SYNTHESIS OF SOME (E)-2-CHLORO-METHOXY-3-[2-(1-INDANOYL)METHYLIDEN]-QUINOLINES AND THEIR CYTOTOXICITY AND ANTIFUNGAL ACTIVITIES

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Abstract: A series of 2-cloro-3-indanoyl-methoxyquinolines **3-29** were prepared in order to investigate their cytotoxicity and antifungal activities. The synthesized compounds were identified by ^{1}H-NMR, IR, MS and micro analysis. Some of them showed a promising cytotoxic activity. The detailed synthesis, spectroscopic and biological data are described.

Introduction

Synthetic and natural α,β-unsaturated carbonyls exhibit remarkable biological activity [1]. Among others, several chalcones and related benzyliden ketones have been reported to possess antifungal [2], antibacterial [3], and cytotoxic (antitumor) activities [4]. Also, we have recently reported the synhesis, antiinflamatory [5], antifungal [6], and antimalarial [7,8] activities of some quinolinylchalcones and (E)-2-chloro-dimethoxy-3-[2′-(1-indanoyl or tetraloyl)methyliden]quinolines [9]. A number of the investigated derivatives were found to be more active than the reference compounds used in the different in vitro test systems. The observed biological activities have been found to be affected by both the ring size and the substitution pattern. The biological activity of α,β-unsaturated carbonyl compounds is considered to be associate with their reactivity with the thiol moiety of critical peptíde and/or proteins. However, recently their antifungal, antiinflamatory and antimalarial activity has been related with the inhibition of chitin synthase-1, and B(1,3)-glucan synthase [6], inhibition of the novo inducible nitric oxide synthase, COX-2 [5], and cystein protease [7,8] respectively. Cytotoxic activity have been related with the inhibition of the cyclin-dependent kinases (CBKs), these enzymes are involved at different stages of cell cycle [10, 11]. The above results prompted us to design and synthesize a series of conformational analogues of (E)-2-qinolynilchalcones to optimize their structure-activity relationships and to develop more potent and selective antitumor agents. Herein, we report the synthesis, systematic investigation of structure-activity relationships of these classes of compounds, and the discovery of remarkably cytotoxicity of compounds **9**, **11**, and **29**.

Results and discussion

The indanones **2a-i** were prepared according to the methods described in the literature [12, 13]. However, for the 4-methoxy-1-indanone it was necessary to made a modification of the reported method, employing NaOH 7.5 N [14]. 2-chloro-3-formyl-methoxyquinolines were obtained by the acetylation of the respective methoxyanilines with acetic acid anhydride at room temperature, to give the related methoxyanilide, which in turn were made to react with the Villsmeyer reagent to give **1a-c** [15]. The final substituted quinolines **3-29** were synthesized by a Claisen-Schmidt condensation between substituted 1-indanones **2a-i** with the respective quinolines (scheme). The purities and structures of the compounds were confirmed by their melting-points, IR, ^{1}H NMR, MS and with exception of compounds **9, 11, 15,** and **29** by elemental analysis. Theoretically, E and Z geometric isomers can be equally formed in the reaction condition used for the synthesis of these compounds. However, the Z configuration is highly unfavourable because of the strong steric interaction between the 2-chloro substituent of the quinoline ring and the carbonyl group. Also, we observed an interesting coupling between the vinyl hydrogen and methylene hydrogens at position 3′ of the indanone ring in the ^{1}HNMR spectra (J values between 2.0 and 2.5 Hz). Due the wide range of activities depicted by chalcone derivatives, we decided to test ours compounds against several strains of fungi. To carry out the antifungal evaluation, concentration of synthesized **3-29** compounds up to 250 μg/ml were incorporated into the growth media. The agar dilution method showed that none of the compounds tested was active against the yeast *C. albicans, C. neoformans, S. cervisae* nor against the filamentous fungi *A. niger, A. fumigatus,* or *A. flavus*. These compounds were also inactive against dermatophytes (MIC > 250 μg/ml).

Compounds **3-29** were tested against 3 cancer cell lines MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) at a concentration of 1.0 x 10^{-4} M. The data reported in Table 2 indicates that some antiproliferative activity was obtained with compounds **9, 11, 29**. In particular, **9** is a prototypic molecule serving as a template for subsequent molecular modification in view of its increased activity and selective toxicity. Despite specific binding assays were not done, we can speculate that the above result further provide evidence for a possible hydrogen binding site around the 7′ position and 1′-carbonyl of the indanone [10,11], and that lipophilic substituents are important on the quinoline moiety for biological activity.

Table 2. In vitro cytotoxic activities of compounds 9, 11 and 29

No	IC_{50} (μg/ml)		
	MCF-7	H-460	SF-268
9	9.9	7.1	25.0
11	58.0	24.0	96.0
29	>100	26.0	>100

MCF-7: Human breast cancer; H-460: Human lung carcinoma; SF-268: Human CNS cancer.

