SYNTHESIS, CHARACTERIZATION AND IN VITRO ANTIFUNGAL EVALUATION OF TETRAHYDROPYRAZOLO[1,5-*c*]QUINAZOLINES

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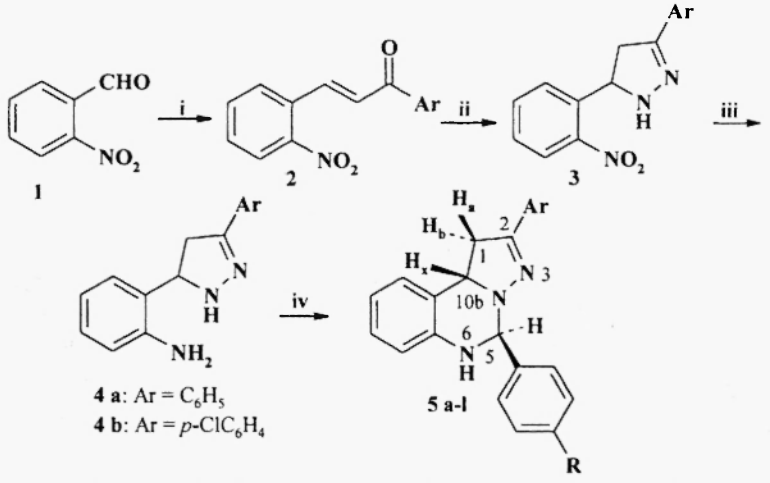
Abstract: The synthesis of a series of new pyrazolo[1,5-*c*]quinazolines starting from 2-nitrobenzaldehyde is described. The structures of the obtained compounds were established by NMR and X-ray diffraction. In contrast with other quinazoline derivatives, these compounds do not show any antifungal *in vitro* activity up to 250 μg/ml.

Introduction

The quinazoline skeleton is an important pharmacophore that occurs frequently in medicinal chemistry literature [1]. Compounds containing the quinazoline ring system show a wide spectrum of biological effects [2-5], some of them displaying interesting antifungal properties [6]. As part of a program aimed to identify novel antifungal agents and on the basis on our current project on synthetic strategies for preparing heterocyclic compounds containing the quinazoline ring, we report the synthesis of a series of tetrahydropyrazolo[1,5-*c*]quinazolines using 2-nitrobenzaldehyde as a starting material. The use of this aromatic aldehyde for the design and synthesis of new quinazoline compounds is a subject of recent interest [7].

Results and Discussion

The synthesis of tetrahydropyrazolo[1,5-*c*]quinazolines (**5**) was performed in four steps. First we synthesized the *o*-nitrochalcones (**2**) [8] by the aldol condensation of *o*-nitrobenzaldehyde (**1**) with acetophenones. Compounds **2** reacted with hydrazine hydrate yielding the 4,5-dihydro-3,5-diarylpyrazoles **3** [7]. The catalytic reduction of **3** afforded the (2-aminophenyl)pyrazoles **4a-b**, which were cyclized with substituted benzaldehydes to form the tricyclic derivates **5a-l** in ethanol with catalytic amounts of acetic acid (Scheme 1). These compounds were characterized by NMR and X-ray diffraction.



i) ArCOMe/AcOH; ii) $N_2H_4.H_2O$/MeOH; iii) Ni-Raney/MeOH, $N_2H_4.H_2O$; iv) 4-RC_6H_4CHO/EtOH/AcOH

Scheme 1

The formation of **5** as unique products of reaction was confirmed by spectroscopy analysis. A detailed analysis of ^1H, ^{13}C, and 2D COSY, HMQC and HMBC NMR spectra in $(CD_3)_2SO$ of compounds **5a-l** revealed the presence of both, a pyrazoline and a quinazoline systems. The CH_2-CH fragment at the pyrazoline ring of the products **5a-l** cause proton signals due to the ABX spin pattern. The resonance of the two non-equivalent methylene protons (δ_{Ha} = 3.24-3.25, δ_{Hb} = 3.34-3.40 ppm) and of the methynic proton (δ_{Hx} = 4.55-5.32 ppm) indicate that this ring was present. The H_a and H_b protons give rise to a doublet and a doublet of doublets respectively (J_{ab} = 15.3-16.2 Hz and J_{bx} = 7.6-8.8 Hz). The NH resonance of the quinazoline ring appears as a hardly resolved doublet at δ = 6.44-6.88 ppm (J_{NH} = 2.0-3.3 Hz) (Table 1).

