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Original article

1,3-Diphenylpyrazoles: synthesis and antiparasitic activities of azomethine derivatives

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Abstract

1,3-Diphenylpyrazole-4-carboxaldehyde and 1-(4-nitrophenyl)-3-phenylpyrazole-4-carboxaldehyde were obtained from the appropriated phenylhydrazones via the Vilsmeier–Haack reaction. These two aldehydes were functionalized by various substituted anilines or substituted benzylamines. Antiparasitic activities of the corresponding azomethines were assessed. In the most cases, nitrated compounds were found to be more efficient than non-nitrated ones against *Plasmodium falciparum*, *Trichomonas vaginalis* and *Leishmania infantum*. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Diphenylpyrazole; Nitrophenylpyrazole; Azomethine; Antiplasmodial activity; Trichmonocidal activity; Leishmanicidal activity

1. Introduction

Pyrazole nucleus [1] has long shown its pharmacological interest as antianxiety [2,3], antipyretic, analgesic and anti-inflammatory drugs [4–6], as well as its good antimicrobial activities [7–11]. The results we had obtained in previous studies about antiparasitic activity in nitroheterocyclic series [12–14] and our will to confirm some structure–activity relationships led us to synthesize the 1,3-diphenylpyrazole-4-carboxaldehyde and the 1-(4-nitrophenyl)-3-phenylpyrazole-4-carboxaldehyde. These two aldehydes were functionalized by condensation with various amines. The antiparasitic activity of the corresponding azomethines was assessed against *Plasmodium falciparum*, *Trichomonas vaginalis* and *Leishmania infantum*.

2. Chemistry

The 1,3-diphenylpyrazole-4-carboxaldehyde **3a** was prepared in two steps. The first one was the reaction between acetophenone and phenylhydrazine. The hydrazone derivative was treated with the Vilsmeier–Haack reagent ($DMF–POCl_3$) leading to the corresponding 4-carboxaldehyde functionalized pyrazole heterocyclic ring in mild operating conditions. Three equivalents of this reagent, instead of two as described by Kira [15], were necessary to obtain the aldehyde **3a** in good yields (see Fig. 1).

In order to prepare the 1-(4-nitrophenyl)-3-phenylpyrazole-4-carboxaldehyde **3b**, we tried, first, to nitrate **3a**. Hurst [16] had shown that the nitration of 3-methyl-1-phenylpyrazole only occurred on the *para* position of the phenyl ring when 1 equiv. of concentrated nitric acid was used. With an excess of nitric acid, the 4-position of the pyrazole ring was also able to be nitrated in a lower yield. We performed such a reaction with **3a**, using 1 equiv. of concentrated nitric acid in concentrated sulfuric acid. The ^{1}H-NMR data of the crude residue has been shown four different CHO proton signals (singlets $\delta = 9.90$, 10.06, 10.09 and 10.13 ppm) which indicated the multiplicity of the nitrated positions. Owing to the number of nitrated compounds obtained, and because of its low solubility, the crude residue was not purified. Thus, the nitrated carboxaldehyde **3b** has been prepared by the pathway

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used to prepare **3a**, starting with *para*-acetophenone instead of acetophenone (see Fig. 1).

The two aldehydes **3a** and **3b** were functionalized by a classical condensation reaction [17] with various substituted anilines and substituted benzylamines in refluxed methanol or ethanol, with traces of concentrated acetic acid, to give the corresponding azomethine derivatives (see Fig. 2 and Table 1).

3. Pharmacology

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. Antileishmanial activity was measured on amastigote forms of *L. infantum* infecting human monocyte-derived macrophages (THP1 cells). $IC_{50\text{-antileishmanial}}$ evaluated the drug concentration that produces a 50% reduction of infected macrophages. Activity against *T. vaginalis* was evaluated versus in vitro parasite culture. $IC_{50\text{-trichomonocidal}}$ was expressed as the drug concentration necessary to induce a 50% decrease of the parasite growth. 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, infected cells or non-infected 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

$TI_{antimalarial} = IC_{50\text{-THP1}}/IC_{50\text{-antimalarial}}$.

The highest concentration assessed against the parasites was 100 μg/mL. In Tables 2–4, the sign '>' indicated that the corresponding product was ineffective against the parasite at such a concentration. 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.

