and to
optimize the antileishmanial activity and antiviral activity of the 6-azathymine as a pharmacophore.

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