SYNTHESIS OF 8-HYDROXYQUINOLINE CHALCONES: TRANS CONFIGURATION, INTRAMOLECULAR HYDROGEN BONDS, BROMINATION, AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Nine (8-Hydroxyquinolin-5-yl)-arylpropenones were synthesized and their structures demonstrated by IR and NMRspectroscopy. These molecules showed transconfiguration and strong intramolecular hydrogen bonding; in the IR spectra of 5-formyl-8-hydroxyquinoline, 5-acetyl-8-hydroxyquinoline, 1-(8-hydroxyquinolin-5-yl)-3-phenylprop-2-en-1-one and 3-(8-hydroxyquinolin-5-yl)-1-phenylprop-2-en-1-one in $CHCl_3$, besides the known intermolecular hydrogen band (~3180 cm^{-1}), we identified the intramolecular hydrogen band OH···N (3460 cm^{-1}); the hydrogen bond peaks shifted to low frequency in proton-donor solutions such as phenol and acetic acid (with respect to 8-hydroxyquinoline) and the bonds were broken in trifluoroacetic acid solutions, due to OH protonation; the apolar solvent CCl_4 and electrophilic substituents in position 5 in the quinoline ring, limited the formation of the intermolecular hydrogen bonds and, therefore, shifted the ~3460 cm^{-1} intramolecular hydrogen band to lower frequencies and made it stronger and sharper. The bromination of 3-(8-hydroxyquinolin-5-yl)-1-(4-tolyl) prop-2-en-1-one occurred on the activated quinoline fragment, producing monobromo and tetrabromo derivatives, instead of bromination on the aliphatic double bond. Three chalcones tested showed strong antifungal activity *in vitro*.

Keywords: 8-hydroxyquinoline, chalcones, bromination, antifungal activity

INTRODUCTION

α,β-unsaturated ketones containing aromatic or heterocyclic radicals (chalcones) have attracted researcher attention mainly due to their high reactivity, which allows the synthesis of new organic compounds.[1,2] Chalcone derivatives have been found with high efficient photoactivity such as luminophores, photo-, thermo-, and electrochromic, compounds and dyes for lasers and others with a variety of physiological activity.[3] Studies have increased on cyclocondensation reactions involving a,b- unsaturated ketones and nitrogen nucleophiles. These reactions are the best method for the synthesis of azoles, azinopyridines, and pyrimidines that are similar to many natural compounds due to their conjugated system with two nonequivalent electrophilic centers that determine the regioselectivity of these reactions.[4] The combination of these electrophilic centers, the hydroxyquinoline and the highly reactive enone fragments, opens a wide perspective for the synthesis of organic compounds with chelating properties.[5,6] The synthesis of 5-formyl- and 5-acetyl-8-hydroxyquinoline has been described;[7,8] the reaction of Reymon and Thiman is traditionally used to obtain 5-formyl-8-hydroxyquinoline,[5,7] but this method has poor reproducibility, possibly because of the tendency of the product to form chelates. On the other hand, 5-formyl- and 5-acetyl-8-hydroxyquinoline easily condense with substituted benzaldehydes and acetophenones in basic-ethanolic medium producing chalcones with high yields.

Literature reports on a,b- unsaturated ketones derived from 8-hydroxyquinoline are scarce;[5,7,9-11] there are reports on derivatives of 5-acetyl-8-hydroxyquinoline and the synthesis of a series of 2-pyrazoline, oxazolines, pyridines, and pyrimidines.[11] This work investigates chalcones derived from 5-formyl- and 5-acetyl-8-hydroxyquinoline, their configuration, interaction with proton donors, bromination reaction, and biological activity.

EXPERIMENTAL

The monitoring of all reactions was performed by thin layer chromatography. The chromatograms were developed on Silufol UV-254 plates, using acetic acid as eluent; UV light or iodine were used to reveal the plates. All reagents were analytical grade (Merck, Darmstadt, Germany).