Table 1. ^1H-NMR Data of **5a-l** (δ values. TMS as the Internal Standard, in DMSO-d_6)

Comp.	NH	1-H_a	1-H_b	5-H	7-H	8-H	9-H	10-H	10b-H_x	Ar		RC_6H_4-	
	(d)	(d)	(dd)	(d)	(dd)	(t)	(t)	(d)	(d)	H_{orto}	H_{meta}	H_{orto}	H_{meta}
5a	6.86	3.25	3.38	6.01	6.63	6.93	6.55	6.87	4.57	7.33*		7.53	7.33
5b	6.81	3.28*		5.96	6.65	6.99	6.52	6.8	4.55	7.30*		7.63	7.15
5c	6.80	3.25*		5.96	6.61	6.70	6.50	6.65	4.57	7.50*		7.43	6.89
5d	6.88	3.25	3.39	6.10	6.70	7.00	6.55	6.85	4.57	7.50*		7.41	7.54
5e	6.44	3.24	3.39	5.72	6.68	6.99	6.75	7.08	5.31	7.45*		8.03	8.33
5f	6.87	3.25	3.39	5.97	6.52	6.85	6.68	6.98	4.58	7.45*		7.47	7.55
5g	6.88	3.25	3.34	6.02	6.85	7.00	6.50	6.60	4.58	7.43	7.64	7.40*	
5h	6.84	3.25	3.30	5.97	6.70	6.95	6.55	6.63	4.57	7.14	7.64	7.40*	
5i	6.85	3.24	3.34	6.10	6.75	6.90	6.60	6.63	4.55	7.16	7.60	7.25	7.35
5j	6.86	3.25	3.34	6.01	6.60	6.67	6.90	6.95	4.57	7.40	7.53	7.43	7.64
5k	6.48	3.24	3.39	5.73	6.68	7.00	6.75	7.10	5.32	7.40	7.50	8.00	8.30
5l	6.89	3.24	3.40	5.98	6.63	6.57	6.95	6.85	4.59	7.64	7.43	7.47	7.54

* Multiplet. CH_3 for **5b**, **5c**, **5h** and **5i** 2.26, 3.72, 2.26 and 3.70 ppm respectively.

In the ^{13}C-nmr spectra, the number of signals belonging to quaternary, tertiary and secondary carbon atoms for compounds **5** were determined using DEPT experiments (see Table 2) and correspond to the proposed structure.

Table 2. ^{13}C NMR chemical shifts (δ in ppm) of compounds **5a-l**.

Comp.	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**
C-1	40.6	40.6	40.5	40.7	40.4	40.7	40.5	40.4	40.3	40.6	41.2	40.6
C-2	150.7	150.6	158.4	151.0	152.8	151.0	149.8	149.7	149.7	150.0	150.4	150.1
C-5	68.8	68.7	68.5	68.3	70.4	68.4	68.9	68.7	68.5	68.5	68.5	68.4
C-6a	141.6	136.4	150.6	132.3	144.6	121.2	142.5	142.5	142.4	121.1	144.6	121.1
C-7	114.0	113.9	112.9	114.1	115.4	114.1	114.0	114.0	113.7	114.2	113.3	114.2
C-8	117.0	117.0	116.9	117.3	118.8	117.3	117.1	117.1	116.9	117.3	116.8	117.4
C-9	127.2	126.2	125.6	127.2	126.5	127.2	127.4	126.5	126.4	127.3	125.9	127.3
C-10	126.6	126.7	126.5	126.6	126.8	126.6	127.0	126.8	126.5	126.6	125.5	126.6
C-10a	121.2	121.3	121.3	121.2	122.7	120.5	121.1	136.4	135.7	142.1	122.7	121.1
C-10b	56.4	56.4	55.0	56.3	62.0	56.3	56.6	56.6	56.3	56.5	56.6	56.5

CH_3 for **5b**, **5c**, **5h** and **5i** 20.5, 113.6, 20.5 and 111.5 ppm respectively.

The molecular structures of four tetrahydropyrazolo[1,5-*c*]quinazolines derivates were confirmed by X-ray diffraction analysis and have been recently reported [9].

These new compounds were assayed for antifungal properties against a panel of 12 fungal strains comprising human opportunistic pathogenic yeasts, hialohyphomycetes as well as dermatophytes with the agar dilution method [10]. Results showed that none of the compounds tested displayed any activity against the fungi tested up to 250 μg/mL, clearly showing that this series of quinazolines are devoid of antifungal properties.

Conclusion

We have described in this paper the preparation of twelve tetrahydropyrazolo[1,5-*c*]quinazolines in high yields by using 2-nitrobenzaldehyde as starting material. Results demonstrate that this synthetic route is highly versatile producing high regioselective quinazolines. Regarding the antifungal properties, this new series of compounds did not show any capacity of inhibiting a panel of 12 human opportunistic pathogenic fungi at concentrations up to 250 μg/ml.

