SYNTHESIS AND PRELIMINARY CYTOTOXIC AND ANTIFUNGAL EVALUATION OF SOME 6-N,N-DIALKYL 2-ARYL-4(3*H*)-QUINAZOLINONE DERIVATIVES

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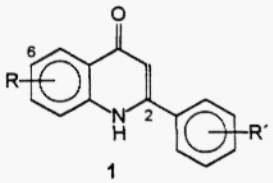
Abstract: Some new 2-aryl-4-quinazolinone derivatives bearing a cyclic amino group at position 6 were prepared and their preliminary cytotoxic and antifungal evaluation is reported. They all showed a moderate cytotoxicity suggesting that not only the oxidation state of the N1-C2 bond is a crucial factor for this kind of biological activity. These quinazolinones were tested against several yeast, filamentous and dermatophyte fungi showing all to be inactive.–

Introduction

For many years, the 4(3*H*)-quinazolinone nucleus has been implicated in diverse structures with a wide range of pharmacological activities, which include analgesic, anesthetic, antibacterial, anticancer, anticonvulsant, antihipertensive, anti-inflamatory, antiviral, antimalarial and antiparasitic, brochodilator, diuretic, muscle relaxant, antisedative and recently, in the designing of novel phosphodiesterase type 5 (PDE5) inhibitors for the treatment of erectile dysfunction and some inhibitors of the thymidylate synthase (TS) enzyme [1-8]. During last decade, a great interest in the isolation, design and synthesis of novel cytotoxic-antitumoral drugs which interfere with the microtubule system has emerged. Specially important examples of this kind of compounds are the 2-aryl-4-quinolones **1** and 2-aryl-naphtiridin-4-ones **2**, both with a relatively simple skeleton [9-12]. Those findings have aimed us for the preparation of some 6-N,N-dialkyl 2-aryl-4(3*H*)-quinazolinone derivatives **7a-i** as possible new cytotoxic agents [13]. Their biological activity toward yeast, filamentous and dermatophyte fungi were also evaluated.



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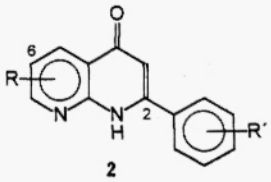

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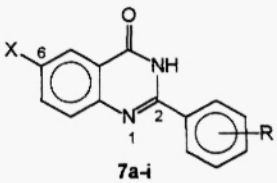


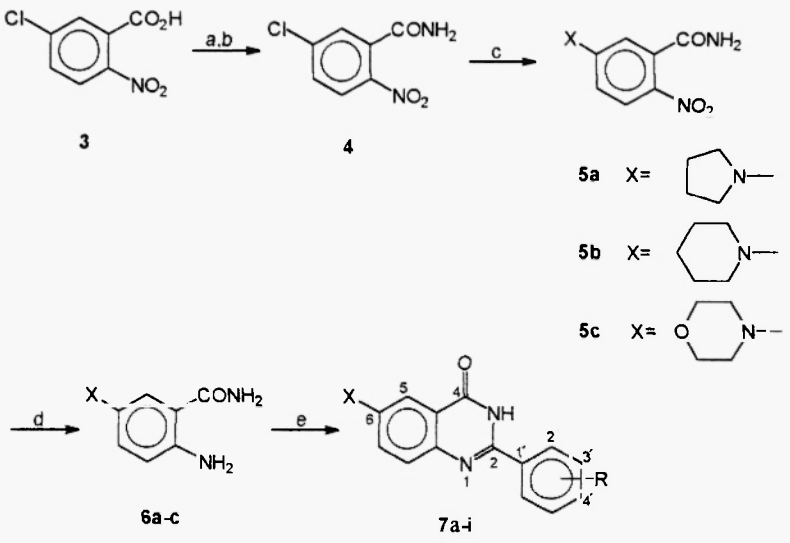
7a-i

Results and discussion

The 2-aryl and alkyl 4(3*H*)-quinazolinones have been prepared by a variety of procedures, which include the classic cyclisation-dehydration of previously synthesized N-acyl anthranilic acid derivatives [14], reaction of ammonia with 4(3-oxazolin)-ones [15], oxidation and cyclisation of substituted 2-amino-benzenenitriles [16], and the direct cyclisation-dehydrogenation of 2-aminobenzamides with benzaldehydes employing sodium hydrogen sulfite in hot DMA [13,17]. Concerning to this last, in a previous paper we described an steric effect attributed to the substituent at position 2′ of the 1,2-dihydro-quinazolinone intermediate, which interfere with the dehydrogenation step in this useful methodology [13].