1. Synthesis of 5-formyl-8-hydroxyquinoline (2)

A solution of 20.32 g (0.140 moles) of 8-hydroxyquinoline in 80 ml of ethanol was mixed with 50 ml of 80% aqueous solution of NaOH and refluxed at 80ºC for 1 hour; then, 27.46 g (0.2300 moles) of chloroform were added dropwise and the mixture refluxed at 80 ºC for 12 hours. Later, the excess of chloroform and ethanol was evaporated, and the residue was diluted with 600 ml of water and acidified with HCl 1% until complete precipitation. The complex mixture on the precipitate was dried, and **2** was extracted with n-hexane.

2. Synthesis of 5-acetyl-8-hydroxyquinoline (3)

A solution of 43.5 g (0.300 moles) of 8-hydroxyquinoline in 40 g of nitrobenzene was added with 25.1 g (0.320 moles) of acetic chloride and produced a yellow precipitate. The mixture was stirred and 100 g (0.75 mol) of $AlCl_3$ was added to dissolve the precipitate. This solution was heated to 70 °C for 12 hours, cooled and crushed ice and 100 ml of 10% HCl was added, which formed two phases: water and nitrobenzene. Nitrobenzene was removed by steam distillation and the aqueous phase was allowed to stand for 8 hours which precipitated the hydrochloride of **3**; the precipitate was dried, dissolved in water, and neutralized with 0.1 M solution of sodium acetate to reprecipitate **3** which was later crystallized with distilled water.

3. Synthesis of 3-(8-hydroxyquinolin-5-yl)-1-phenylprop-2-en-1-one (4)

A solution of 2.80 g (0.0161 moles) of 5-formyl-8-hydroxyquinoline in 50 ml of hot concentrated hydrochloric acid was added with 4.00 g (0.0333 mol) of acetophenone; the solution was stirred for 8 hours and a precipitate (**4**) was obtained, which was washed with ether, neutralized with aqueous 0.1 M sodium acetate, and recrystallized from ethanol. Compounds **5-10** were obtained likewise but changing acetophenone for the corresponding reagent (Table 1).

4. Synthesis of 1-(8-hydroxyquinolin-5-yl)-3-phenylprop-2-en-1-one (11)

A solution of 0.370 g (0.00214 moles) of 5-acetyl-8-hydroxyquinoline, 0.210 g (0.00198 moles) of benzaldehyde and 5 ml of concentrated HCl was left for 6 hours in a closed flask stirring every half hour by opening the flask lid. The precipitate obtained was filtered, dissolved in water and neutralized with 0.1 M sodium acetate to reprecipitate **11**. Compounds **12-17** were obtained likewise but changing benzaldehyde for the corresponding reagent (Table 1).

5. Bromination of 3-(8-hydroxyquinolin-5-yl)-1-(4-tolyl) prop-2-en-1-one (5)

A solution of 200 mg of **5** in 10 ml of chloroform was added with a solution of 8 ml of chloroform with 280 mg of bromine, stirring at 50 °C. Then more bromine solution was added until the solution was colored brown; a yellow product precipitated (**19**) which was filtered and washed with hot ethanol. The filtrate plus ethanol were mixed and a red crystal (**18**) precipitated by shaking.

6. Characterization of the chalcones

The chalcones and their precursors were identified by ^{1}H NMR and IR spectroscopy. NMR spectra were taken on a Varian Mercury VX-200 (200 MHz) in DMSO-d_6 solutions. IR spectra were taken on an IR-75 Specord instrument. Melting temperatures were determined in glass capillaries. Elemental analysis was performed to determine nitrogen (bromine indirectly) by the Dumas method.[12] Potential biological activity was calculated with the computer system PASS (Prediction of Activity Spectra for Substances). PASS provides simultaneous prediction of several hundreds of biological activity