The THP1 cells were employed in the toxicity assays, because of their use in the culture of the amastigote forms of *Leishmania* so the products could be tested against the parasites only at a concentration lower than the IC_{50} against THP1 cells.

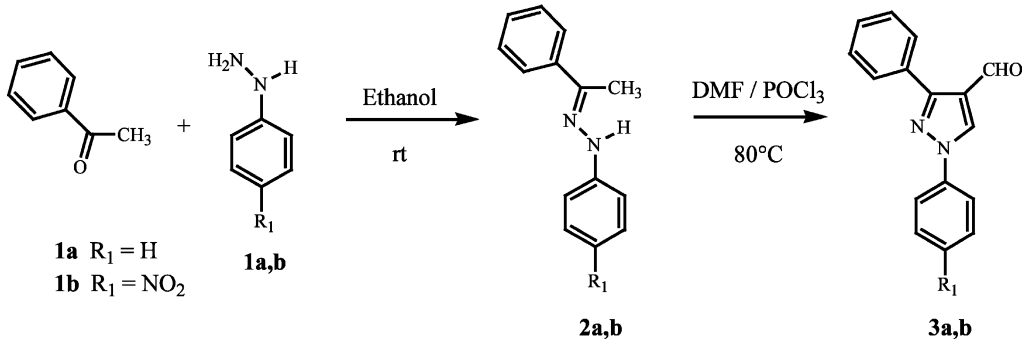


Fig. 1. Synthesis of 1,3-diphenylpyrazole-4-carboxaldehyde (**3a**) and 1-(4-nitrophenyl)-3-phenylpyrazole-4-carboxaldehyde (**3b**).

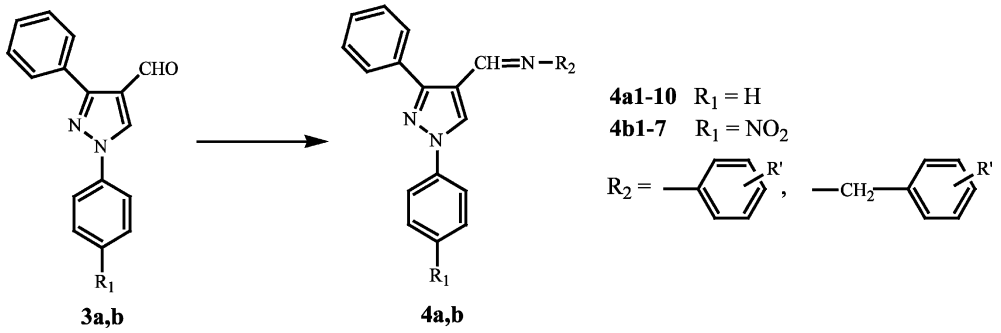
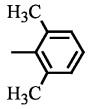
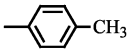
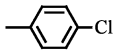
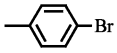
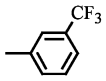
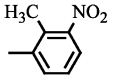
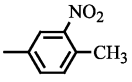
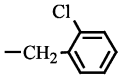
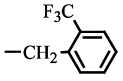
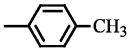
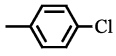
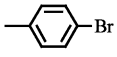
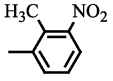
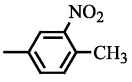
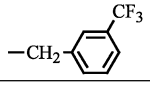


Fig. 2. Reactions of **3a**–**b** with various substituted anilines or substituted benzylamines.

