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Antiparasitic activity of highly conjugated pyrimidine-2,4-dione derivatives

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Abstract

4-[2-(1,3-Dimethyl-5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)vinyl]benzaldehyde was synthesized in four steps from 6-methyl-1*H*,3*H*-pyrimidine-2,4-dione. This aldehyde was functionalized by various substituted anilines or substituted benzylamines. Antiparasitic activities of the corresponding azomethines were assessed against *Plasmodium falciparum*, *Trichomonas vaginalis* and *Leishmania infantum* compared to their toxicity versus human cells.

Keywords: Antiparasitic activity; Pyrimidine-2,4-dione derivatives; Toxicity; THP1 cells; Azomethines

1. Introduction

The pyrimidine-2,4-dione ring is frequently encountered in many drugs used for the treatment of hypothyroidy, hypertension, cancer chemotherapy or HIV infection [1]. According to our interest toward nitrated heterocycles and their significant antiparasitic activity [2–4], we report here the synthesis of a new azomethine series after subjecting the 4-[2-(1,3-dimethyl-5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)vinyl]benzaldehyde to classical reactions. Their antiparasitic activity versus *Plasmodium falciparum*, *Trichomonas vaginalis* and *Leishmania infantum* was assessed and compared to their toxicity against THP1 cells.

2. Experimental

2.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Bruker ARX 200 spectrometer. The ^{1}H chemical shifts were reported as parts per million downfield from tetramethylsilane (Me_4Si). Absorptions were reported with the following notations: s, singlet; d, doublet; t, triplet; q, quartet; m, a more complex multiplet or overlapping multiplets. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM). Microanalyses for C, H, N were performed by the Service de Microanalyse de l'Université de Pharmacie de Châtenay-Malabry and the results for C, H, N elements were within $\pm$0.4% of the calculated values, unless otherwise indicated.

2.1.1. 1,3,6-Trimethyl-1H,3H-pyrimidine-2,4-dione (*1*)

A solution of 2,4-dihydroxy-6-methylpyrimidine-2,4-dione (15 g, 119 mmol) in 140 ml of 4 N NaOH was heated at 70 °C. Then, dimethylsulfate (40 ml, 422 mmol) was added dropwise. The reaction mixture was then heated for 1 h at 80 °C. After cooling at room temperature (r.t.), the solution was neutralized with concentrated acetic acid and extracted with chloroform. The chloroformic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo leading to a white solid crystallized from ethanol in 70% yield

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(12.8 g), m.p. 112 °C (lit. m.p. 112 °C [6]). ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, C-CH_3), 3.34 (s, 3H, N–CH_3), 3.41 (s, 3H, N–CH_3), 5.63 (s, 1H, H_5).

*2.1.2. 1,3,6-Trimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**2**)*

1,3,6-Trimethyl-1*H*,3*H*-pyrimidine-2,4-dione (**1**) (15 g, 97.3 mmol) was dissolved into concentrated sulfuric acid (50 ml) previously cooled at 0 °C. Fuming nitric acid (40 ml), cooled at 0 °C, was then added dropwise. The solution was stirred at a such temperature for 1 h and poured slowly over crushed ice. The yellow precipitate was filtered, dried in vacuo over P_2O_5 and crystallized from ethanol to give the nitrated derivative **2** in 86% yield (16.7 g). Yellow solid, m.p. 153 °C (lit. m.p. 150 °C [7]). ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, C–CH_3), 3.37 (s, 3H, N–CH_3), 3.53 (s, 3H, N–CH_3).

