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Synthesis and analgesic properties of new 4-arylhydrazone 1-H pyrazole [3,4-b] pyridine derivatives [1]

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Abstract

Based on the principle of bioisosterism, a successful strategy in the planning of new drugs, we describe in this work the synthesis and the analgesic activity of the new functionalized arylcarbaldehyde 4-(1-phenyl-3-methylpyrazolo[3,4-b]pyridine) hydrazone derivatives 5a–m. These derivatives (5a–m) were synthesized in ca. 45% overall yield, using 4-(1-phenyl-3-methylpyrazolo[3,4-b]pyridinyl) hydrazine 6, as key intermediate, by applying classical synthetic methods to construct the aryl-hydrazone unit at C-4 of the heterocyclic system. Compound 6 was prepared from the corresponding 4-chloro-(*N*-phenyl-3-methylpyrazolo[3,4-b]pyridine) derivative 7 in very high yield. The antinociceptive activity of these new compounds 5a–m was evaluated by a test of abdominal contortions induced by 0.6% acetic acid solution i.p. in albino mice. The compounds 5f, 5g, 5j and 5k were strongly active showing a good analgesic profile.

Keywords: Arylcarbaldehyde 4-(1-phenyl-3-methyl-pyrazolo[3,4-b]pyridine) hydrazone; Pyrazolo[3,4-b]pyridine derivative; Synthesis; Analgesic property; Bioisosterism

1. Introduction

In a research program for the design, synthesis and pharmacological evaluation of new heterocyclic compounds structurally planned as isosters of known inhibitors of arachidonic acid cascade (CAA) enzymes, we have recently described the synthesis and biological activity of functionalized heterocyclic arylamine 1, arylhydrazine 2 (Freitas et al., 1993) and acylarylhydrazone 3 (Pereira et al., 1991; Matheus et al., 1991, Silveira et al., 1991) belonging to nitrogen containing heterocyclic derivatives as *N*-phenylpyrazole (1,2,3) (Freitas and Barreiro, 1992) and pyrazolo[3,4-b]pyridine 4 (Dias et al., 1994) (Scheme 1). These compounds have presented an important analgesic profile measured using the classical acetic acid-induced constrictions bioassay (Miranda et al., 1993). Otherwise, in the classical model of carrageenan-induced rat paw edema (CIRPE) (Higs et al., 1960), using indomethacin as standard, these compounds possessed little or no antiinflammatory activity (Barreiro et al., 1993). These pharmacological results indicated that the hydrazone functionality in these derivatives could be an important pharmacophore moiety and prompted us to propose, as a new attractive synthetic target, the 4-arylhydrazonyl pyrazolo[3,4-b]pyridine (5) derivatives. This new class of compounds was designed based on the classical isosterism concept between aromatic rings (Barreiro, 1991) and

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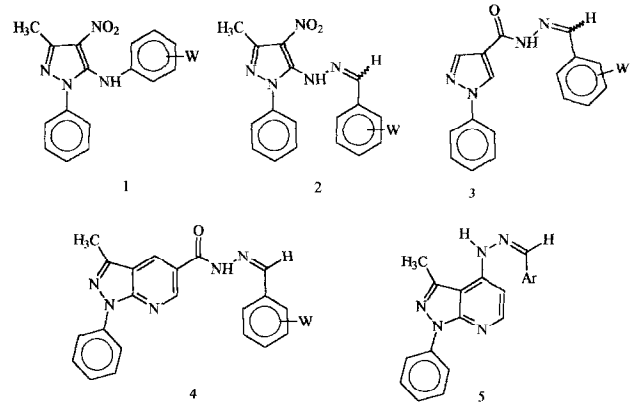


Scheme 1.

planned as a probable isoster of the corresponding 5-arylhydrazonylpyrazole series 2, previously described (Freitas and Barreiro, 1994). These derivatives (5) present a more important hydrophobic character than pyrazole 2, due to the presence of the pyrazolo[3,4-b]pyridine system. Preliminary structural calculations on derivatives 1 and 2, using the molecular mechanics determinations (Albuquerque et al., 1993), indicated an appropriate distance between the substituents at C-4 (NO_2) and C-5 (NH-) of pyrazole rings (e.g. 1 and 2) favoring the participation hydrogen intramolecular bond (Rodrigues et al., 1993), introducing a certain level of conformational restriction in the hydrazone moiety in these derivatives (1 and 2). Therefore we decided to investigate the possible role of that structural characteristic in the activity, to view the presence of pyrazolo[3,4-b]pyridine system in the new proposed compounds 5, to abolish the eventual participation of any intramolecular hydrogen bond involving the arylhydrazone framework, representing an important information about the structure–activity relationships and to study the eventual role of arylhydrazone moiety as a pharmacophore for the activity in this series of derivatives.