Table 1
Azomethine derivatives of the aldehydes **3a** and **3b**

Compound	R_2	Yield (%)	Formula F.W.	M.p. (°C)
4a1[a]		66	$C_{23}H_{19}N_3$ 337.42	130 (isopropanol)
4a2		45	$C_{24}H_{21}N_3$ 351.44	146 (ethanol)
4a3[b]		60	$C_{23}H_{19}N_3$ 337.42	131 (isopropanol)
4a4[c]		56	$C_{22}H_{16}ClN_3$ 357.84	161 (ethanol)
4a5		59	$C_{22}H_{16}BrN_3$ 402.29	157 (ethanol)
4a6		42	$C_{23}H_{16}F_3N_3$ 391.39	133 (washed by ether)
4a7		60	$C_{23}H_{18}N_4O_2$ 382.41	188 (ethanol)
4a8		56	$C_{23}H_{18}N_4O_2$ 382.41	204 (ethanol)
4a9		45	$C_{23}H_{18}ClN_3$ 371.86	131 (isopropanol)
4a10		36	$C_{24}H_{18}F_3N_3$ 405.42	99 (pentane)
4b1		62	$C_{23}H_{18}N_4O_2$ 382.41	194 (ethyl acetate)
4b2		80	$C_{23}H_{18}N_4O_2$ 382.41	192 (ethanol)
4b3		70	$C_{22}H_{15}ClN_4O_2$ 402.83	194 (ethanol)
4b4		53	$C_{22}H_{15}BrN_4O_2$ 447.28	191 (ethanol)
4b5		52	$C_{23}H_{17}N_5O_4$ 427.41	219 (ethanol)
4b6		50	$C_{23}H_{17}N_5O_4$ 427.41	190 (ethanol)
4b7		46	$C_{24}H_{17}F_3N_4O_2$ 450.41	105 (ethanol/water)

[a] Litt. [18]: yield = 45%, m.p. = 125-127°C. [b] Litt. [18]: yield = 74%, m.p. = 128-130°C.
[c] Litt. [18]: yield = 56%, m.p. = 163-165°C.

4. Results and discussion

The Table 2 displayed the antiplasmodial activity of aldehyde derivatives. The azomethines synthesized from the non-nitrated aldehyde **3a** have been shown a lower activity compared with aldehyde **3a**, excepted for compound **4a10**, substituted by a 2-trifluoromethylbenzyl group, which exhibits a TI of 4.9. Among the derivatives from the nitrated aldehyde **3b** (**4b1**–**7**), compounds **4b2**, **4b5** and **4b7**, respectively, substituted by a

p-tolyl, a 2-methyl-3-nitrophenyl or a 3-trifluoromethylbenzyl group, have been shown a better antiplasmodial activity compared with **3b**. The TI of the most active compound **4b7** was 20.2. The IC_{50} observed for **4a10** and **4b7** have confirmed the interest of a trifluoromethylbenzyl substituent for antiplasmodial activity as observed previously in nitroisoquinolines series [12].

The Table 3 reported the assessment of trichomonacidal activity. Similarly to antiplasmodial activity, functionalization of aldehyde **3a** had not enhanced the efficiency against the *T. vaginalis* strain. Nitrated derivatives are more interesting than **4a1**–**10** derivatives, but their low solubility in dimethylsulfoxyde as solvent did not allow to assess higher concentrations on THP1 cells, which explain the limit values reported for their TI. These azomethines (**4b1**–**7**) have been shown a similar activity compared with aldehyde **3b**.

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

Compound	IC_{50} (μM) *P. falciparum*	Toxicity versus human cells (THP1 cells)		Therapeutic index
		Toxicity LC_{50} (μM)	Antiproliferative activity IC_{50} (μM)	
3a	32	>201	>201	>6.3
4a1	>60	50	47	
4a2	56	137	74	1.3
4a3	>60	56	56	
4a4	48	140	70	1.5
4a5	>50	27	3	
4a6	>50	28	13	
4a7	>52	65	13	
4a8	>52	105	4	
4a9	>54	13	13	
4a10	50	246	246	4.9
3b	68	>340	>340	5
4b1	>262	>262	262	
4b2	35	>262	262	7.5
4b3	122	>248	>248	>2
4b4	>224	>224	>224	
4b5	35	>234	>234	>6.7
4b6	61	>234	>234	>3.8
4b7	11	>222	>222	>20.2

Table 3
Trichomonocidal activity against TVR87 *T. vaginalis* strain

Compound	IC_{50} (μM) *T. vaginalis*	Toxicity versus human cells (THP1 cells)		Therapeutic index
		Toxicity LC_{50} (μM)	Antiproliferative activity IC_{50} (μM)	
3a	50	>201	>201	>4
4a1	>148	50	47	
4a2	>142	137	74	
4a3	>148	56	56	
4a4	>140	140	70	
4a5	>124	27	3	
4a6	>76	28	13	
4a7	>130	65	13	
4a8	>130	105	4	
4a9	>134	13	13	
4a10	>246	246	246	
3b	51	>340	>340	>6.6
4b1	130	>262	262	2
4b2	65	>262	262	4
4b3	122	>248	>248	>2
4b4	33	>224	>224	>6
4b5	81	>234	>234	>3
4b6	61	>234	>234	>3.8
4b7	55	>222	>222	>4