*2.1.3. 6-[2-(4-Diethoxymethylphenyl)-vinyl]-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**3**)*

Compound **2** (5 g, 25.1 mmol) and 4-(diethoxymethyl)benzaldehyde (15.7 g, 75.3 mmol) were added to a mixture of pyridine (2.5 ml) and ethanol (25 ml) and heated at 60–65 °C for 3 h. Then, the reaction mixture was poured over water and extracted with chloroform. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The crude residue was purified by chromatography on a silica gel column, eluting with petroleum ether–ethyle acetate (1:1) to give 7.8 g (80%) of yellow crystals, m.p. 167 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ 1.00 (m, 6H, $2CH_2CH_3$), 3.15 (s, 3H, N–CH_3), 3.24 (s, 3H, N–CH_3), 3.37 (m, 4H, $2CH_2CH_3$), 5.27 [s, 1H, $CH(OEt)_2$], 6.49 (d, $J = 16.4$ Hz, 1H, ethylenic H), 6.77 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.42 (d, $J = 8.3$ Hz, 2H, CH–phenyl), 7.63 (d, $J = 8.3$ Hz, 2H, CH–phenyl).

*2.1.4. 4-[2-(1,3-Dimethyl-5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-vinyl]-benzaldehyde (**4**)*

Water (10 ml) and 37% hydrochlorid acid (5 ml) were added to a solution of **3** (3 g, 7.7 mmol) in 30 ml of THF. The reaction mixture was stirred at r.t. for 24 h. The residue was filtered and dried in vacuo over P_2O_5. The yellow precipitate was, then, purified by chromatography on a silica gel column, eluting with petroleum ether–ethyle acetate (1:1) and crystallized from ethanol yielding 1.7 g (70%) of a yellow solid, m.p. 177 °C. ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 3H, N–CH_3), 3.42 (s, 3H, N–CH_3), 6.75 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.05 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.60 (d, $J = 8.3$ Hz, 2H, CH–phenyl), 7.90 (d, $J = 8.3$ Hz, 2H, CH–phenyl), 10.01 (s, 1H, CHO).

2.1.5. General procedure for the preparation of aldimines

A solution of amine (0.96 mmol, 1.5 equiv.) in ethanol (5 ml) was added to a solution of aldehyde **4** (0.2 g, 0.64 mmol) in ethanol (5 ml). The reaction mixture was refluxed for 2 h.

Azomethines which precipitated in boiling ethanol were isolated by filtration and purified by washing with boiling ethanol.

For azomethines which precipitated after cooling the reaction mixture at r.t., the solvent was removed in vacuo and the crude residue washed with water and crystallized from ethanol.

For other azomethines the solvent was removed in vacuo and was dissolved in chloroform. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was crystallized from ethanol.

*2.1.5.1. 1,3-Dimethyl-5-nitro-6-(2-{4-[(4-trifluoromethylbenzylimino)-methyl]-phenyl}-vinyl)-1H,3H-pyrimidine-2,4-dione (**5a**).* Yellow crystals, m.p. 140 °C, yield 76% (230 mg). ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3H, N–CH_3), 3.42 (s, 3H, N–CH_3), 4.82 (s, 2H, CH_2N), 6.65 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.02 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.41 (d, $J = 8.0$ Hz, 2H, N–benzyl), 7.45 (d, $J = 8.2$ Hz, 2H, CH–phenyl), 7.57 (d, $J = 8.0$ Hz, 2H, N–benzyl), 7.78 (d, $J = 8.2$ Hz, 2H, CH–phenyl), 8.38 (s, 1H, CH=N).

*2.1.5.2. 1,3-Dimethyl-5-nitro-6-(2-{4-[(3-trifluoromethylbenzylimino)-methyl]-phenyl}-vinyl)-1H,3H-pyrimidine-2,4-dione (**5b**).* Yellow crystals, m.p. 156 °C, yield 68% (205 mg). ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3H, N–CH_3), 3.45 (s, 3H, N–CH_3), 4.82 (s, 2H, CH_2N), 6.67 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.01 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.39–7.58 (m, 6H, 4H N–benzyl, 2H CH–phenyl), 7.78 (d, $J = 8.2$ Hz, 2H, CH–phenyl), 8.37 (s, 1H, CH=N).