Experimental

Melting points were determined in a Buchi Melting Point Apparatus and are uncorrected. The ^{1}H- and ^{13}C nmr spectra were run on a Bruker DPX 300 spectrometer operating at 300 MHz and 75 MHz respectively, using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. The mass spectra were scanned on a Hewlett Packard HP Engine-5989 spectrometer (equipped with a direct inlet probe) a operating at 70 eV. The elemental analysis have been obtained using a LECO CHNS-900 equipment. The compounds **2** and **3** were prepared according to reported methods [7,8].

General procedure for the synthesis of 5-(2-aminophenyl)-3-aryl-4,5-dihydropyrazoles **4**.

To a methanolic solution (30 mL) of **3** (5 mmol) and catalytic amounts of Raney nickel was slowly added hydrazine hydrate (2 mL). The mixture was heated at reflux until the consumption of the starting material was completed. The solution was then filtered and the aminopyrazolines **4** were filtered and recrystallized from ethanol.

5-(2-Aminophenyl)-3-phenyl-4,5-dihydropyrazole **4a**.

This compound was obtained according to general procedure as white crystals, mp 138 °C, yield 95%; ^{1}H NMR: δ = 3.48 (d, 2H, H-4); 4.30 (s, 2H, NH_2); 4.97 (td, 1H, H-5); 6.08 (d, 1H, NH); 7.00-8.00 (m, 9H, H-Ar).

Anal. Calcd. for $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.71; H, 6.35; N, 17.78.

5-(2-Aminophenyl)-3-(4-chlorophenyl)-4,5-dihydropyrazole **4b**.

This compound was obtained according to general procedure as white crystals, mp 133 °C, yield 97%; ^{1}H NMR: δ = 3.61 (d, 2H, H-4); 4.32 (s, 2H, NH_2); 5.25 (td, 1H, H-5); 6.82 (d, 1H, NH); 7.43 (d, 2H, H-*o*); 7.64 (d, 2H, H-*m*); 7.70-8.05 (m, 4H, H-Ar.).

Anal. Calcd. for $C_{15}H_{14}ClN_3$: C, 66.30; H, 5.19; N, 15.46. Found: C, 66.35; H, 5.26; N, 15.38.

General procedure for the synthesis of 2,5-diaryl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazolines **5a-l**.

A solution of equimolar amounts (16 mmol) of compound **4** and benzaldehyde in ethanol (8 mL) was treated with acetic acid (1mL). The mixture was stirred at room temperature for 10 minutes. The resulting solid was collected and recrystallized from ethanol.

2,5-Diphenyl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5a**.

This compound was obtained according to general procedure as white crystals, mp 172 °C, yield 80%; MS: *m/z* (%) = 325 (M^+, 100), 222 (36), 221 (34), 180 (24), 145 (47), 119 (28), 104 (24), 77 (33), 51 (19).

Anal. Calcd. for $C_{22}H_{19}N_3$: C, 81.20; H, 5.89; N, 12.91. Found: C, 81.19; H, 5.80; N, 12.86.

2-Phenyl-5-(4-Tolyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5b**.

This compound was obtained according to general procedure as white crystals, mp 213 °C, yield 86%; MS: *m/z* (%) = 339 (M^+, 100), 235 (33), 206 (22), 145 (31), 119 (32), 105 (74), 91 (39), 77 (45), 51 (22).

Anal. Calcd. for $C_{23}H_{21}N_3$: C, 81.38; H, 6.24; N, 12.38. Found: C, 81.47; H, 6.12; N, 12.31.

5-(4-Methoxphenyl)-2-Phenyl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5c**.

This compound was obtained according to general procedure as white crystals, mp 217 °C, yield 84%; MS: *m/z* (%) = 355 (M^+, 95), 210 (12), 121 (100), 91 (16), 77 (32), 51 (17).

Anal. Calcd. for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.76; H, 5.88; N, 11.74.

5-(4-Chlorophenyl)-2-phenyl-1,5,6,10b-tetrahydropirazolo[1,5-*c*]quinazoline **5d**.

This compound was obtained according to general procedure as white crystals, mp 248 °C, yield 89%; MS: *m/z* (%) = 359 (M^+, 100), 256 (31), 255 (25), 206 (22), 145 (42), 119 (30), 77 (26), 51 (14).

Anal. Calcd. for $C_{22}H_{18}ClN_3$: C, 73.43; H, 5.04; N, 11.68. Found: C, 73.48; H, 5.11; N, 11.60.

5-(4-Nitrophenyl)-2-phenyl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5e**.

This compound was obtained according to general procedure as pale yellow crystals, mp 228 °C, yield 95%; MS: *m/z* (%) = 370 (M^+, 100), 353 (34), 267 (43), 251 (19), 206 (23), 145 (43), 119 (31), 77 (18).