Table 4
Antileishmanial activity against MHOM/FR/78/LEM75 *L. infantum* strain (amastigote forms)

Compound	IC_{50} (μM) *L. infantum*	Toxicity versus human cells (THP1 cells)		Therapeutic index
		Toxicity LC_{50} (μM)	Antiproliferative activity IC_{50} (μM)	
3a	>201	>201	>201	
4a1	>47	50	47	
4a2	>74	137	74	
4a3	14	56	56	4
4a4	>70	140	70	
4a5	>3	27	3	
4a6	13	28	13	1
4a7	>13	65	13	
4a8	>4	105	4	
4a9	>13	13	13	
4a10	>246	246	246	
3b	>340	>340	>340	
4b1	>262	>262	262	
4b2	>262	>262	262	
4b3	12.4	>248	>248	>20
4b4	>224	>224	>224	
4b5	>233	>234	>234	
4b6	23	>234	>234	>10.2
4b7	>222	>222	>222	

Results of antileishmanial activity were presented in Table 4. Most of the compounds assessed, including aldehydes **3a** and **3b**, were ineffective against *L. infantum* at the highest concentration assessed. Among the non-nitrated derivatives, **4a3** showed a TI of 4. Nitrated azomethine **4b6** substituted by a 4-methyl-3-nitrophenyl moiety presented a TI upper than 10. The most effective compound is the 4-chlorophenyl substituted azomethine **4b3**, which exhibited a TI upper than 20.

5. Conclusions

The derivatives of this 1,3-diphenylpyrazole series have shown modest antiparasitic activities. Their low solubility did not allow to assess higher concentrations on THP1 cells, which limit the in vitro TI values. According to the mutagenicity of nitro derivatives, further studies related to the assessment of mutagenic potentialities of this series will be necessary to complete this pharmacological evaluation.

6. Experimental

6.1. Chemistry

Melting points (m.p.) 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*.

6.1.1. Synthesis of the hydrazones *2a* and *2b*

Concentrated acetic acid (1 mL) and phenylhydrazine hydrochloride (**1a**) (3 g, 20 mmol) or 4-nitrophenylhydrazine (**1b**) (3.68 g, 24 mmol) were added to a solution of acetophenone (2.88 g, 24 mmol) in 90 mL of ethanol. Then, the reaction mixture was refluxed for 1 h at room temperature (r.t.). The precipitate was filtered and washed with ethanol. After drying in vacuum over P_2O_5, 8.05 g (92%) of **2a** or 4.24 g (83%) of **2b** were obtained.

Acetophenone phenylhydrazone (**2a**): yellow solid, m.p. = 104 °C (litt. 105 °C [19]). ^{1}H-NMR ($CDCl_3$) δ 2.24 (s, 3H, CH_3), 7.20–7.46 (m, 9H, $H_{3,4,5}$ 1-phenyl, $H_{2,3,4,5,6}$ 3-phenyl, NH), 7.81–7.85 (m, 2H, $H_{2,6}$ 1-phenyl).

Acetophenone 4-nitrophenylhydrazone (**2b**): orange solid, m.p. = 183 °C (litt. 185 °C [19]). ^{1}H-NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 7.21 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 7.37–7.46 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.79–7.84 (m, 3H, $H_{2,6}$ 3-phenyl, NH), 8.21 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl).

6.1.2. Vilsmeier–Haack reaction

Dimethylformamide (2.58 g, 35.3 mmol) and $POCl_3$ (5.4 g, 35.3 mmol) were previous separately cooled at

0 °C before being stirred at such temperature. A solution of **2a** (3 g, 11.76 mmol) or **2b** (3 g, 11.76 mmol) in DMF (3 mL) was added dropwise to the reaction mixture which was, then, warmed at r.t. and heated at 70–80 °C for 5 h. After cooling at r.t., the mixture was basified with a cool saturated K_2CO_3 solution. The precipitate was filtered, strongly washed with water and crystallized from ethanol, yielding 95% (2.78 g) of **3a** or 94% (3.25 g) of **3b**.