The synthesis of 4-quinazolinones **7a-i** is depicted in **Scheme 1**. Starting with comercially available 5-chloro-2-nitrobenzoic acid **3**, we firstly synthesized the corresponding 2-nitrobenzamide **4**, which was further reacted with the appropriate cyclic alkylamine affording desired 5-N,N-dialkyl substituted 2-nitrobenzamide **5a-c**, that upon catalytic hydrogenation gave the key intermediate 2-aminobenzamide **6a-c**. In a final step, **6a-c** were treated directly as crude with an appropriate substituted benzaldehyde using 1.5 eq of sodium hydrogen sulfite in dimethylacetamide (DMA) at 160°C for three hours giving the expected quinazolinones **7a-i**.

Scheme 1. Synthesis of 6-N,N-dialkyl 2-aryl-4(3*H*)-quinazolinone derivatives



Conditions: a. $SOCl_2$ 5 eq / benzene, DMF cat., reflux, 12 h; b. 25% aq. NH_3, rt, 1 h;
c. pyrrolidine, 5 eq, reflux, 45 min.; d. H_2, 5% Pd / C, EtOH, 3 h;
e. substituted benzaldehydes, $NaHSO_3$ 1.5 eq, DMA, 160°C, 3 h

Preliminary Cytotoxic Evaluation

The preliminary cytotoxic evaluation of synthesized compounds **7a-i** was carried out employing lymphocyte cell cultures, measuring the incorporation of [^{3}H]Timidine [18]. The results are outlined in **Table 1** and they were expressed in *dpm* (disintegrations per minute), thus, a lower *dpm* value indicates a higher cytotoxic activity.

The cytotoxic and antitumor effects of 2,3-dihydro-2-aryl-4-quinazolinones were reported near 30 years ago [20,21]. Also, 2-styrylquinazoline-4(3*H*)-ones have demonstrated to be a highly cytotoxic class of compounds against several cancer cells lines and it seemed that inhibition of tubulin polymerization is their mechanism of action [11]. As seen (**Table 1**), the quinazolinones here reported were moderate cytotoxic against lymphocyte cells; the most cytotoxic compound was **7g**, bearing a 6-piperidine ring and a 4′-methyl group, it was 3.2 and 5.1 fold more potent than its 6-pirrolidino (**7e**) and 6-morpholino (**7i**) analogues respectively. A 3′-methoxy group seems to be better than a 3′-chloro for the cytotoxic effect in 6-pirrolidino substituted compounds (**7c** *vs.* **7b**). Contrary to that suggested for related 6-pirrolidino-2-aryl-quinolones, a 4′-chloro group increases the observed cytotoxic effect to that seen for its 3′-methoxy counterpart (**7d** *vs.* **7c**) [11]. The diminished activity of **7i** as compared with its 1,2-dihydro derivative **7f** suggest that the

Table 1.Cytotoxic Evaluation of compounds **7a-i** in linfocite cell cultures (incorporation of [^{3}H]Timidine expressed in disintegrations per minute, *dpm*)