Experimental

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H-NMR spectra were recorded using a Jeol Eclipse 270 MHz spectrometer and are reported in ppm downfield from $CHCl_3$ residual. Mass spectra were obtained a Varian Saturn GC/MS 2080 workstation. Elemental analyses were performed by Atlantic Microlab (Norcross, GA. USA), and analytical results were within ± 0.4 % of predicted values for all compounds.

General procedure for the synthesis of 2-chloro-3-formyl-methoxyquinoline derivatives 1a-c

Compounds **1a-c** were similarly prepared as reported in the literature [15]

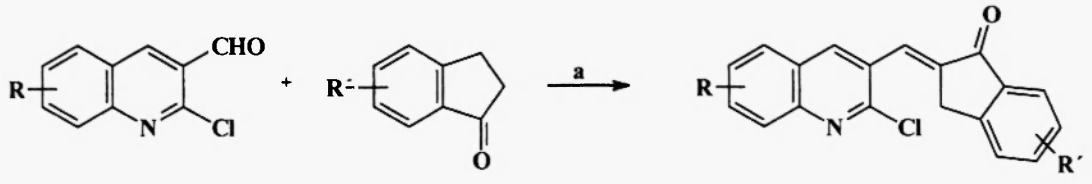
General procedure for the synthesis of 1-indanone derivatives 2a-i

Compounds **2b, 2c-i** were similarly prepared as reported in the literature [12-14]

General procedure for the synthesis of (E)-2-chloro-3-[2´(1-indanoyl)methyliden]quinoline derivatives 3-29

A solution of an appropriately substituted 1-indanone (5 mmol) and substituted 2-chloro-3-formylquinoline (5 mmol), NaOH one pellet in MeOH 10 mL was stirred at room temperature for 12 h. Formation of a precipitate, generally accompanied by a color change in the reaction mixture, is indicative for product formation. The solid product was filtered off and recrystallized from a minimum amount of solvent (EtOAc) and dried under vacuum at 50 °C to afford derivatives **3-29** (Table 1, 3).

Scheme.



1a-c **2a-i** **3-29**

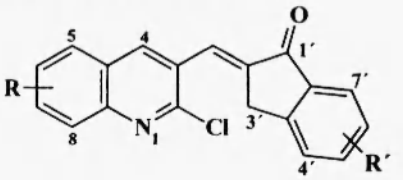
a: NaOH, MeOH, r.t.

Table 1. Yields, melting points, infrared, mass spectra data of compounds 3-29

No	Yield %	m.p °C	IR cm^{-1}CO	MS(EI) m/z
3	77	236-237	1693	395[a]-360[b]
4	45	270-272	1689	409[a]-374[b]
5	50	268-270	1708	425[a]-390[b]
6	68	168-170	1686	425[a]-390[b]
7	56	228	1686	425[a]-390[b]
8	96	246	1689	439[a]-404[b]
9	31	280	1685	455[a]-420[b]
10	94	260	1683	455[a]-420[b]
11	35	238	1702	455[a]-420[b]
12	56	232	1689	335[a]-300[b]
13	52	200	1690	349[a]-314[b]
14	71	228	1699	365[a]-330[b]
15	27	217-219	1689	365[a]-330[b]
16	59	246	1688	365[a]-330[b]
17	77	230	1673	379[a]-344[b]
18	36	211-212	1689	395[a]-360[b]
19	50	274	1702	395[a]-360[b]
20	53	212-214	1693	395[a]-360[b]
21	72	240	1702	335[a]-300[b]
22	76	202-204	1687	349[a]-314[b]
23	40	272-274	1700	365[a]-330[b]
24	97	250-252	1687	365[a]-330[b]
25	83	253-255	1695	365[a]-330[b]
26	90	274	1691	379[a]-344[b]
27	65	250	1699	395[a]-360[b]
28	98	236-238	1693	395[a]-360[b]
29	51	154-156	1693	395[a]-360[b]

[a] M^+; [b] M^+-Cl, *Anal.* Calc. (found). **9**: $C_{24}H_{22}O_6NCl$ C:63.23 (62.88); H: 4.86 (4.80); N: 3.07 (3.23). **11**: $C_{24}H_{22}O_6NCl$ C:63.23 (62.27); H: 4.86 (5.01); N: 3.07 (2.98). **15**: $C_{21}H_{16}O_3NCl$ C:68.95 (68.90); H:4.41(4.37); N: 3.83 (3.80). **29**:$C_{22}H_{18}O_4NCl$ C: 66.75 (66.93); H: 4.58(4.67); N: 3.54 (3.48).

Table 3. ^{1}H-NMR spectroscopic data with assignments, for the compounds 3-29.