*2.1.5.3. 1,3-Dimethyl-5-nitro-6-(2-{4-[(2-trifluoromethylbenzylimino)-methyl]-phenyl}-vinyl)-1H,3H-pyrimidine-2,4-dione (**5c**).* Yellow crystals, m.p. 195 °C, yield 60% (180 mg). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H, N–CH_3), 3.50 (s, 3H, N–CH_3), 5.01 (s, 2H, CH_2N), 6.70 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.08 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.37 (dd, $J = 7.7$ and 7.2 Hz, 1H, N–benzyl), 7.51–7.69 (m, 5H, 3H N–benzyl, 2H CH–phenyl), 8.40 (s, 1H, CH=N).

*2.1.5.4. 1,3-Dimethyl-5-nitro-6-(2-{4-[(1-phenylethylimino)-methyl]-phenyl}-vinyl)-1H,3H-pyrimidine-2,4-dione (**5d**).* Yellow crystals, m.p. 148 °C, yield 71% (190 mg). ^{1}H NMR ($CDCl_3$) δ 1.54 (d, $J = 6.6$ Hz, 3H, CH-CH_3), 3.37 (s, 3H, N–CH_3), 3.45 (s, 3H, N–CH_3), 4.50 (q, $J = 6.6$ Hz, 1H, CH–CH_3), 6.60 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.00 (d, $J = 16.4$ Hz, 1H, ethylenic H), 7.12–7.39 (m, 5H, N–benzyl), 7.43 (d, $J = 8.2$ Hz, 2H, CH–phenyl), 7.75 (d, $J = 8.2$ Hz, 2H, CH–phenyl), 8.29 (s, 1H, CH=N).

*2.1.5.5. 6-(2-{4-[(2-Chlorobenzylimino)-methyl]-phenyl}-vinyl)-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**5e**).* Yellow crystals, m.p. 184 °C, yield 66% (185 mg). ^{1}H NMR ($CDCl_3$) δ 3.36 (s, 3H, N–CH_3), 3.44 (s, 3H, N–CH_3), 4.86 (s, 2H, CH_2N), 6.63 (d, J = 16.4 Hz, 1H, ethylenic H), 7.00 (d, J = 16.4 Hz, 1H, ethylenic H), 7.12–7.22 (m, 2H, N–benzyl), 7.28–7.36 (m, 2H, N–benzyl), 7.46 (d, J = 8.2 Hz, 2H, CH–phenyl), 7.77 (d, J = 8.2 Hz, 2H, CH–phenyl), 8.35 (s, 1H, CH=N).

*2.1.5.6. 6-(2-{4-[(4-Chlorophenylimino)-methyl]-phenyl}-vinyl)-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**5f**).* Yellow crystals, m.p. 250 °C, yield 70% (190 mg). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H, N–CH_3), 3.51 (s, 3H, N–CH_3), 6.71 (d, J = 16.4 Hz, 1H, ethylenic H), 7.06 (d, J = 16.4 Hz, 1H, ethylenic H), 7.16 (d, J = 8.7 Hz, 2H, CH–phenyl), 7.38 (d, J = 8.7 Hz, 2H, CH–phenyl), 7.55 (d, J = 8.0 Hz, 2H, N–phenyl), 7.94 (d, J = 8.2 Hz, 2H, N–phenyl), 8.44 (s, 1H, CH=N).

*2.1.5.7. 6-(2-{4-[(2,6-Dichlorophenylimino)-methyl]-phenyl}-vinyl)-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**5g**).* Yellow crystals, m.p. 252 °C, yield 61% (180 mg). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H, N–CH_3), 3.50 (s, 3H, N–CH_3), 6.73 (d, J = 16.4 Hz, 1H, ethylenic H), 7.03–7.20 (m, 2H, ethylenic H and N–phenyl), 7.36 (dd, J = 8.5 and 1.5 Hz, 2H, N–phenyl), 7.55 (d, J = 8.2 Hz, 2H, CH–phenyl), 7.93 (d, J = 8.2 Hz, 2H, CH–phenyl), 8.44 (s, 1H, CH=N).