Anal. Calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.31; H, 4.83; N, 15.16.

5-(4-Bromophenyl)-2-phenyl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5f**.

This compound was obtained according to general procedure as white crystals, mp 263 °C, yield 92%; MS: *m/z* (%) = 407/405 (M^+, 92/100), 300 (22), 206 (30), 145 (73), 119 (69), 104 (36), 77 (95), 51 (62).

Anal. Calcd. for $C_{22}H_{18}BrN_3$: C, 65.36; H, 4.49; N, 10.39. Found: C, 65.23; H, 4.39; N, 10.28.

2-(4-Chlorophenyl)-5-phenyl-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5g**.
This compound was obtained according to general procedure as white crystals, mp 189 °C, yield 81%; MS: *m/z* (%) = 359 (M^+, 100), 222 (35), 206 (21), 145 (49), 104 (39), 91 (50), 77 (52), 51 (42).
Anal. Calcd. for $C_{22}H_{18}ClN_3$: C, 73.43; H, 5.04; N, 11.68. Found: C, 73.46; H, 5.05; N, 11.65.

2-(4-Chlorophenyl)-5-(4-methylphenyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5h**.
This compound was obtained according to general procedure as white crystals, mp 234 °C, yield 81%; MS: *m/z* (%) = 373 (M^+, 100), 236 (28), 145 (22), 119 (19), 105 (88), 91 (26), 77 (18), 51 (10).
Anal. Calcd. for $C_{23}H_{20}ClN_3$: C, 73.89; H, 5.39; N, 11.24. Found: C, 73.83; H, 5.33; N, 11.31.

2-(4-Chlorophenyl)-5-(4-methoxylphenyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5i**.
This compound was obtained according to general procedure as white crystals, mp 236 °C, yield 80%; MS: *m/z* (%) = 391/389 (M^+, 20/63), 210 (10), 122 (10), 121 (100), 77 (10), 91.
Anal. Calcd. for $C_{23}H_{20}ClN_3O$: C, 70.85; H, 5.17; N, 10.78. Found: C, 70.89; H, 5.23; N, 10.72.

2,5-bis(4-Chlorophenyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5j**.
This compound was obtained according to general procedure as white crystals, mp 206 °C, yield 85%; MS: *m/z* (%) = 393 (M^+, 100), 282 (22), 255 (42), 233 (17), 145 (49), 119 (49), 77 (16), 51 (8).
Anal. Calcd. for $C_{22}H_{17}Cl_2N_3$: C, 67.01; H, 4.35; N, 10.66. Found: C, 67.09; H, 4.31; N, 10.59.

2-(4-Chlorophenyl)-5-(4-nitrophenyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5k**.
This compound was obtained according to general procedure as pale yellow crystals, mp 199 °C, yield 90%; MS: *m/z* (%) = 406/404 (M^+, 39/100), 387 (38), 357 (26), 266 (33), 267 (61), 251 (32), 204 (27), 179 (27), 145 (83), 119 (72).
Anal. Calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.20; H, 4.28; N, 13.78.

5-(4-Bromophenyl)-2-(4-Chlorophenyl)-1,5,6,10b-tetrahydropyrazolo[1,5-*c*]quinazoline **5l**.
This compound was obtained according to general procedure as white crystals, mp 214 °C, yield 85%; MS: *m/z* (%) = 439 (M^+, 100), 300 (35), 282 (35), 240 (26), 233 (22), 204 (22), 171 (34), 145 (73), 119 (59), 77 (24), 51 (11).
Anal. Calcd. for $C_{22}H_{17}ClBrN_3$: C, 60.22; H, 3.91; N, 9.58. Found: C, 60.19; H, 3.81; N, 9.48.

Microorganisms and media

The microorganisms used for the fungistatic evaluation were purchased from the American Type Culture Collection (Rockville, MD, USA): *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATCC 26934, *Aspergillus niger* ATCC 9029 and *Trichophyton mentagrophytes* ATCC 9972. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30°C. Cell suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 viable yeast cells/mL [11]. Dermatophytes: *Microsporum canis* C 112, *Trichophyton rubrum* C 113, *Epidermophyton floccosum* C 114 and *Microsporum gypseum* C 115 as well as *Candida tropicalis* C131 are clinical isolates and were kindly provided by CEREMIC, Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures [11] and adjusted to 10^6 spores with colony forming ability/mL.

Antifungal assays.

The antifungal activity was evaluated with the agar dilution method by using Sabouraud-chloramphenicol agar for both yeast, hialohyphomycetes and dermatophyte species as previously described [12]. Stock solutions of compounds (10 mg/mL in DMSO) were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 0.10 to 250 μg/mL MIC for each compound was defined as the lowest concentration that produces no visible fungal growth after the incubation time.

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