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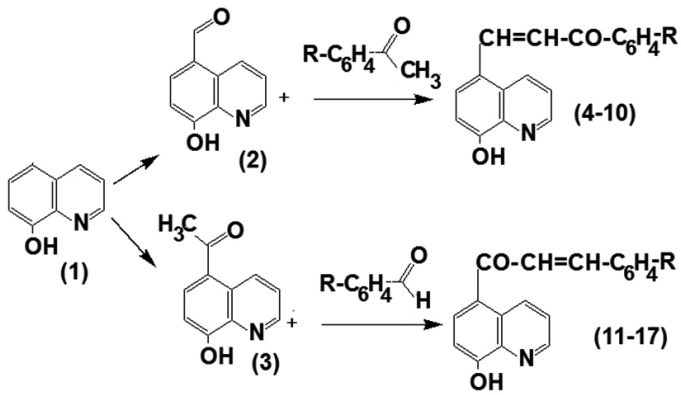
types for any drug-like compound. The prediction is based on the analysis of structure-activity relationships of the training set including more than 30000 known biologically active compounds.[13] Biological activity in-vitro was calculated for three chalcones in the Universidad del Valle (Colombia) using a broth microdilution method to determine the minimum inhibitory concentration (NCCLS, 2003).[14]

RESULTS AND DISCUSSION

Nine new chalcones were synthesized (**5-10**, **12**, **16**, **18** and **19**, Table 1). The synthesis of **2** and **3** was described long ago,[7,8] but we have found difficult to reproduce reaction yields, especially those of the aldehyde. Compounds **2** and **3** were easily condensed with substituted benzaldehydes and acetophenones in basic ethanolic medium producing chalcones **4-17** with 60-80% yields (Scheme 1). The yield of the synthesis of **2** was increased from 10.5% [7] to 18%.

Nitrogen analyses corresponded to those calculated. Melting temperatures for our compounds had no significant differences from those in the literature[6,8] (Table 1), which corroborates the identification of those already reported. The structures of **4-17** were demonstrated by IR and NMR. The IR spectra showed the covalent vibrational bands associated with the OH groups (~ 3300 cm^{-1}), carbonyl groups (1645-1655 cm^{-1}), and the medium intensity band often at 1100 cm^{-1}, characteristic of the deformation vibration of C-H bonds of trans-vinylene groups,[15] which indicates a trans configuration for the vinylene protons.



Scheme 1

Table 1. Physico-chemical properties of the a,b-unsaturated ketones of the 8-hydroxyquinoline series.

Compound	R	Formula	Melting Temperature [1]	Yield %/hours[2]	IR (KBr), cm^{-1}	
			°C		C=O	O-H
2	-	$C_{10}H_7NO_2$	173°C	18%	1684	3430 3120
3	-	$C_{11}H_9NO_2$	112°C	17%	1659	3270
4	H	$C_{18}H_{13}NO_2$	165	70/8	1656	3304
5	CH_3	$C_{19}H_{15}NO_2$	162-65	28/7	1656	3175
6	OCH_3	$C_{19}H_{15}NO_3$	155-7	70/8	1652	3188
7	$N(CH_3)_2$	$C_{20}H_{18}N_2O_2$	210	23/15	1644	3312
8	Cl	$C_{18}H_{12}ClNO_2$	170-2	70/4	1652	3288
9	Br	$C_{18}H_{12}BrNO_2$	186	70/10	1652	3288
10	NO_2	$C_{18}H_{12}N_2O_4$	205-7	20/22	1652	3250
11	H	$C_{18}H_{13}NO_2$	139-40	60/6	1652	3320
12	CH_3	$C_{19}H_{15}NO_2$	180	79/7	1652	3320
13	OCH_3	$C_{19}H_{15}NO_3$	188	62/9	1648	3156
14	$N(CH_3)_2$	$C_{20}H_{18}N_2O_2$	184	65/11	1652	3284
15	Cl	$C_{18}H_{12}ClNO_2$	185	72/7	1652	3316
16	Br	$C_{18}H_{12}BrNO_2$	197-205	70/11	1656	3308
17	NO_2	$C_{18}H_{12}N_2O_4$	217-24	80/17	1660	3312
18	CH_3 [3]	$C_{19}H_{14}NO_2Br$	220	70/1	1656	3312
19	CH_3 [4]	$C_{19}H_{11}NO_2Br_4$	240	20/1	1656	3416