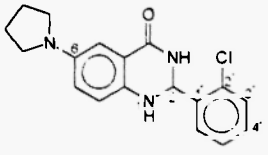
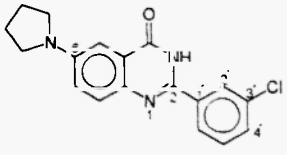
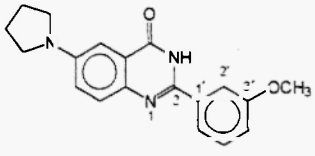
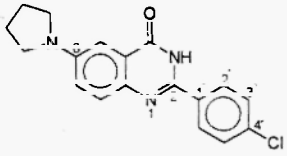
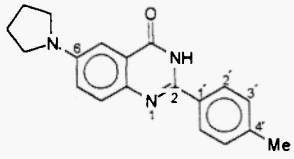
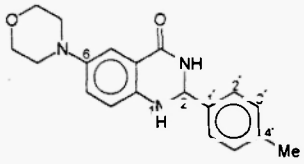
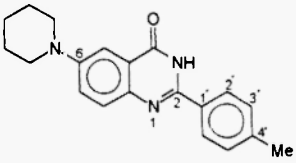
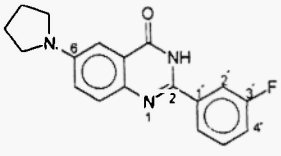
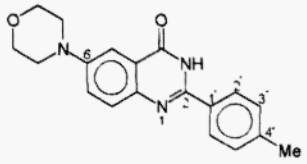
Structure	Compd	Formula	*dpm*
	**7a**	$C_{18}H_{18}ClN_3O$	88.4
	**7b**	$C_{18}H_{16}ClN_3O$	53.0
	**7c**	$C_{19}H_{19}N_3O_2$	46.4
	**7d**	$C_{18}H_{16}ClN_3O$	31.8
	**7e**	$C_{19}H_{19}N_3O$	73.7
	**7f**	$C_{19}H_{21}N_3O_2$	92.5
	**7g**	$C_{20}H_{21}N_3O$	23.0
	**7h**	$C_{18}H_{16}FN_3O$	nt*

Table 1 (*Continued...*)

		**7i**	$C_{19}H_{19}N_3O_2$	117.0
-		**Colchicine**	-	5.0

* nt = *not tested*

oxidation state of the N1-C2 bond in these compounds may be important for observed biological activity, but it is not a crucial factor for cytotoxicity, thus the most active compound **7g** possess a double bond between those positions. The promptly evaluation of these and some other new quinazolinones against various cancer tumor cell lines and of their antitubulin polymerization activity will give us a better understanding about the mode of action of them. Those future assays will be very important because it was previously suggested that the N1-C2 double bond accounts for elimination of the antitubulin activity of 2-phenyl-4(3*H*)-quinazolinone [22].

Antifungal Evaluation

Due the wide range of activities depicted by quinazolinones, we decided to test ours against several strains of fungi. To carry out the antifungal evaluation, concentration of synthesized quinazolinones up to 250 μg/mL were incorporated into growth media according to reported procedures [19]. The agar dilution method showed that none of the compounds tested was active against the yeast *Candida albicans*, *Criptococcus neoformans*, *Saccharomyces cerevisae* nor against the filamentous fungi *Aspergillus niger*, *Aspergillus fumigatus*, or *Aspergillus flavus* (see **Table 2**). These quinazolinones were also inactive against dermatophytes (MICs > 250 μg/mL) (**Table 3**).

Table 2. MIC Values (μg/mL) for Antifungal Activities of synthesized quinazolinones against Yeast and Filamentous Fungi

	Yeast			Filamentous		
compd	a	b	c	d	e	f
7a*	-	-	-	-	-	-
7b	>250	>250	>250	>250	>250	>250
7c*	-	-	-	-	-	-
7d	>250	>250	>250	>250	>250	>250
7e*	-	-	-	-	-	-
7f	>250	>250	>250	>250	>250	>250
7g	>250	>250	>250	>250	>250	>250
7h	>250	>250	>250	>250	>250	>250
7i	>250	>250	>250	>250	>250	>250

[a] *C. albicans*, [b] *S.cerevisae*, [c] *C. neoformans*, [d] *A. fumigatus*, [e] *A. niger*, [f] *A. flavus*. * Not tested