2. Materials and methods

2.1. Chemistry

Infrared (IR) spectra were obtained with a Perkin-Elmer 1600 spectrometer by using potassium bromide plates. The mass spectra were obtained with a GC/VG Micromass 12 at 70 eV. Nuclear magnetic resonance (^{1}HNMR and ^{13}CNMR) spectra were determined in dimethylsulfoxide (DMSO-d_6) containing ca. 1% TMS as an internal standard with an AC 200 Brucker and VXR 300 Varian and GEMINI 300 Varian spectrophotometers. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad.

The progress of all reactions was monitored by TLC which was performed on 2.0 cm × 6.0 cm aluminium sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light (254–265 nm). Solvents used in the reactions were generally distilled before use.

The compounds 4-chloroester pyrazolo [3,4-b] pyridine 8 (Dias et al., 1994), 4-hydroxypyrazolo [3,4-b] pyridine 10 (Bare et al., 1989), were obtained according to the reported methods.

2.1.1. 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazine (6)

An ethanolic solution of (7) (4 g (14 mM) in 50 ml of ethanol) was heated under stirring until complete dissolution. Then, 14 ml of hydrazine hydrate were added to the reaction vessel. The reaction mixture was still stirred at reflux for 3 h, when TLC analysis showed the end of the reaction. The product (6) in 85% yield, was isolated by concentration of reaction mixture under reduced pressure and cold water addition to furnish (6) as an yellow light solid, mp 170–171°C. IR (KBr): (N-H) 3322; 1591; 879–678. cm^{-1}; ^{1}H NMR (300 MHz): 8.32 (dd, J = 11Hz and 2.77 Hz, 2H, H-2′); 8.2 (d, J = 5.5 Hz, 1H, H-6); 7.59 (s, 1H, N-H), 7.46 (dd, J = 11Hz, 1H, H-3′); 7.21 (dd, J = 11Hz and 152,4 (C-4); 149.6 (C-6); 141.0 (C-8); 139.7 (C-1′); 128.6 (C-3); 128.3 (C-3′); 124.2 (C-4′); 119.7 (C-2′); 103.3 (C-9); 97.3 (C-5); 14.9 ppm (CH_3).

2.1.2. General procedure to obtain aryl-4-(1-phenyl-3-methyl pyrazolo [3,4-] pyridine) hydrazones (2)

To an ethanolic solution of 0.21 g (78 mM) of hydrazine derivative (6) (in 35 ml of absolute ethanol) was added an adequate amount of aldehyde (78 mM) and a few drops of hydrochloric acid as a catalyst. The end of the reaction was observed by TLC and the hydrazones (5a–m) were isolated by concentration of the reaction mixture under reduced pressure and addition of cold water to give the desired product as a colored precipitate in ca. 92% yield (Table 1).

2.1.3. Benzaldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazone (5a)

IR (KBr): (N = H) 2706; 1528; 956–680 cm^{-1}; ^{1}H NMR (300 MHz): 10.7 (ls, 1H, N-H); 8.6 (s, 1H, H-11); 8.3 (d, J = 6.0 Hz, 1H, H-6); 8.1 (dd, J = 8.1 Hz, 2H, H-2′); 7.8 (d, J = 7.0 Hz, 2H, H-2″); 7.5 (m, 5H, H-3′ and H-3″); 7.3 (dd, J = 11.4 Hz, 1H, H-4′); 7.2 (d, J = 6.0 Hz, 1H, H-5); 2.8 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz): 148,1 (C-4); 147.9 (C-11); 147.2 (C-6); 142.2 (C-8); 138.7

(C-1′); 134.4 (C-1″); 130.0 (C-4″) 129.9 (C-3 e C-3′); 129.2 (C-3″); 127.1 (C-4′); 125.9 (C-2″); 121.2 (C-2′); 103.9 (C-9); 99.8 (C-5); 15.7 ppm (CH_3); MS (70 eV) m/z 327 (100%); 223 (40%); 209 (30%); 193 (9%); 22 (29%).