*2.1.5.8. 6-(2-{4-[(4-Bromophenylimino)-methyl]-phenyl}-vinyl)-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**5h**).* Yellow crystals, m.p. 244 °C, yield 62% (185 mg). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H, N–CH_3), 3.51 (s, 3H, N–CH_3), 6.74 (d, J = 16.4 Hz, 1H, ethylenic H), 7.06–7.12 (m, 3H, ethylenic H and N–phenyl), 7.51 (d, J = 8.7 Hz, 2H, N–phenyl), 7.56 (d, J = 8.2 Hz, 2H, CH–phenyl), 7.74 (d, J = 8.2 Hz, 2H, CH–phenyl), 8.44 (s, 1H, CH=N).

*2.1.5.9. 6-(2-{4-[(3-Bromophenylimino)-methyl]-phenyl}-vinyl)-1,3-dimethyl-5-nitro-1H,3H-pyrimidine-2,4-dione (**5i**).* Yellow crystals, m.p. 248 °C, yield 38% (115 mg). ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3H, N–CH_3), 3.46 (s, 3H, N–CH_3), 6.67 (d, J = 16.4 Hz, 1H, ethylenic H), 6.94–7.06 (m, 2H, ethylenic H and N–phenyl), 7.18–7.24 (m, 1H, N–phenyl), 7.43–7.56 (m, 3H, CH–phenyl and N–phenyl), 7.61 (d, J = 7.6 Hz, 1H, N–phenyl), 7.90 (d, J = 8.2 Hz, 2H, CH–phenyl), 8.31 (s, 1H, CH=N).

*2.1.5.10. 1,3-Dimethyl-6-(2-{4-[(2-methyl-3-nitrophenylimino)-methyl]-phenyl}-vinyl)-5-nitro-1H,3H-pyrimidine-2,4-dione (**5j**).* Yellow crystals, m.p. 103 °C, yield 52% (150 mg). ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3), 3.43 (s, 3H, N–CH_3), 3.51 (s, 3H, N–CH_3), 6.76 (d, J = 16.4 Hz, 1H, ethylenic H), 7.08–7.15 (m, 2H, ethylenic H and N–phenyl), 7.33 (dd, J = 8.2 and 8.0 Hz, 1H, N–phenyl), 7.58 (d, J = 8.3 Hz, 2H, CH–phenyl), 7.73 (d, J = 7.9 Hz, 1H, N–phenyl), 7.96 (d, J = 8.3 Hz, 2H, CH–phenyl), 8.36 (s, 1H, CH=N).

*2.1.5.11. 1,3-Dimethyl-6-(2-{4-[(4-methyl-3-nitrophenylimino)-methyl]-phenyl}-vinyl)-5-nitro-1H,3H-pyrimidine-2,4-dione (**5k**).* Yellow crystals, m.p. 116 °C, yield 49% (140 mg). ^{1}H NMR ($CDCl_3$) δ 2.62 (s, 3H, CH_3), 3.44 (s, 3H, N–CH_3), 3.51 (s, 3H, N–CH_3), 6.78 (d, J = 16.4 Hz, 1H, ethylenic H), 7.13 (d, J = 16.4 Hz, 1H, ethylenic H), 7.36–7.41 (m, 2H, N–phenyl), 7.58 (d, J = 8.3 Hz, 2H, CH–phenyl), 7.85 (d, J = 1.9 Hz, 1H, N–phenyl), 7.95 (d, J = 8.3 Hz, 2H, CH–phenyl), 8.36 (s, 1H, CH=N).