Table 3. MIC Values (μg/mL) for Antifungal Activities of synthesized quinazolinones against Dermatophytes

compd	a	b	c	d	e
7a*	-	-	-	-	-
7b	>250	>250	>250	>250	>250
7c*	-	-	-	-	-
7d	>250	>250	>250	>250	>250
7e*	-	-	-	-	-
7f	>250	>250	>250	>250	>250
7g	>250	>250	>250	>250	>250
7h	>250	>250	>250	>250	>250
7i	>250	>250	>250	>250	>250

[a] *T. rubrum*, [b] *T. mentagrophytes*, [c] *E. floccosum*, [d] *M.gypseum*, [e] *M. canis*, * *Not tested.*

Experimental

Chemistry

Melting points were determined with a Fischer-Johns micro hot-stage apparatus and are uncorrected. IR spectra were recorded on a BIO-Rad FT/IR 6000 Excalibur Series spectrometer as potassium bromide discs. ^{1}H NMR spectra were measured either with a JEOL Eclipse (270 MHz) or a JEOL Eclipse Plus (400 MHz) spectrometer; δ values in ppm relative to tetramethylsilane are given. When reported, mass spectra were recorded on a Varian Saturn 2000 Mass Selective Detector with EI(70 eV) connected to a gas chromatograph Varian Chrompack CP-38000 (GC/MS/MS) Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA , USA); the results fell in the range ± 0.4% of the required theoretical values. Silica gel plates ALUGRAM® SIL G/UV$_{254}$ (Macherey-Nagel GmbH & Co., Germany) were used for TLC testing. Reagents were obtained from Aldrich (Milwaukee, MI, USA) or Merck (Darmstadt, Germany) and used without further purification. Solvents were distilled prior to use. 5-chloro-2-nitrobenzamide **4** was synthesized according to the literature [23]. 2-nitro-5-(1-pyrrolidino)-benzamide **5a**, 2-amino-5-(1-pyrrolidino)-benzamide **6a** and the corresponding 6-N,N-dialkyl 2-aryl-4(3*H*)-quinazolinones **7a-e**, **7h** were prepared following the procedure previously reported by us [13].

2-nitro-5-(1-Piperidino)-benzamide (**5b**).- A mixture of **4** (3.00 g, 15 mmol) and piperidine (6.4 g, 75 mmol, 5 eq.) was heated under reflux for 45 min., cooled to room temperature and then poured into crushed ice-water. The yellow solid was filtered off, washed with water, dried under vacuo and recrystallized from hot EtOH (yield: 6.44 g , 81 %); mp: 207 °C *d.* IR (KBr, cm^{-1}): ν = 1654 (C=O). ^{1}H NMR (DMSO d_6/ TMS): δ = 1.58 (m, 6H, CH_2), 3.47 (m, 4H, CH_2), 6.80 (d, 1H, 6-H, J= 2.7 Hz), 6.96 (dd, 1H, 4-H, J= 2.7 Hz, J= 9.3 Hz), 7.50 (s, 1H, NH_2), 7.85 (s, 1H, NH_2), 7.92 (d, 1H, 3-H, J= 9.3 Hz) . (Found: C, 57.71; H, 6.16; N, 16.68 %. $C_{12}H_{15}N_3O_3$ requires C, 57.82; H, 6.07; N, 16.86 %).

2-nitro-5-(1-Mopholino)-benzamide (**5c**).- A similar procedure as for **5b**. The yellow solid was filtered off, washed with water, dried under vacuo and recrystallized from hot EtOH (yield: 6.11 g , 75 %); mp: 260-261 °C. IR (KBr, cm^{-1}): ν = 1656 (C=O). ^{1}H NMR (DMSO d_6/ TMS): δ = 3.40 (t, 4H, CH_2), 3.72 (t, 4H, CH_2), 6.89 (d, 1H, 6-H, J=

2.9 Hz), 7.03 (dd, 1H, 4-H, J= 2.9 Hz, J= 9.3 Hz), 7.55 (s, 1H, NH_2), 7.89 (s, 1H, NH_2), 7.97 (d, 1H, 3-H, J= 9.3 Hz). (Found: C, 52.43; H, 5.08; N, 16.87 %. $C_{11}H_{13}N_3O_4$ requires C, 52.59; H, 5.21; N, 16.73 %).