2.1.4. ortho-Nitrobenzaldehyde 4-(1-phenyl-3-methyl-pyrazolo-[3,4-b]-pyridine) hydrazone (5b)

IR (KBr): (N-H) 3349; 1586; 845–682 cm^{-1}; ^1H NMR (300 MHz): 10.8 (s, 1H, N-H); 8.9 (s, 1H, H-11); 8.4 (dd, 2H, H-2′); 8.3 (dd, 2H, H-4″); 8.2 (d, J = 5.6 Hz, 1H, H-6); 7.8 (dd, 1H, H-3″); 7.7 (dd, 1H, H-6″); 7.6 (dd, 2H, H-3′); 7.4 (dd, 1H, H-4′); 7.3 (d, J = 5.6 Hz, 1H, H-5); 2.9 ppm (s, 3H, CH_3; ^{13} C NMR (75 MHz): 152.2 (C-4); 149.7 (C-11); 147.8 (C-6″); 146.2 (C-6); 141.2 (C-8); 139.3 (C-1″), 138.7 (C-3″); 133.3 (C-4″); 129.9 (C-1″); 128.9 (C-3″); 128.4 (C-3); 127.9 (C-2″) 125.1 (C-4′); 124.4 (C-5″); 120.2 (C-2″); 103.9 (C-9); 100.1 (C-5); 15.7 ppm_3); MS (70 eV) m/z 372 (100%); 242 (13%); 223 (31%); 209 (38%); 193 (12%); 77 (26%).

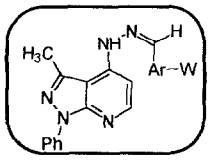
2.1.5. para-Nitrobenzaldehyde 4-(1-phenyl-3-methyl-pyrazolo[3,4-b] pyridine) hydrazone (5c)

IR (KBr): (N-H) 3344; 2831; 1593; 844–690 cm^{-1}; ^{1} NMR (300 MHz): 11.2 (sl, N-H); 8.7 (s, 1H, H-11); 8.2 (3H; dd, H-6′, H-4); 8.0 (d, J = 8.1 Hz, 2H, H-3″); 7.9 (d, J = 8.1 Hz, 2-H″); 7.5 (dd, J = 7.5 Hz, 2H, H-3′); 7.3 (dd, J = 7.5 Hz, 1H, H-4′); 7.2 (d, J = 6.0 Hz, 1H, H-5), 2.8 ppm (3H, CH_3); ^{13}C NMR (75 MHz): 144.8 (C-4′); 143.5 (C-4 and C-11); 140.9 (C-6); 139.3 (C-1″), 137.5 (C-6); 135.5 (C-1′); 126.0 (C-3 e C-3′); 124.6 (C-4′); 123.2 (C-2″); 120.9 (C-3″), 118.5 (C-2″); 101.4 (C-9); 97.4 (C-5); 12.6 ppm (CH_3); MS (70 eV) m/z: 372 (100%); 342 (32%); 223 (70%); 209 (29%); 193 (12%); 168 (11%); 77 (43%).

2.1.6. ortho-Trifluoromethylbenzaldehyde 4-(1-phenyl-3-methylpyrazolo[3,4-b]pyridine) hydrazone (5d)

IR (KRr): (N-H) 2720; 1588; 910–670 cm^{-1}; ^1H NMR (200 MHz): 11.7 (sl, 1H, N-H); 8.9 (s, 1H, H-11); 8.3 (dd, J = 6.3 Hz, H-3″); 8.3 (d, J = 6.3 Hz, 1H, H-6); 8.1 (dd,

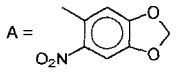
Table 1
Arylcarbaldehyde 4-(1-phenyl-3-methyl-1-H pyrazolo [3,4-b]pyridine) hydrazones

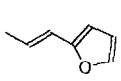


5

Compound	Substituents		Molecular formula [a]	Yield (%)	Molecular weight	Melting point (°C) [b]
	Ar	W				
5a	C_6H_4	H	$C_{20}H_{17}N_5O$	94	327	211
5b	C_6H_4	*o*-NO_2	$C_{20}H_{16}N_6O_2$	97	372	230–232
5c	C_6H_4	*p*-NO_2	$C_{20}H_{16}N_6O_2$	98	372	260
5d	C_6H_4	*o*-CF_3	$C_{21}H_{16}N_5F_3$	93	395	243–245
5e	C_6H_4	*p*-CF_3	$C_{21}H_{16}N_5F_3$	95	395	221–222
5f	C_6H_4	*p*-OCH_3	$C_{21}H_{19}N_5O$	94	357	241–245
5g	C_6H_4	*p*-F	$C_{20}H_{16}N_5F$	94	345	238–240
5h	C_6H_4	*p*-CN	$C_{21}H_{16}N_6$	90	352	265–266
5i	C_6H_3	OCH_2O	$C_{21}H_{17}N_5O_2$	93	371	231-233
5j	A [c]		$C_{21}H_{16}N_6O_4$	96	416	260–261
5k	2-furyl		$C_{18}H_{15}N_5O$	95	318	231–235
5l	2-furyl	5-NO_2	$C_{18}H_{15}N_6O_3$	93	420	242–244
5m	B [c]		$C_{20}H_{16}N_5O$	90	343	230–232