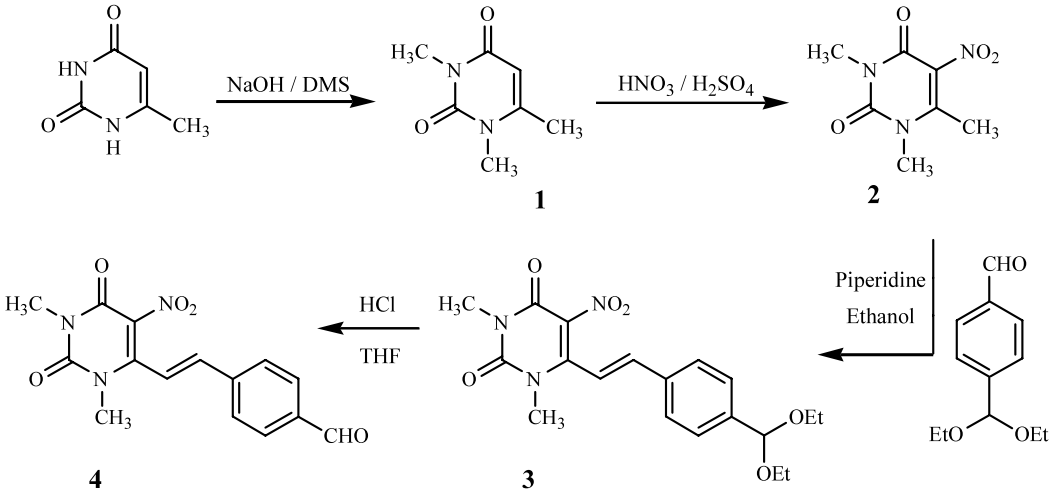
2.2. Pharmacology

2.2.1. Antiplasmodial activity

Assays were performed against a chloroquine-resistant strain (W2) of *P. falciparum* maintained in continuous culture according to the methodology described by Trager and Jensen [9]. Parasites were cultivated in group A+ human erythrocytes and suspended at a 4% hematocrit in RPMI 1640 medium supplemented with Hepes, $NaHCO_3$, 10% A+ human serum and Neomycin (Sigma, St Louis, MO) at 37 °C in a gas mixture of 5% O_2–6% CO_2–90% N_2; RPMI 1640, Hepes, $NaHCO_3$ were obtained from Gibco–BRL (Paisley, Scotland).

Assays were performed in triplicate in 96 well tissue culture plates (Nunc Brand products, Fisher, Paris, France) containing 200 μl of W2 asynchronous parasite cultures at 2% of parasitemia and 2% hematocrit, and 5 μl of the appropriate drug or drug combination dissolved in DMSO (dimethylsulfoxide, Sigma, St Louis, MO). Negative controls treated by solvent (DMSO).

2.2.1.1. Flow cytometric assessment of parasitemia [10,11]. Parasitemia was evaluated after 48 h by a flow cytometric method derived from the protocol published by Wyatt et al. [10] and adapted to *Plasmodium* strain by Van der Heyde et al. [11]. Parasite growth was assessed by a flow cytometric method in order to determine the number and viability of the intraerythrocytic *P. falciparum* on the basis of the ability of the parasite to take up and metabolize hydroethidine (HE) into ethidium, a DNA-binding fluorochrome. After incubation with hydroethidine, parasitized and uninfected erythrocytes were all identified on the basis of fluorescence intensity and size. For HE staining, a stock solution of HE (10 mg/ml) (Interchim, Montluçon, France) in DMSO was prepared and stored at −20 °C. The culture medium was removed from each



Scheme 1.

well of parasite culture plates. Two hundred microliters of HE diluted at 1/200 in phosphate buffered saline (PBS; Sigma, St Louis, MO) was added to each well and incubated for 20 min at 37 °C in the dark. Parasites were then washed twice in PBS by centrifugation at $400 \times g$ for 5 min and were resuspended in a final volume of 1 ml of PBS in the tubes for fluorescence-activated cell sorter (FACS) analysis. Flow cytometry data acquisition and analysis were performed on a FACSort instrument (Becton-Dickinson, San Jose, CA). The detectors for forward and side scatter of the FACSort were set to E-01 and 250, respectively, and both detectors were set to the logarithmic scale. The FL2 detectors were also adjusted to bring events within the detection range of the instrument (generally 459 for FL2). Both infected and uninfected erythrocytes were gated in the analysis and the percentage of parasitemia (number of infected erythrocytes/total erythrocytes $\times 100$) was determined using the LYSIS II program (Becton-Dickinson). Ten thousand cells were used for data acquisition.