1,3-Diphenylpyrazole-4-carboxaldehyde (**3a**): beige needles, m.p. = 145–147 °C (litt. 140 °C [15]), ^{1}H-NMR ($CDCl_3$) δ 7.29–7.56 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.77–7.85 (m, 4H, $H_{2,6}$ 1-phenyl, $H_{2,6}$ 3-phenyl), 8.59 (s, 1H, H_5), 10.11 (s, 1H, CHO). Anal. Calc. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28. Found: C, 77.28; H, 4.94; N, 11.19%.

1-(4-Nitrophenyl)-3-phenylpyrazole-4-carboxaldehyde (**3b**): brown solid, m.p. = 210–212 °C (litt. 210 °C [15]), ^{1}H-NMR ($CDCl_3$) δ 7.52–7.57 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.80–7.85 (m, 2H, $H_{2,6}$ 3-phenyl), 8.03 (d, $J = 9.1$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.41 (d, $J = 9.1$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.67 (s, 1H, H_5), 10.09 (s, 1H, CHO). Anal. Calc. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.61; H, 3.96; N, 13.99%.

6.1.3. General procedure for preparation of aldimines

Compound **3a** (0.5 g, 2.02 mmol) was dissolved in boiling methanol (15 mL). Then, 0.2 mL of concentrated acetic acid and 2.22 mmol of amine derivative were added and the reaction mixture was refluxed for 7 h. After cooling at r.t., the precipitate was isolated by filtration, washed with water and crystallized.

Compound **3b** (0.5 g, 1.70 mmol) was dissolved in boiling ethanol (40 mL). Then, 0.2 mL of concentrated acetic acid and 1.87 mmol of amine derivative were added and the reaction mixture was refluxed for 7 h. After cooling at r.t., the precipitate was isolated by filtration, and washed with water and crystallized.

*6.1.3.1. (1,3-Diphenylpyrazol-4-ylmethylene)-o-tolylamine (**4a1**).* White crystals, m.p. = 130 °C (litt. 125–127 °C [18]). ^{1}H-NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 6.88 (dd, $J = 6.8$ Hz, $J = 1.7$ Hz, 1H, H_3 N-phenyl), 7.11–7.24 (m, 2H, $H_{4,5}$ N-phenyl), 7.32–7.55 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.73–7.79 (m, 2H, $H_{2,6}$ 3-phenyl), 7.82–7.88 (m, 2H, $H_{2,6}$ 1-phenyl), 8.44 (s, 1H, H_5), 8.66 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{19}N_3$: C, 81.87; H, 5.68; N, 12.45. Found: C, 81.75; H, 5.59; N, 12.53%.

*6.1.3.2. (2,6-Dimethylphenyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a2**).* Brown crystals, m.p. = 146 °C. ^{1}H-NMR ($CDCl_3$) δ 2.21 (s, 6H, $2CH_3$), 6.92–7.10 (m, 3H, $H_{3,4,5}$ N-phenyl), 7.37–7.56 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.69–7.76 (m, 2H, $H_{2,6}$ 3-phenyl), 7.83–7.90 (m, 2H, $H_{2,6}$ 1-phenyl), 8.30 (s, 1H, H_5), 8.74 (s, 1H, CH=N). Anal. Calc. for $C_{24}H_{21}N_3$: C, 82.02; H, 6.02; N, 11.96. Found: C, 81.90; H, 6.09; N, 11.93%.

*6.1.3.3. (1,3-Diphenylpyrazol-4-ylmethylene)-p-tolylamine (**4a3**).* Beige crystals, m.p. = 131 °C (litt. 128–130 °C [18]). ^{1}H-NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 7.10 (d, $J = 8.4$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.20 (d, $J = 8.4$ Hz, 2H, $H_{2,6}$ N-phenyl), 7.41–7.56 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.70–7.76 (m, 2H, $H_{2,6}$ 3-phenyl), 7.81–7.86 (m, 2H, $H_{2,6}$ 1-phenyl), 8.54 (s, 1H, H_5), 8.67 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{19}N_3$: C, 81.87; H, 5.68; N, 12.45. Found C: 81.92; H, 5.70; N, 12.41%.