[a] Elemental analyses are in full agreement with the calculated values.
[b] EtOH/H_2O was used as crystallization solvent.
[c]

A = 

B = 

J = 7.4 Hz, 2H, H-2″); 7.8 (m 2H, H-4″ and H-5″); 7.7 (dd, J = 6.3 Hz, 1H, H-6″); 7.6 (dd, J = 7.3 Hz, 2H, H-3′), 7.4 (dd, J = 7.4 Hz, 1H, H-4′); 7.3 (d, J = 6.3 Hz, 1H, H-5); 2.8 ppm (s, 3H, CH_3); ^{13}C NMR (50 MHz): 148.2 (C-11), 146.5 (C-4); 146.0 (C-6); 142.5 (C-5″); 142.5 (C-8); 138.1 (C-1′); 132.1 (C-1″); 130.0 (C-2″); 129.3 (C-3 e C-3″); 127.2 (C-4″); 126.9 (C-3″); 126.7 (C-6″); 126.3 (C-4′); 126.0 (CF_3); 121.9 (C-2′); 104 (C-9); 100.3 (C-5); 15.7 ppm (CH_3); MS (70 eV) m/z: 395 (100%); 223 (55%); 209 (30%); 77 (15%).

2.1.7. para-Trifluoromethylbenzaldehyde 4-(1-phenyl-3-methylpyrazolo[3,4-b]pyridine) hydrazone (5e)

IR (KBr): (N-H) 2924; 1592; 834–691 cm^{-1}; ^{1}H NMR (200 MHz): 10.6 (s, 1H, N-H); 8.3 (s, 1H, H-11); 7.9 (d, J = 6.7 Hz, 1H, H-6); 7.8 (dd, J = 8.8 Hz, 2H, H-2′); 7.6 (d, J = 6.7 Hz, 2H, H-3″); 7.4 (d, J = 6.7 Hz, 2H, H-2″); 7.2 (dd, J = 8.8 Hz, 2H, H-3′); 7.1 (dd, J = 8.8 Hz, 1H, H-4′); 6.9 (d, J = 6.7 Hz, 1H, H-5); 2.3 ppm (s, 3H, CH_3); ^{13}C NMR (50 MHz): 149.0 (C-4); 147.5 (C-11); 146.5 (C-6); 144.0 (C-1″); 142.0 (C-8); 138.0 (C-1′); 129.5 (C-4″); 129.0 (C-3-C-3′); 127.0 (C-2″); 126.0 (C-4, CF_3); 125.5 (C-3″); 121.0 (C-2′); 103.5 (C-9); 99.9 (C-5); 15.5 ppm (CH_3); EM (70 eV) m/z: 395 (100%, 223 (54%), 209 (4%; 193 (10%); 77 (21%).

2.1.8. para-Methoxybenzaldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazone (5f)

IR (KBr) (N-H)2601; 16080; 916–680 cm^{-1}; ^{1}H NMR (300 MHz): 11.4 (sl, 1H, N-H); 8.7 (s, 1H, H-11); 8.3 (dd, J = 5.4 Hz, 2H, H-6); 8.1 (dd, J = 7.20 Hz, 1H, H-2″); 7.9 (d, J = 8.5 Hz, 2H, H-2″); 7.7 (dd, J = 7.32 Hz, 2H, H-4′); 7.6 (dd, J = 7.20 and 7.32 Hz, 2H, H-3′); 7.4 (d, J = 5.4 Hz, 1H, H-5); 7.2 (d, J = 8.50 Hz, 2H, H-3″); 3.95 (s, 3H, OCH3); 2.9 ppm (s, 3H, CH3); ^{13}C NMR (75 MHz): 160.9 (C-4″); 148.9 (C-4 and C-11); 148.3 (C-6); 143.4 (C-8); 137.5 (C-1′); 129.3 (C-3′ and C-3); 128.8 (C-1″); 127.2 (C-4′); 126.3 (C-2″); 2.5 (C-2′); 114.5 (C-3″); 103.7 (C-9); 99.7 (C-5); 55.0 (OCH3); 15.9 ppm (CH3); MS (70 eV) m/z: 357 (100%); 223 (24%); 209 (7%); 77 (14%).