The antimalarial activity of derivatives was expressed by the inhibitory concentrations 50% (IC_{50}), representing the drug concentration that induced a 50% parasitemia decrease compared to control culture. IC_{50} were calculated by non-linear regression analysis processed on dose–response curves by the Table Curve software (Jandel Scientific, Paris, France).

2.2.2. Activity versus T. vaginalis

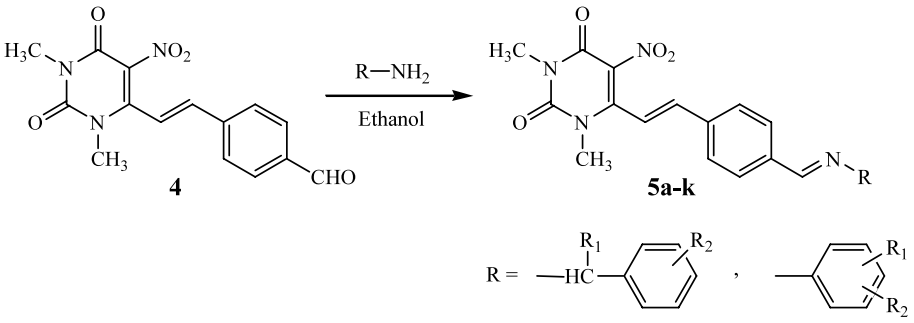
The antiproliferative activity of derivatives versus parasites of the genus *Trichomonas* was assessed on the referenced strain of *T. vaginalis* (TVR87) maintained in continuous culture in *Trichomonas* medium TM 161 (Oxoid) supplemented with 8% heat-inactivated horse serum (Eurobio, Paris, France) [12]. Parasites in late log-phase were incubated at an average of 10^4 cells/ml and a range of product concentrations were aseptically incorporated into duplicate cultures. After a 48-h incubation period at 37 °C, viable *Trichomonas* were identified and counted microscopically on the basis of their aspect and motility.

2.2.3. Antileishmanial activity versus promastigotes [13,14]

Experiments were performed on the referenced strain *L. infantum* MHOM/FR/78/LEM75. Promastigotes in the late log phase were incubated in RPMI medium supplemented with 12% foetal calf serum (Eurobio, Paris, France), at an average of 10^5 cells/ml. Various concentrations of active compounds were aseptically dissolved in DMSO (final concentration less than 1.1% volume/volume) and incorporate in duplicate cultures. After a 48-h incubation period at 25 °C, parasite growth was estimated by counting promastigotes with haemacytometer. The IC_{50} represented the concentration of drug that induced a decrease of 50% promastigotes compared to a control culture.

2.2.4. Toxicity on THP1 cells

In vitro toxicity and antiproliferative activity of azomethine derivatives were assessed on human monocytes (THP1 cells) maintained in RPMI medium (Eurobio, Paris, France) supplemented with 10% foetal calf serum (Eurobio, Paris, France) at 37 °C in 5% CO_2 and replicated every 7 days. Human monocytes in late log phase culture (10^5 cells/ml) were treated in duplicate assays with various concentrations of drugs and incubated in RPMI medium during 72 h at 37 °C with 5% CO_2 [14]. At the end of the incubation period, cell growth and viability were estimated by flow cytometry after staining by propidium iodide. The antiproliferative activity of chemical compounds was determined by the inhibitory concentration IC_{50} that represented the concentration of chemical products that induced a 50%



Scheme 2.