*6.1.3.4. (4-Chlorophenyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a4**).* Yellow solid, m.p. = 161 °C (litt. 163–165 °C [18]). ^{1}H-NMR ($CDCl_3$) δ 7.11 (d, $J = 8.7$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.34 (d, $J = 8.7$ Hz, 2H, $H_{2,6}$ N-phenyl), 7.39–7.55 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.69-7.78 (m, 2H, $H_{2,6}$ 3-phenyl), 7.80-7.87 (m, 2H, $H_{2,6}$ 1-phenyl), 8.49 (s, 1H, H_5), 8.66 (s, 1H, CH=N). Anal. Calc. for $C_{22}H_{16}ClN_3$: C, 73.84; H, 4.51; N, 11.74. Found: C, 73.76; H, 4.47; N, 11.80%.

*6.1.3.5. (4-Bromophenyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a5**).* Yellow powder, m.p. = 157 °C. ^{1}H-NMR ($CDCl_3$) δ 7.05 (d, $J = 8.7$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.38 (d, $J = 8.7$ Hz, 2H, $H_{2,6}$ N-phenyl), 7.42–7.54 (m, 6H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl), 7.71–7.75 (m, 2H, $H_{2,6}$ 3-phenyl), 7.82–7.86 (m, 2H, $H_{2,6}$ 1-phenyl), 8.49 (s, 1H, H_5), 8.66 (s, 1H, CH=N). Anal. Calc. for $C_{22}H_{16}BrN_3$: C, 65.68; H, 4.01; N, 10.45. Found: C, 65.69; H, 4.06; N, 10.34%.

*6.1.3.6. (1,3-Diphenylpyrazol-4-ylmethylene)-(3-trifluoromethylphenyl)-amine (**4a6**).* White crystals, m.p. = 133 °C. ^{1}H-NMR ($CDCl_3$) δ 7.30–7.38 (m, 2H, N-phenyl), 7.40–7.57 (m, 8H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl, 2H, N-phenyl), 7.72–7.78 (m, 2H, $H_{2,6}$ 3-phenyl), 7.83–7.88 (m, 2H, $H_{2,6}$ 1-phenyl), 8.53 (s, 1H, H_5), 8.68 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{16}F_3N_3$: C, 70.58; H, 4.12; N, 10.74. Found: C, 70.57; H, 4.11; N, 10.77%.

*6.1.3.7. (2-Methyl-3-nitrophenyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a7**).* Beige solid, m.p. = 188 °C. ^{1}H-NMR ($CDCl_3$) δ 2.52 (s, 3H, CH_3), 7.08 (d, $J = 7.7$ Hz, 1H, H_6 N-phenyl), 7.30–7.56 (m, 7H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl, H_6 N-phenyl), 7.67 (d, $J = 7.7$ Hz, 1H, H_4 N-phenyl), 7.72–7.77 (m, 2H, $H_{2,6}$ 3-phenyl), 7.83–7.87 (m, 2H, $H_{2,6}$ 1-phenyl), 8.41 (s, 1H, H_5), 8.67 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65. Found: C, 72.25; H, 4.69; N, 14.52%.

*6.1.3.8. (4-Methyl-3-nitrophenyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a8**).* Beige solid, m.p. = 204 °C. ^{1}H-NMR ($CDCl_3$) δ 2.60 (s, 3H, CH_3), 7.33–7.54 (m, 8H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl, $H_{5,6}$ N-phenyl), 7.70–7.77 (m, 3H, $H_{2,6}$ 3-phenyl, H_2 N-phenyl), 7.83–7.87 (m, 2H, $H_{2,6}$ 1-phenyl), 8.53 (s, 1H, H_5), 8.67 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65. Found: C, 72.30; H, 4.71; N, 14.71%.

*6.1.3.9. (2-Chlorobenzyl)-(1,3-diphenylpyrazol-4-ylmethylene)-amine (**4a9**).* White crystals, m.p. = 131 °C. ^{1}H-NMR ($CDCl_3$) δ 4.86 (s, 2H, CH_2), 7.18–7.54 (m, 10H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl, $H_{3,4,5,6}$ N-benzyl), 7.67–7.74 (m, 2H, $H_{2,6}$ 3-phenyl), 7.77–7.83 (m, 2H, $H_{2,6}$ 1-phenyl), 8.50 (s, 1H, H_5), 8.57 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{18}ClN_3$: C, 74.29; H, 4.88; N, 11.30. Found: C, 74.27; H, 4.91; N, 11.19%.