2.1.9. para-Fluorobenzaldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazone (5g)

IR (KBr): (NH) 3250; 2369; 1524; 956–811 cm^{-1}; ^{1}H NMR (300 MHz): 11.2 (sl, 1H, N-H); 8.8 (s, 1H, H-11); 8.3 (d, J = 6.3 Hz, 1H, H-6); 8.1 (dd, J = 7.8 Hz, 2H, H-2′); 8.0 (dd, J = 11 Hz and 5.8 Hz, 2H, H-2″); 7.7 (dd, J = 7.8 Hz and 7.6 Hz, 2H, H-3′); 7.5 (dd, J = 7.6 Hz, 1H, H-4′); 7.4 (dd, J = 8.1 and 9.0 Hz, 2H, H-3″); 7.3 (d, J = 6.3 Hz, 1H, H-5); 2.9 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz): 161.4 (C-4″); 148.5 (C-4); 147.5 (C-11); 146.3 (C-6); 142.7 (C-8); 137.8 (C-1′); 130.5 (C-1″); 129.2 (C-3′ and C-3); 126.7 (C-4′); 121.9 (C-2′); 116.1 (C-2″); 115.8 (C-3″); 103.8 (C-9); 99.8 (C-5); 15.7 ppm (C-10); MS (70 eV) m/z: 345 (100%); 223 (35%); 209 (3%); 77 (10%).

2.1.10. para-Cyanobenzaldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazone (5h)

IR (KBr); (NH) 3300; 2604; 1528 cm^{-1}; ^{1}H NMR (200 MHz): 8.3 (s, 1H, H-11); 8.0 (d, J = 6.5 Hz, 1H, H-6); 7.8 (dd, J = 8.0 Hz, 2H, H-2′); 7.7 (d, J = 8.0 Hz, 1H, H-3″); 7.6 (d, J = 8.0 Hz, 1H, H-2″); 7.2 (dd, J = 8.0 Hz, 2H, H-3′); 7.1 (dd, J = 8.0 Hz, 1H, H-4′); 7.0 (d, J = 6.5 Hz, 1H, H-5); 2.5 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz) 147.7 (C-4); 146.8 (C-11); 144.5 (C-6); 142.2 (C-8); 138.2 (C-1′ and C-1″); 132.3 (C-3″); 129.2 (C-3′ and C-3″); 127.4 (C-2″); 126.3 (C-4′); 121.5 (C-2′); 118.7 (CN); 11.8 (C-4″); 104.5 (C-9); 100.1 (C-5); 15.5 ppm (C-10); MS (70 eV) m/z: 352 (100%); 256 (33%); 223 (58%); 193 (3%); 129 (41%); 77 (34%); 73 (54%).

2.1.11. 3′,4′-Methylenedioxybenzaldehyde 4-(1-phenyl-3-methylpyrazolo [3,4-b]pyridine) hydrazone (5i)

IR (KBr): (NH) 3300; 2900; 1527; 754–695.5. cm^{-1}; ^{1}H NMR (300 MHz): 8.5 (s, 1H, H-11); 8.3 (d, J = 6.0 Hz, 1H, H-6); 8.2 (dd, J = 7.8 Hz, 2H, H-2′); 7.6 (dd, J = 7.8 and 8.1 Hz, 2H, H-3′); 7.5 (d, 1H, H-5″); 7.4 (dd, 1H, H-4′); 7.3 (dd, 1H, H-6″); 7.26 (d, 1H, H-2″); 7.1 (d, J = 6.0 Hz, 1H, H-5); 6.2 (s, 2H, O-CH_2-O) 2.9 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz): 149.5 (C-4 and C-11); 148.2 (C-6); 147.9 (C-4″); 146.2 (C-3″); 143.0 (C-8); 137.4 (C-1′); 129.0 (C-3′); 128.0 (C-1″); 126.8 (C-4′); 123.3 (C-6″); 122.3 (C-2′); 108.2 (C-2″); 105.1 (C-5″); 103.6 (C-9); 101.3 (O-CH_2-O); 99.7 (C-5); 15.4 ppm (C-10); MS (70 eV) m/z: 371 (100%); 223 (26%); 209 (6%); 177 (18%).