Table 1
Azomethine derivatives of the aldehyde **4**

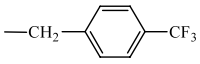
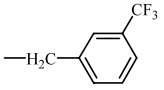
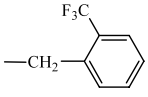
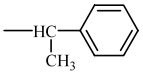
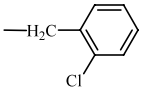
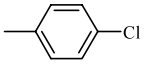
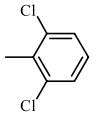
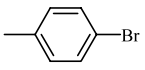
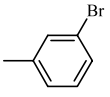
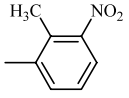
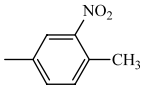
N°	R	m.p. (°C)	Yield (%)	Molecular formula F.W.
5a		140	76	$C_{23}H_{19}F_3N_4O_4$ 472,42
5b		156	68	$C_{23}H_{19}F_3N_4O_4$ 472,42
5c		195	60	$C_{23}H_{19}F_3N_4O_4$ 472,42
5d		148	71	$C_{23}H_{22}N_4O_4$ 418,45
5e		184	66	$C_{22}H_{19}ClN_4O_4$ 438,86
5f		250	70	$C_{21}H_{17}ClN_4O_4$ 424,84
5g		252	61	$C_{21}H_{16}Cl_2N_4O_4$ 459,28
5h		244	62	$C_{21}H_{17}BrN_4O_4$ 469,29
5i		248	38	$C_{21}H_{17}BrN_4O_4$ 469,29
5j		103	52	$C_{22}H_{19}N_5O_6$ 449,42
5k		116	49	$C_{22}H_{19}N_5O_6$ 449,42

Table 2
Antiplasmodial activity against W2 *P. falciparum* strain

Comp.	*P. falciparum* IC_{50} (μM)	Toxicity versus human cells (THP1 cells)		Therapeutic index (TI)
		IC_{50} (μM)	LC_{50} (μM)	
4	15	32	320	2
5a	10.5	105	> 105	10
5b	10.5	10.5	105	
5c	2.1	8	40	4
5d	12	12	24	
5e	5.6	11.3	11.3	2
5f	12	59	59	5
5g	21	108	> 108	5
5h	43	> 106	> 106	> 2.4
5i	21	53	53	5
5j	44	> 111	> 111	> 2.5
5k	15	> 56	> 56	> 4

Table 3
Antileishmanial activity against MHOM/FR/78/LEM75 *L. infantum* strain

Comp.	*L. infantum* IC_{50} (μM)	Toxicity versus human cells (THP1 cells)		Therapeutic index (TI)
		IC_{50} (μM)	LC_{50} (μM)	
3	3.2	32	320	10
5a	105	105	> 105	
5b	10.5	10.5	105	
5c	2.1	8	40	3.8
5d	12	12	24	
5e	2.2	11.3	11.3	5
5f	12	59	59	5
5g	> 108	108	> 108	
5h	> 106	> 106	> 106	
5i	21.3	53	53	2.5
5j	55.6	> 111	> 111	> 2
5k	11.1	> 56	> 56	> 5

decrease of cell growth compared to the control culture. Toxicity versus human cells was measured by the lethal concentration LC_{50} representing the concentrations of chemical compounds that produced a 50% cell death.

3. Results and discussion

3.1. Chemistry

The required aldehyde **4** was obtained in a four steps process (Scheme 1), starting from 6-methyl-1*H*,3*H*-pyrimidine-2,4-dione. The first step performed the *N*-alkylation of 1*H*,3*H*-pyrimidine-2,4-dione ring by dimethylsulfate (DMS) while the reaction mixture was maintained alkaline [5,6]. Kromov–Borisov performed the nitration stirring **1** in a mixture of concentrated nitric acid and phosphorus pentoxide for 18 h at −6 °C to lead to 1,3,6-trimethyl-5-nitropyrimidine-2,4-dione (**2**) in 72% yield [5]. Senda isolated **2** in 93% yield, stirring **1** in a mixture of concentrated nitric and sulfuric acids for 1 h at 0 °C [7]. Thus, we used this second procedure owing to its better yield in **2** and its easier workup.

1,3,6-Trimethyl-5-nitro-1*H*,3*H*-pyrimidine-2,4-dione (**2**) reacted by base-catalyzed condensation of **1** with 4-diethoxymethylbenzaldehyde to give 6-[2-(4-diethoxymethylphenyl)vinyl]-1,3-dimethyl-5-nitro-1*H*, 3*H*-pyrimidine-2,4-dione (**3**) in high yield. A such reaction has previously reported in our laboratory on a 5-nitroimidazole substrate [8]. After deprotection, the styryl compound **3** led to the 4-[2-(1,3-dimethyl-5-nitro-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)vinyl]benzaldehyde **4** (Scheme 1).