*6.1.3.10. (1,3-Diphenylpyrazol-4-ylmethylene)-(2-trifluoromethylbenzyl)-amine (**4a10**).* White crystals, m.p. = 99 °C. ^{1}H-NMR ($CDCl_3$) δ 4.95 (s, 2H, CH_2), 7.30–7.58 (m, 10H, $H_{3,4,5}$ 1-phenyl, $H_{3,4,5}$ 3-phenyl, $H_{3,4,5,6}$ N-benzyl), 7.62–7.73 (m, 2H, $H_{2,6}$ 3-phenyl), 7.79–7.84 (m, 2H, $H_{2,6}$ 1-phenyl), 8.49 (s, 1H, H_5), 8.57 (s, 1H, CH=N). Anal. Calc. for $C_{24}H_{18}F_3N_3$: C, 71.10; H, 4.48; N, 10.36. Found: C, 71.17; H, 4.54; N, 10.30%.

*6.1.3.11. [1-(4-Nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-o-tolylamine (**4b1**).* Yellow crystals, m.p. = 194 °C. ^{1}H-NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 6.88 (dd, $J = 7.5$ Hz, $J = 1.8$ Hz, 1H, H_3 N-phenyl), 7.13–7.22 (m, 3H, $H_{4,5,6}$ N-phenyl), 7.47-7.54 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.74–7.78 (m, 2H, $H_{2,6}$ 3-phenyl), 8.05 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.40 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.44 (s, 1H, H_5), 8.76 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65. Found: C, 72.25; H, 4.75; N, 14.67%.

*6.1.3.12. [1-(4-Nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-p-tolylamine (**4b2**).* Brown needles, m.p. = 192 °C. ^{1}H-NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 7.10 (d, $J = 8.4$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.21 (d, $J = 8.4$ Hz, 2H, $H_{2,6}$ N-phenyl), 7.47–7.57 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.70–7.76 (m, 2H, $H_{2,6}$ 3-phenyl), 8.03 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.39 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.53 (s, 1H, H_5), 8.76 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65. Found: C, 72.26; H, 4.73; N, 14.69%.

*6.1.3.13. (4-Chlorophenyl)-[1-(4-nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-amine (**4b3**).* Yellow crystals, m.p. = 194 °C. ^{1}H-NMR ($CDCl_3$) δ 7.12 (d, $J = 8.7$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.35 (d, $J = 8.7$ Hz, 2H, $H_{2,6}$ N-phenyl), 7.50–7.58 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.71–7.75 (m, 2H, $H_{2,6}$ 3-phenyl), 8.04 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.39 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.50 (s, 1H, H_5), 8.76 (s, 1H, CH=N). Anal. Calc. for $C_{22}H_{15}ClN_4O$: C, 65.59; H, 3.75; N, 13.91. Found: C, 65.65; H, 3.78; N, 13.76%.

*6.1.3.14. (4-Bromophenyl)-[1-(4-nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-amine (**4b4**).* Yellow needles, m.p. = 191 °C. ^{1}H-NMR ($CDCl_3$) δ 7.06 (d, $J = 8.7$ Hz, 2H, $H_{3,5}$ N-phenyl), 7.48-7.56 (m, 5H, $H_{3,4,5}$ 3-phenyl, $H_{2,6}$ N-phenyl), 7.70–7.75 (m, 2H, $H_{2,6}$ 3-phenyl), 8.04 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.40 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.50 (s, 1H, H_5); 8.76 (s, 1H, CH=N). Anal. Calc. for $C_{22}H_{15}BrN_4O$: C, 59.08; H, 3.38; N, 12.53. Found: C, 59.18; H, 3.47; N, 12.39%.

*6.1.3.15. (2-Methyl-3-nitrophenyl)-[1-(4-nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-amine (**4b5**).* Yellow solid, m.p. = 219 °C. ^{1}H-NMR ($CDCl_3$) δ 2.52 (s, 3H, CH_3), 7.08 (d, $J = 7.5$ Hz, 1H, H_6 N-phenyl), 7.33 (dd, $J = 8.0$ Hz, $J = 7.5$ Hz, 1H, H_5 N-phenyl), 7.51–7.53 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.67–7.76 (m, 3H, $H_{2,6}$ 3-phenyl, H_4 N-phenyl), 8.06 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.40 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.42 (s, 1H, H_5), 8.78 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{17}N_5O_4$: C, 64.63; H, 4.01; N, 16.39. Found C, 64.48; H, 4.02; N, 16.21%.