2.1.12. 3′,4′-Methylenedioxy-6′-nitrobenzaldehyde 4-(1-phenyl-3-methylpyrazolo[3,4-b] pyridine) hydrazone (5j)

IR (KBr): (NH) 3454; 2659; 1521; 910–696 cm^{-1}; ^{1}H NMR (300 MHz): 8.9 (s, 1H, H-11); 8.2 (d, J = 5.7 Hz, 1H, H-6); 8.1 (dd, J = 7.8 Hz, 2H, H-2′); 7.6 (dd, 2H, H-3′); 7.5 (dd, 2H, H-4′); 7.5 (d, 1H, H-5″); 7.5 (d, 1H, H-3″); 7.3 (d, 1H, H-2″); 7.2 (d, J = 5.7 Hz, 1H, H-5); 6.3 (s, 2H, O-CH_2-O); 2.8 (s, 3H, CH_3O-); 2.8 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz) 151.7 (C-4 and C-11); 149.3 (C-6″); 148.5 (C-4″); 147.9 (C-3″); 146.9 (C-6); 142.7 (C-8); 138.8 (C-1′); 130.5 (C-1″); 129.0 (C-3′); 125.6 (C-4′); 120.8 (C-2′); 105.2 (C-2′); 104.9 (C-5″); 103.9 (C-9) 103.6 (O-CH2-O); 100.3 (C-5); 15.7 ppm (C-10); MS (70 eV) m/z: 416 (100%); 386 (24%); 239 (43%); 224 (95%); 209 (100%); 193 (24%); 167 (25%); 152 (43%); 77 (84%).

2.1.13. 2-Furfuraldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine)hydrazone (5k)

IR (KBr): (NH) 3361; 2916; 1592; 907–807 cm^{-1}; ^1H NMR (300 MHz) 8.2 (dd, 4H, H-2′, H-4′); 7.5 (dd, 2H, H-3′); 7.2 (dd, 2H, H-4′); 6.8 (dd, 2H, H-2″); 6.6 (dd, 2H, H-3″); 7.8 ppm; (s, 1H, H-11); ^{13}C NMR (75 MHz): 152.6 (C-4); 149.3 (C-11); 145.6 (C-1″); 144.0 (C-4″); 141.2 (C-8); 133.8 (C-1′, C-6); 128.4 (C3, C3′); 124.5 (C-4′); 119.9 (C-2′); 111.7 (C-2′); 111.2 (C-3″); 103.7 (C-9); 99.5 (C-5); 15.5 ppm (C-10); MS (70 eV) m/z: 420 (23%); 405 (11%); 362 (100%); 330 (31%); 223 (49%); 209 (16%); 193 (15%); 77 (25%).

2.1.14. 5-Nitro-2-furfuraldehyde 4-(1-phenyl-3-methyl pyrazolo [3,4-b] pyridine) hydrazone (5l)

IR (KBr): (NH) 3330; 2890; 1580; 911–815 cm^{-1}; ^1H NMR (300 MHz): 11.0 (sl, 1H, NH); 8.5 (dd, 2H, H-2′); 7.6 (dd, 2H, H-3′); 7.4 (dd, 2H, H-2″); 7.3 (dd, 2H, H-4′); 7.9 (d, 2H, H-3″); 7.8 ppm (s, 1H, H-11); ^{13}C NMR (75 MHz): 152.4 (C-4); 151.5 (C-4′); 149.0 (C-11); 146.6 (C-1″); 141.3 (C-8); 131.2 (C-1′, C-6); 128.8 (C-3, C-3′); 125.0 (C-4′); 120.2 (C-2′); 115.0 (C-3″); 113.7 (C-2″); 104.0 (C-9); 100.4 (C-5); 16.2 ppm (C-10); MS (70 eV) m/z 420 (23%); 405 (11%); 362 (100%); 330 (31%); 223 (49%); 209 (10%); 193 (15%); 77 (25%).

2.1.15. β-(2-Vinyl)furfuralaldehyde 4-(1-phenyl-3-methyl-pyrazolo [3,4-b] pyridine) hydrazone (5m)