2-Amino-5-(1-piperidino)-benzamide (**6b**) and 2-Amino-5-(1-morpholino)-benzamide (**6c**).- To a solution of either **5b** (1 g, 4.01 mmol) or **5c** (1g, 3.98 mmol) in dry ethanol (100 mL) was added 5% Pd/C (50 mg), and the mixture was hydrogenated at room temperature (40 psi) for 3 h. Then it was filtered through celite, the solvent was evaporated and washed with dry benzene (Yield of crude **6b**: 0.67 g, 76 %), (Yield of crude **6c**: 0.86 g, 98 %). These products were then used as crude for the next step.

2-(Substituted phenyl)-6-(alkylamino)-4-quinazolinones (**7f**, **7g** and **7i**).

General Procedure:

Sodium hydrogen sulfite (0.31 g, 3.0 mmol, 1.5 eq.) is added to a solution of the substituted benzaldehyde (2.0 mmol) and the corresponding 2-aminobenzamide (2.0 mmol) in dimethylacetamide (6 mL). The mixture was heated with stirring at 160 °C for 3 h, cooled to room temperature and poured into crushed ice-water. The precipitate was filtered, washed twice with water and dried under vacuo.

2-(4-Methylphenyl)-6-(1-morpholino)-1,2-dihydro-4(1*H*)-quinazolinone (**7f**).-(Yield: 0.53 g, 82 %, recrystallized from DMF/H_2O); mp: 240-243 °C . IR (KBr, cm^{-1}): ν = 1670 (C=O). ^1H NMR (DMSO d_6/ TMS): δ = 2.37 (s, 3H, CH_3), 3.25 (t, 4H, CH_2), 3.72 (m, 4H, CH_2), 6.50 (s, 1H, 2-H), 6.73 (d, 1H, 8-H, J=8.9 Hz), 7.07 (dd, 1H, 7-H, J=2.2 Hz, J= 8.9 Hz), 7.20 (d, 1H, 5-H, J= 2.2 Hz), 7.20-7.42 (m, 3H, NH, 3'-H, 5'-H), 7.48-7.62 (m, 2H, 2'-H, 6'-H), 8.05 (bs, 1H, NH). (Found: C, 70.32, H, 6.69; N, 12.73 % . $C_{19}H_{21}N_3O_2$ requires C, 70.57; H, 6.55; N, 12.99 %).

2-(4-Methylphenyl)-6-(1-piperidino)-4(3*H*)-quinazolinone (**7g**).- (Yield: 0.54 g, 84 %, recrystallized from DMF/H_2O). mp: 257 °C *d* . MS (EI, m/z): 319 [M^+, 100 %]. IR (KBr, cm^{-1}): ν = 1670 (C=O). ^1H NMR (DMSO d_6 / TMS): δ =1.57-1.62 (m, 6H, CH_2), 2.37 (s, 3H, CH_3). 3.26-3.35 (m, 4H, CH_2), 7.32 (d, 2H, 3'-H, 5'-H, J=8.2 Hz), 7.43 (d, 1H, 5-H, J=2.0 Hz), 7.57 (dd, 1H, 7-H, J= 2.0 Hz, J= 8.2), 8.06 (d, 1H, 8-H., J= 8.2 Hz), 12.27 (bs, 1H, NH). (Found: C, 75.06; H, 6.89; N, 12.98 %. $C_{20}H_{21}N_3O$ requires C, 75.21; H, 6.63., N, 13.16 %).