[1] 4 (173);9 11 (143-144),9 (102-104);11 13 (193-194);9 14 (191-192),9 (185-186);11 15 (180-181);11 17 (224).9
[2] Yield after recrystallization from ethanol (4-12, 14-19) and methanol (13)/reaction time
[3] Monobromination product of 5: 3-(7-bromo-8- hydroxyquinolin-5-yl)-1-(4-tolyl)prop-2-en-1-one (18)
[4] Tetrabromination product of 5: 3-(3,4,6,7-tetrabromo-8-hydroxyquinolin-5-yl)-1-(4- tolyl)prop-2-en-1-one (19)

One objective of this research was to study chalcones basicity by measuring the signal change of the hydroxyl group band due to hydroxyl protonation when adding a proton donor (phenol, trifluoroacetic acid and acetic acid); we also analyzed the IR spectra of **1-3** to compare with those of the chalcones.

The chelating ability 8-hydroxyquinoline is well known.[16] In its IR spectrum in KBr,[17] 8-hydroxyquinoline shows a broad band at ~3179 cm^{-1}. The IR spectra of compounds **1** and **2** in KBr (Table 2) showed two bands, one assigned to the intermolecular (~3140 cm^{-1}) and other to the intramolecular hydrogen bond $OH^{-}N$ (~3440 cm^{-1}). This intramolecular band has been demonstrated for 8-hydroxyquinoline N-oxide[18] and we are demonstrating it for **2-4** and **11**. In CCl_4, the band at 3140 cm^{-1} of **1** and **2** disappeared, and the intramolecular band shifted to lower frequencies and became intense and sharp, with respect to **1**, (peak width at half height ~7 cm^{-1}) because CCl_4 is an apolar solvent that limits the formation of intermolecular hydrogen bonds; the intensity of the band at 3416 cm^{-1} of solutions of **1** in CCl_4 increased with concentration. The bands at ~3140 and ~3440 cm^{-1} of **3**, **4** and **11** in KBr (Table 2) overlapped into a wide band centered at ~3300 cm^{-1}.

As expected, the intramolecular band increased in intensity and sharpness in **2**, **3**, **4** and **11** in $CHCl_3$ (not shown in Table 2) and CCl_4 and shifted to low frequency (~3360 cm^{-1}), with respect to **1**; this behavior was probably due to the increased acidity of the OH group caused by the electroreceptor carbonyl group in **2**, **3**, **4** and **11** compared to **1**; this group makes the OH hydrogen more positive which strengthen the intramolecular hydrogen bond.

Table 2. Frequency of vibrations of CO and OH groups in the IR spectra of a,b-unsaturated ketones of the 8-hydroxyquinoline series in KBr and CCl_4 solution with additions of proton donors and without them.

	KBr		CCl_4		CCl_4 + Phenol		$CCl_4 + CH_3COOH$		$CCl_4 + CF_3COOH$	
	γ_{CO} (cm^{-1})	γ_{OH} (cm^{-1})	γ_{CO} (cm^{-1})	g_{OH} (cm^{-1})	γ_{CO} (cm^{-1})	g_{OH} (cm^{-1})	γ_{CO} (cm^{-1})	g_{OH} (cm^{-1})	γ_{CO} (cm^{-1})	g_{OH} (cm^{-1})
1		3453 3160		3416		3416		3419	1689 1675 1630	3070
2	1684	3430 3120	1689	3366	1689	3363	1689	3363	1692 1675 1630	3426 3089
3	1659	3270	1669	3359	1669	3356	1669	3356	1689 1675 1630	3433 3092
4	1656	3304	1665	3346	1659	3440	**	**	1685 1645 1635	3440
11	1652	3320	1665	3386	1665	3390	1665	3385	1655	3296