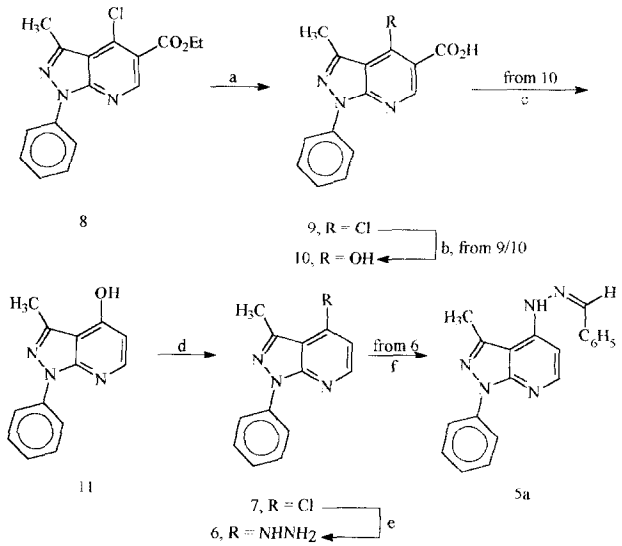
IR (KBr): (NH) 3100–3300; 1590; 900–805 cm^{-1}; ^1H NMR (300 MHz): 11.1 (sl, 1H, NH); 8.2 (dd, 2H, H-2′); 7.6 (dd, 2H, H-3′); 7.2 (dd, 2H, H-4′); 7.5 (dd, 2H, H-2′); 6.9 (dd, 2H, H-3″); 8.4 (dd, 2H, H-4″); 7.9 ppm (s, 1H, H-11); ^{13}C NMR (75 MHz): 151.7 (C-4); 147.9 (C-11); 147.6 (C-1″); 146.0 (C-4″); 144.3 (C-6); 142.5 (C-8); 138.3 (C-1′); 129.1 (C-3, C-3′); 125.6 (C-4′); 121.5 (C-2′); 112.5 (C-2″); 111.9 (C-3″); 103.7 (C-9); 99.7 (C-5); 16.3 ppm (C-10); MS (70 eV) m/z: 343 (100%); 223 (23%); 172 (10%); 77 (10%).

2.2. Pharmacology

Antinociceptive activity was assayed using the mouse abdominal constriction test. Albino mice of both sexes weighing 18–22 g were used. Abdominal constrictions were induced by i.p. injection of an 0.6% acetic acid solution. The number of abdominal constrictions was recorded during 30 min after the i.p. injection, and compared with the control. Activity was expressed as percent of inhibition. The results are expressed as ± standard error mean for number n of animals. These results were analyzed statistically by Student's t-test with the level of significance set at $p < 0.05$. Dipyrone (in saline) was used as a standard and the compounds 2a–m were administered p.o. in a dose of 100 μmol/kg, dissolved in 5% Arabic gum, 1 h before testing.

3. Results and discussion

Among the several possible synthetic approaches to the derivatives 5, the synthetic route employed was based on the previously described synthesis of 4, which depicted the chloro derivative 7 as key intermediate to 5 (Scheme 2). This compound could be prepared from the described chloro-ester 8, obtained previously in high overall yield in our laboratory, as a precursor of compound 4 (Dias et al., 1994). Our initial purpose was to synthesize the 4-chloro derivative 7 by ester hydrolysis of 8 to obtain the halocarboxylic acid 9, which could then be decarboxylated by heating (Alvim, 1992) to produce the key intermediate 7. This approach caused an unexpected problem. Hydrolytic treatment of 8, in a mixture of hydrochloric and acetic acid at reflux, by 3 h, produced, in 90% yield, a mixture of the desired 4-cholorocarboxylic acid 9 and the corresponding 4-hydroxycarboxylic acid 10, possessing an hydroxyl group at the C-4 position (Bare et al., 1989). We were able to convert this mixture of compounds into only 4-hydroxy derivative 10, obtained in 87% yield, by employing a longer reflux time using the same experimental hydrolytic conditions. This acid 10 could then be decarboxylated by heating with polyphosphoric acid (PPA) to produce 11. Subsequently, treatment of the decarboxylated product 11 with phosphorous oxychloride produced, in 80% yield, the desired 4-halodecarboxylated product 7. The next step in



Scheme 2. (a) HCl, AcOH, reflux, 3h (90%). (b) HCl, AcOH, reflux, 8 h (87%). (c) H_3PO_4, reflux, 10 h (73%). (d) $POCl_3$, reflux, 3 h (80%). (e) $NH_2NH_2 \cdot H_2O$, reflux, EtOH, 3 h (85%). (f) PhCHO, EtOH, HCl (cat.), r.t., 2 h, (94%).

the planned route was conversion of 7 into the hydrazine 6, which could be obtained in 85% yield by nucleophilic displacement of the chloride ion at the C-4 position with a small excess of hydrazine hydrate, in ethanol solution at reflux, producing the desired 4-(*N*-phenyl-3-methyl pyrazolo[3,4-b]pyridine) hydrazine (6) (Scheme 2). Finally, the 4-arylpyrazolo[3,4-b]pyridine hydrazone derivatives 5a–m (Table 1) could be prepared from 6 in ca. 92% yield by acid-catalyzed condensation with requisite aldehydes 11, in ethanol, at room temperature, using hydrochloric acid as catalyst (Alvim, 1992).

The choice of the substituent pattern in the aldehyde counterpart was defined in order to introduce a significant diversity in the hydrophobic character of the final derivatives permitting a future study of SAR.