*6.1.3.16. (4-Methyl-3-nitrophenyl)-[1-(4-nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-amine (**4b6**).* Brown solid, m.p. = 190 °C. ^{1}H-NMR ($CDCl_3$) δ 2.61 (s, 3H, CH_3), 7.34–7.35 (m, 2H, $H_{5,6}$ N-phenyl), 7.51-7.59 (m, 3H, $H_{3,4,5}$ 3-phenyl), 7.70–7.78 (m, 2H, $H_{2,6}$ 3-phenyl), 7.95 (s, 1H, H_2 N-phenyl), 8.05 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.41 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.53 (s, 1H, H_5), 8.78 (s, 1H, CH=N). Anal. Calc. for $C_{23}H_{17}N_5O_4$: C, 64.63; H, 4.01; N, 16.39. Found: C, 64.54; H, 3.92; N, 16.46%.

*6.1.3.17. [1-(4-Nitrophenyl)-3-phenylpyrazol-4-ylmethylene]-(3-trifluoromethylbenzyl)-amine (**4b7**).* Yellow solid, m.p. = 105 °C. ^{1}H-NMR ($CDCl_3$) δ 4.83 (s, 2H, CH_2), 7.51–7.60 (m, 7H, $H_{3,4,5}$ 3-phenyl, $H_{2,4,5,6}$ N-phenyl), 7.69–7.73 (m, 2H, $H_{2,6}$ 3-phenyl), 8.00 (d, $J = 9.2$ Hz, 2H, $H_{2,6}$ nitrophenyl), 8.41 (d, $J = 9.2$ Hz, 2H, $H_{3,5}$ nitrophenyl), 8.50 (s, 1H, H_5), 8.66 (s, 1H, CH=N). Anal. Calc. for $C_{24}H_{17}F_3N_4O_2$: C, 64.00; H, 3.80; N, 12.44. Found: C, 63.83; H, 3.87; N, 12.30%.

6.2. Pharmacology

6.2.1. Antiplasmodial activity

Assays were performed against a chloroquino-resistant strain (W2) of *P. falciparum* maintained in continuous culture according to the methodology described by Trager [20]. 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, USA) 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 dimethylsulfoxide (DMSO, Sigma). Negative controls treated by solvent (DMSO) and positive controls containing a range of chloroquine (Sigma) concentrations were added to each set of experiments.

6.2.1.1. Flow cytometric assessment of parasitemia. Parasitemia was evaluated after 48 h by a flow cytometric method derived from the protocol published by Wyatt et al. [21] and adapted to *Plasmodium* strain by Van der Heyde et al. [22]. 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 HE, 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 well of parasite culture plates. Two hundred microliters of HE diluted at 1/200 in phosphate buffered saline (PBS; Sigma) 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 × 100) was determined using the LYSIS II program (Becton-Dickinson). 10 000 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 with 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).

6.2.2. Activity versus Trichomonas 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) [23]. Parasites in late log-phase were incubated at an average of 10^4 cells per mL and a range of product concentrations was 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.

6.2.3. Antileishmanial activity

Experiments were performed on the referenced strain *L. infantum* MHOM/FR/78/LEM75. Intracellular amastigote cultures were performed in human-derived macrophages (THP1) cells according to the methodology described by Ogunkolade et al. [24]. Maturation of adherent macrophages was performed by treating exponentially-growing monocytes (10^5 cells per mL) with 1 μM phorbol myristate acetate (Sigma). After a 48 h incubation period at 37 °C in chamber-slides (Fisher), cells were rinsed with fresh medium and suspended in RPMI medium containing stationary-phase *Leishmania* promastigotes (cells/promastigotes ratio = 1/10). After a 24 h incubation period at 37 °C, promastigotes were removed by four successive watches with fresh medium. Adapted dilutions of drugs were added in duplicate chambers and cultures were incubated for 96 h at 37 °C. Cells were stained with 10% Giemsa stain (Eurobio). The percentage of infected macrophages in each assay was determined microscopically at 1000 times magnification. The IC_{50} represented the concentration of drug that induced a decrease of 50% of infected human monocytes compared with a control culture.

6.2.4. 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) supplemented with 10% foetal calf serum (Eurobio) at 37 °C in 5% CO_2 and replicated every 7 days. Human monocytes in late log phase culture (10^5 cells per 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 [25]. 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 in-

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

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