** Not obtained due to solubility problems

The addition of proton donors such as phenol and acetic acid did not change the IR spectra of **1**, **2** and **3**. With the addition of trifluoroacetic acid in CCl_4, the intramolecular hydrogen bond peak (3440 cm^{-1}) disappeared from the IR spectrum of **1** due to OH protonation, and the intermolecular hydrogen bond peak shifted to low frequency (~3070 cm^{-1}). With **2** and **3**, besides the intermolecular hydrogen bond peak (3089-3092 cm^{-1}), one appeared between 3426-3433 cm^{-1}, probably caused by the association trifluoroacetic acid-carbonyl group, which formed a hydroxyl group. In all experiments with trifluoroacetic acid, we observed a complex spectrum in the region of the carbonyl group (1630-1692 cm^{-1}), identical for **1**, **2** and **3**, due to carbonyl protonation.

It was difficult to add proton donors to chalcones **5-10**, **12-1**7 due to their low solubility in CCl_4 which hindered the interaction with proton donors. Therefore, Table 2 only shows data for compounds without substitution on the benzene ring, **4** and **11;** these data suggests that the nature of the interaction of **4-17** with proton donors might be similar to that discussed previously for **2** and **3**.

The number of aliphatic and aromatic protons calculated from the area of the peaks in the spectra (see supplementary material) correlated well with the number of protons expected from the molecular formulas of the compounds synthesized. Proton signals, especially the vinyl proton signals, were identified by comparison with those of 8-hydroxyquinoline (**1**); vinyl protons appeared as a pair of well defined doublets with spin-spin coupling constants between 15.3 and 15.9 Hz, which shows the trans configuration and geometry of these protons relative to each other in chalcones **4-17**. We compared the change in chemical shift of quinoline protons of chalcones **4-17** to the same signals in compounds **2** and **3**; the introduction of the enonic fragment (-C=C-CO-) produced a signal shift between 0.57 and 1.81 ppm in protons 4H for chalcones **4-10** compared to **2** (Table 3). This shift shows that the double bond was close to the 4H proton and exerted a strong effect on it. Similarly, when chalcones **11-17**, wherein the 4H proton approaches the carbonyl group, are compared to **3**, this effect is minor. This spatial effect is also exerted on the β proton of the enonic fragment of chalcones **4-10** moving its signal to high field. This can be explained only if the quinolinic fragment and the double bond had an anti orientation, i.e. the pyridine fragment is the closest to the enonic b proton.

The bromination of **5** produced **18** and **19,** that were substituted on the quinolinic fragment; using elemental analysis and NMR, **18** was identified as the 7-bromoderivative (the signal of proton 7 at 7.18 ppm disappeared, Table 3) and 19 was identified as the 3,4,6,7-tetrabromoderivative of 5 (the signals of protons 3, 4, 6, and 7 at 8.91, 7.66, 8.75, 8.33 and 7.18 ppm, respectively, disappeared, Table 3); there was not addition of bromine to the C=C double bond due to the strong activating influence of the 8-hydroxy group on the quinolinic fragment, which led to replacement of the quinolinic protons by bromine; the double bond was brominated only after the aromatic ring. Furthermore, the first proton replaced by bromine was the one in position 7 on the ring, ortho to the hydroxyl group, which is the active position due to activation by this group.

Biological activity. PASS software was used to calculate the biological activity; in general, all studied compounds showed theoretical biological activity; **6** and **13**, for example, were found to be mucomembranous protectors, antiseborrheic, and inhibitors of amine dehydrogenase, glutathione thiolesterase, feruloyl esterase, alkane 1-monohydrogenase, and indanol dehydrogenase for a probability of more than 78%. Antifungal activity was measured in vitro for **5, 12** and **14;** the minimal inhibitory concentrations (MIC) in μg/mL for ten fungi (Table 4) showed antifungal activity for these compounds; compound 14 showed the lowest minimal inhibitory concentrations between these three compounds.