In fact, hydrazone derivatives may exist in two possible diastereomeric forms due to the nitrogen-carbon double bond (Newkome and Bhacca, 1971). Unfortunately, we do not have direct evidence for a particular configuration in this series of derivatives, but careful examination of the pattern of methynic group CH=N in the ^{1}H NMR spectra (Lynch et al., 1988) indicates the presence of a major configurational isomer (> than 95%), preliminary considered as *Z*-isomer, with a basis in the observed chemical shift (Karabatsos and Taler, 1963; Karabatsos et al., 1963). The ^{13}C NMR spectra (Gordon and Sojka, 1984) also indicate that these derivatives are not a uniform mixture of the two possible isomers. In these spectra we are able to detect only two downfield resonances for the carbonyl and hydrazone carbons. These observations also eliminated the possibility that the double bond has migrated to a diazo-like species (Szileigyl et al., 1984; Sanchez and Rossi, 1995), which would not present the olefinic carbon atom resonance in the ^{13}C NMR spectrum. For instance, preliminary molecular mechanics calculations indicate that the *Z*-isomer is more stable (ca. 8 kcal/mol) than the *E*-isomer (Rodrigues et al., 1993). These results lead us to regard the *Z*-isomer as the principal diastereoisomer in our series of compounds 5a-m. These findings seem to indicate that in the condensation step between hydrazine 6 with the different aldehydes 11 a thermodynamic control was operating in favor of the diastereoisomeric product.

3.1. Pharmacology

The evaluation of analgesic properties of these derivatives was carried out by the mouse writhing method (Wittle, 1964). The compounds 5f (Ar = Ph, W = p-OMe), 5g (Ar = Ph, W = p-F), 5j (Ar, W = A), and 5k (Ar = 2-furyl) (Table 2) showed an activity similar to dipyrone used as standard, suggesting that the antinociceptive activity depends on the presence of arylhydrazone moiety rather than on the nature of the substituent or the aromatic hydrazone ring. The more active derivative 5j presenting an activity similar to standard, possessed a methylenedioxy unit at C-3′/C-4′ and a nitro group at C-6′. The weak analgesic activity presented by compounds 5b (Ar = Ph, W = *o*-nitro) and 5c (Ar = Ph, W = *p*-nitro) seems to indicate that an oxygenated function as substituent of the arylhydrazone ring improves the analgesic activity, as observed with 5f

Table 2
In vivo effect of arylcarbaldehyde 4-(1-phenyl-3-methyl)-pyrazolo[3,4-b]pyridine derivatives (5a–m) and dipyrone in the inhibition of abdominal constrictions induced by acetic acid (0.6% i.p.) in mice

Compound	Concentration	*n* [a]	Contortion number	Inhibition	Relative activity [b]
Control		57	93.75 + 9.59 *		
Propylene glycol	5000	21	90.28 + 8.99 *	3.0	
5a	100	18	77.11 + 8.55 *	15.6	0.26
5b	88	10	79.30 + 9.96 *	15.4	0.26
5c	88	8	83.25 + 12.16	11.2	0.20
5d	83	22	73.59 + 8.73 *	22.6	0.40
5e	83	15	79.93 + 6.47 *	15.0	0.26
5f	92	10	49.10 + 8.29 *	48.5	0.86
5g	95	8	53.00 + 10.4	43.5	0.77
5h	93	6	89.93 + 5.76	< 1.0	
5i	88	8	83.75 + 6.23 *	11.0	0.20
5j	79	10	41.03 + 6.85 *	56.5	1.00
5k	103	15	47.30 + 8.40 *	51.0	0.90
5l	78	8	94.00 + 6.75	< 1.0	
5m	96	8	96.79 + 5.59	< 1.0	
Dipyrone	100	9	39.11 + 5.79 *	56.0	1.00

[a] *n* = number of animals.
[b] The value of relative activity was obtained by comparison with the dipyrone, attributed activity equal to 1.0.
* $P < 0.05$.

and 5k. For instance, the fact that neither member of this series was active in the carrageenan-induced edema model (Higs et al., 1960) was a favorable finding from our point of view, since this model correlates best with the acidic class of NSAI agents suggesting that the analgesic response of these active compounds perhaps is due to the reduction of the antinociceptive impulse without action over AAC, at the same level as NSAI agents.

In conclusion, the synthetic methodology used in this work was shown to be efficient to obtain these new 4-arylhydrazone-1-H pyrazolo [3,4-b] pyridine derivatives 5 in high yield. The pharmacological results indicate an antinociceptive activity for some compounds and we conclude that this series could represent a new pharmacophoric tool for the development of more efficacious analgesics.

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