Then, the benzaldehyde derivative **4** was functionalized by a classical condensation reaction with various

Table 4
Trichomonocidal activity against TVR87 *T. vaginalis* strain

Comp.	*T. vaginalis* IC_{50} (μM)	Toxicity versus human cells (THP1 cells)		Therapeutic index (TI)
		IC_{50} (μM)	LC_{50} (μM)	
4	22.2	32	320	1.4
5a	78	105	> 105	1.3
5b	38	10.5	105	
5c	38	8	40	
5d	16.7	12	24	
5e	7	11.3	11.3	1.6
5f	42.3	59	59	1.4
5g	> 106	108	> 108	
5h	> 106	> 106	> 106	
5i	2	53	53	26.5
5j	> 111	> 111	> 111	
5k	40	> 56	> 56	> 1.4

substituted anilines and substituted benzylamines in refluxed ethanol, to give the corresponding azomethine compounds in good yields (Scheme 2, Table 1).

3.2. Antiparasitic activity

Antimalarial activity was assessed against a chloroquine resistant strain of *P. falciparum* (W2) maintained in continuous culture in human erythrocytes. $IC_{50\text{-antimalarial}}$ represented the drug concentration able to induce a 50% decrease of infected erythrocytes (Table 2). Antileishmanial activity was measured on the referenced strain *L. infantum* (MHOM/FR/78/LEM75). $IC_{50\text{-antileishmanial}}$ was determined as the concentration of drug necessary to inhibit 50% of parasite growth (Table 3).

Antitrichomonal activities were assessed on the referenced strain *T. vaginalis* (TVR87). $IC_{50\text{-trichomonocidal}}$ was expressed as the drug concentration necessary to induce a 50% decrease of the parasite growth (Table 4). Toxicity versus human cells was assessed against THP1 cells: $IC_{50\text{-THP1}}$ and $LC_{50\text{-THP1}}$ expressed the drug concentrations required to induce respectively a 50% reduction of cell growth and a 50% cell viability drop. IC_{50} were calculated by non linear regression analysis of dose–response curves representing the number of viable parasites and THP1 cells according to drug concentrations and were expressed as the mean values of three independent experiments. A therapeutic index (TI) was calculated for each parasite according to the following formula: $TI_{trichomonocidal} = IC_{50\text{-THP1}}/IC_{50\text{-trichomonocidal}}$, $TI_{antileishmanial} = IC_{50\text{-THP1}}/IC_{50\text{-antileishmanial}}$ and $TI_{antimalarial} = IC_{50\text{-THP1}}/IC_{50\text{-antimalarial}}$. In Tables 2–4, the sign ‘ > ’ indicated that the corresponding product was ineffective against the parasite at the highest concentration assessed. Concerning the toxicity against THP1 cells, the sign ‘ > ’ indicated that higher concentrations of the corresponding compound were not able to be assessed owing to it’s low solubility.

Most of the compounds of these series have shown a more or less interesting antiparasitic activity compared to their cytotoxicity on THP1 cells. The lower solubility in DMSO of some derivatives explain the limit values reported for the IC_{50} on THP1 cells. Higher concentrations could not be assessed.

Concerning the activity against the *Plasmodium falciparum* W2 strain, the functionalization increases the therapeutic index values for seven azomethines among these series (Table 2). The best therapeutic index was obtained for compound **5a** which confirmed the interest of a trifluoromethylbenzyl substituant for antiplasmodial activity as shown in previous studies [2].

The functionalization of aldehyde **4** decreases the leishmanicidal activity: the best therapeutic index (10) was observed with **4** (Table 3).

The results observed on *T. vaginalis* show only one interesting product: compound **5i** which presents 26.5 as therapeutic index value (Table 4).

The derivatives of this new azomethines series displayed antiparasitic activities modulated by their amine moiety. The low solubility in dimethylsulfoxyde of several compounds did not allow to assess higher concentrations on human cells which limited their in vitro therapeutic index values.

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