Table 3. NMR spectra of the a,b-unsaturated ketones of the 8-hydroxyquinoline series

№	d, (ppm)*									
	Quinolinic fragment					enon		aryl		
	2 H	3 H	4 H	6 H	7 H	α-H	β-H	ortho	meta	CH_3
1	8.83	7.52	8.30	7.40	7.06					
2	8.96	7.76	9.55	8.17	7.22					
3	8.89	7.68	9.39	8.28	7.12					2.67
4	8.90	7.67	8.74	8.33	7.18	8.45	7.98	8.13	7.58**	
5	8.91	7.66	8.75	8.33	7.18	8.43	7.93	8.08	7.36	2.39
6	8.92	7.67	8.75	8.32	7.19	8.42	7.94	8.19	7.08	3.84
7	8.91	7.48	7.74	8.24	7.17	8.36	7.90	8.07	6.77	2.99
8	8.93	7.67	8.77	8.38	7.20	8.48	7.95	8.21	7.64	
9	8.96	7.80	8.80	8.35	7.26	8.47	7.93	8.12	7.75	
10	9.11	7.86	8.98	8.60	7.38	8.69	8.14		8.57	
11	8.92	7.84	9.24	8.35	7.16	7.73	7.66	7.71	7.44**	
12	8.92	7.72	9.23	8.34	7.17	7.76	7.63	7.73	7.25	2.34
13	8.92	7.71	9.21	8.3	7.17	7.64	7.64	7.80	7.00	3.80
14	8.86	7.64	9.19	8.18	7.12	7.58	7.42	7.63	6.72	2.97
15	8.91	7.88	9.24	8.36	7.15	7.77	7.65	7.70	7.49	
16	8.98	7.92	9.49	8.44	7.28	7.86	7.65	7.79	7.64	
17	8.99	7.84	9.44	8.50	7.29	8.03	7.74	8.26	8.11	
18	8.96	7.73	8.79	8.65		8.39	8.08	8.15	7.38	2.40
19	9.05					8.46	8.00	8.10	7.39	2.40

* Spin-spin coupling constants (Hz): 2H 1.2-1.8 and 3.7-4.3; 3H 3.7-4.3 and 8.5-8.8; 4H 1.2-1.5 and 7.3-8.9; 6H 7.9-8.6; 7H 7.0-8.6; αH 14.7-15.9; βH 15.3-15.9; ortho 5.3-8.6; meta 6.1-9.2

** Overlapped signals of para and meta protons

Table 4. Antifungal activity for conventional fungi. Data are the minimal concentrations to inhibit fungi growth/minimal fungicide concentrations, in μg/mL.

№	Ca	Ct	Sc	Cn	Afl	Afu	An	Mg	Tr	Tm
5	125/ >250	250/ >250	125/ >250	31.2/ >250	125/ >250	125/ >250	125/ >250	125/ 250	62.5/ 125	62.5/ 125
12	125/ >250	250/ >250	125/ >250	125/ >250	250/ >250	250/ >250	250/ >250	125/ 250	125/ 250	125/ 250
14	62.5/ >250	125/ >250	62.5/ >250	62.5/ >250	125/ >250	125/ >250	125/ >250	62.5/ 62.5	62.5/ 125	62.5/ 125

Ca: *Candida albicans;* Ct: *Candida tropicalis;* Sc: *Saccharomyces cerevisiae;* Cn: *Cryptococcus neoformans;* Afl: *Aspergillus flavus;* Afu: *Aspergillus fumigatus;* An: *Aspergillus niger;* Mg*: Microsporum gypseum;* Tr: *Trichophyton rubrum;* Tm: *Trichophyton mentagrophytes.*

CONCLUSIONS

Nine new chalcones were synthesized and their melting temperatures were obtained; their structures were demonstrated by IR, NMR and elemental analysis.

1. These chalcones showed trans configuration and strong intramolecular hydrogen bonds; these intramolecular bonds were demonstrated for **1-4** and **11**; the hydrogen bonds remained in proton-donor solutions of phenol and acetic acid and were broken only in trifluoroacetic acid solutions. These chalcones have a strong potential as chelating agents in analytical chemistry.
2. The bromination of these chalcones occurred on the quinoline fragment and not on the double bond due to activation of the rings by the hydroxyl group.
3. The chalcones showed an important antifungal activity.

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