2-(4-Methylphenyl)-6-(1-morpholino)-4(3*H*)-quinazolinone (**7i**).- This compound was obtained only when the heating was elevated to 180 °C, at lower temperatures the 1,2-doble bond was not formed and the dihydro compound **7f** predominates (Yield: 0.57 g, 88 %, recrystallized from DMF/H_2O). mp: 318 °C *d*. IR (KBr, cm^{-1}): ν = 1668 (C=O). ^1H NMR (DMSO d_6 / TMS): δ =2.40 (s, 3H, CH_3), 3.24 (t, 4H, CH_2), 3.78 (t, 4H, CH_2), 7.34 (d, 2H, 2'-H, 6'-H, J=8.1 Hz), 7.45 (d, 1H, 5-H, J=2.6 Hz), 7.58 (dd, 1H, 7-H, J= 2.6 Hz, J= 8.8 Hz), 7.62 (d, 1H, 8-H, J= 8.8 Hz), 8.07 (d, 2H, 3'-H, 5'-H, J= 8.1 Hz), 12.25 (bs, 1H, NH). (Found: C, 70.84; H, 5.79; N, 13.20 %. $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.07 %)

Biological Evaluation

Cytotoxicity

The preliminary cytotoxic effect of compounds **7a-i** was determined using a lymphocyte cell culture, as previously described [18]. These lymphocytes were isolated from the spleen of Hoffman rats (100 to 200g) in sterile conditions Eritrolysis was made in a solution of NH_4Cl. Cultures, 3.10^5 cells / mL MEM (Sigma), were supplemented with $NaHCO_3$ and 5-10% of fetal serum (FCS) (Gibco). Also, there were added the antibiotics penicillin (10 IU/mL) and streptomycin (100 μg/mL). They were incubated for 24 hours at 37°C in a CO_2 atmosphere (5%). After washing, cells were transfered to a fresh medium, supplemented with FCS and phytohemaglutinine (PHA) and then incubated at 37°C for a further 48 hours. For the determination of the antiproliferative effects of different compounds, solutions of 10 μg/mL each were

added to a vial containing 1 mL of the above mentioned lymphocyte suspension (3.10^5 cells / mL). Controls were made using Colchicine (10 μg/mL), under similar conditions. After 48 hours, 2 μCi/mL of [^{3}H] Thymidine (specific activity 2 Ci/min) (NEM) were added to each vial and cultured for 4 hours.

Antifungal Assays

The following microorganisms used for the fungistatic evaluation were purchased from American Type Culture Collection (Rockville, MD): *C. albicans* ATCC 10231, *S. cerevisiae* ATCC 9763, *C. neoformans* ATCC 32264, *A. flavus* ATCC 9170, *A. fumigatus* ATCC 26934, and *A. niger* ATCC 9029. Strains were grown on Saboureaud chloramphenicol agar slants for 48 h at 30 °C. Cell suspensions in sterile distilled H_2O were adjusted to give a final concentration of 10^6 viable yeast cells/mL. Dermathophytes: *M. canis* C112, *T. rubrum* C 115 are clinical isolates and were kindly provided by CEREMIC, Centro de Referencia Micologica, Facultad de Ciencias Bioquimicas y Farmaceuticas, Suipacha 531-(2000)-Rosario, Argentina. *T. Mentagrophytes* was ATCC 9972. Organisms were maintained on slopes of Saboureaud dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent plemorphic transformations. Spore suspensions were obtained according to reported procedures and adjusted to 10^6 spores with colony forming ability per mililiter [24].

The fungistatic activity of compounds **7a-i** was evaluated with the agar dilution method by using Saboureaud-chloramphenicol agar for both yeast and dermatophyte species. The assays were carried out in 96 well microtiter plates Stock solutions of compounds in DMSO were diluted to give serial twofold dilutions that were added to each medium, resulting in concentrations ranging from 0.10 to 250 μg/mL. The final concentration of DMSO in the assay did not exceed 2%. Using a micropipette, an inoculum of 5 μL of the yeast cell or spore suspension was added to each Saboureaud-chloramphenicol agar well. The antifungal agents ketoconazol (Janssen Pharmaceutica) and amphotericin B (Sigma Chemical Co.) were included in the assay as positive controls. Drug-free solution was also used as blank control. The plates were incubated 24, 48 or 72 h at 30 °C (according to the control fungus growth) up to 15 days for dermatophyte strains. MIC was defined as the lowest compound concentration showing no visible fungal growth after incubation time.

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