No	R′	H-4 (s 1H)	H-5	H-6	H-7	H-8	CH= (t 1H)	H-3′ (d 2H)	H-4′	H-5′	H-6′	H-7′	CH_3 (s)	OCH_3 (s)
3	H[a]	8.62				7.15 s	8.05	4.04	7.65 t	7.6 d	7.44 t	7.42 d		3.99; 3.99; 4.14
4	4′-Me	8.64				7.16 s	8.04	3.89		7.43 d	7.34 t	7.75 d	2.40	3.98; 3.99; 4.14
5	4′-OMe	8.65				7.17 s	8.05	3.99		7.08 d	7.41 t	7.53 d		3.93; 4.00; 4.14
6	5′-OMe	8.58				7.15 s	7.96	3.96	6.96 d		6.94 dd	7.85 d		3.89; 3.97; 3.98; 4.14
7	6′-OMe	8.61				7.15 s	8.02	3.98	7.44 d	7.2dd		7.35 d		3.86; 3.97; 3.98; 4.14
8	5′,6′-OCH_2O	8.56				7.14 s	7.93	3.91	6.92 s			7.25 s	6 08CH_2	3.97; 3.98; 4.13
9	4′,7′-OMe	8.59				7.15 s	7.95	3.94		6.77 d	7.02 d			3.86; 3.97; 3.98; 4.12
10	5′,6′-OMe	8.58				7.14 s	7.96	3.95	6.97 s			7.32 s		3.93; 3.97; 3.98; 4.23
11	4′,5′-OMe	8.62				7.15 s	7.99	3.96			6.98 d	7.71 d		3.93; 3.98; 4.00; 4.13
12	H[b]	8.34	7.7 d	7.22dd		7.39 d	8.05	4.07	7.62 t	7.55 d	7.44 t	7.92 d		3.94
13	4′-Me	8.35	7.76 d	7.23dd		7.34 d	8.04	3.88		7.44 d	7.32 t	7.8 d	2.42	3.94
14	4′-OMe	8.37	7.79 d	7.22dd		7.33 d	8.03	3.89		7.07 d	7.40 t	7.55 d		3.93; 3.94
15	5′-OMe	8.30	7.74 d	7.23dd		7.33 d	7.96	3.96	6.97 d		6.94 dd	7.85 d		3.89; 3.93
16	6′-OMe	8.34	7.76 d	7.22dd		7.34 d	8.04	3.96	7.43 d	7.19dd		7.35 d		3.86; 3.94
17	5′,6 -OCH_2O	8.30	7.74 d	7.22dd		7.34 d	7.96	3.94	6.91 s			7.28 s	6 08CH_2	4 08
18	4′,7′-OMe	8.35	7.89 d	7.20dd		7.38 d	8.04	3.95		7.01 d				
19	5′,6′-OMe	8.27	7.71 d	7.21dd		7.30 d	7.93	3.97		6.94 s	7.32 s			3.92
20	4′,5′-OMe	8.35	7.70 d	7.22dd		7.32 d	7.98	3.97			7.0 d	7.80 d		3.94; 3.95
21	H[c]	8.31	7.14 d		7.34dd	7.90 d	8.02	4.05	7.7 t	7.6 d	7.45 t	7.42 t		3.96
22	4′-Me	8.29	7.16 d		7.35dd	7.91 d	8.01	3.86		7.43 d	7.34 t	7.8 d	2.42	3.97
23	4′-OMe	8.27	7.12 d		7.38dd	7.89 d	8.00	3.97		7.08 d	7.41 t	7.56 d		3.93; 3.95
24	5′-OMe	8.27	7.11 d		7.38dd	7.89 d	7.96	3.98	6.96 d		6.95dd	7,86 d		3.90; 3.95
25	6′-OMe	8.30	7.13 d		7.36dd	7.91 d	8.02	3.97	7.43 d	7.2dd		7.38d		3.87; 395
26	5′,6′-OCH_2O	8.24	7.11 d		7.39dd	7.89 d	7.93	3.92	6.89 s			7.27 s	6 09CH_2	3.94
27	4′,7′-OMe	8.28	7.15 d		7.38dd	7.88 d	7.94	3.85		6.78 d	7.04 d			3.92; 3.96
28	5′,6′-OMe	8.25	7.09 d		7.38dd	7.89 d	7.93	3.97	6.94 s			7.32 s		3.93, 3.94
29	4′,5′-OMe	8.31	7.04 d		7.40dd	7.89 d	7.97	3.95			7.01 d	7.70 d		3.94; 3.96; 3.98

R: [a]3-11 (5,6,7-OMe); [b]12-20 (7-OMe); [c]21-29 (6-OMe). s. singlet; d. doblet; t. triplet.

Biological activity

Antifungal Assays

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. Dermathophytes: *M. canis* C112, *T. rubrum* C115, *T. Mentagrophytes* ATCC 9972. The fungistatic activity of compounds **3-29** was evaluated with the agar dilution method by using Saboureaud-chloramphenicol agar for both yeast and dermatophyte species [16]. The assays were carried out in 98 well microtiter plates. Stock solutions of compounds in DMSO were diluted to give serial twofold dilutions that were added to each medium, resulting in concentration 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 and amphotericin B were included in the assay as positive controls. Drug-free solution was also used as a 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 dermathophyte strains. MIC was defined as the lowest compound concentration showing no visible fungal growth after incubation time.

Citotoxicity Assays

In the current protocol [17], each cell line: MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS), was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration of 1×10^{-4} M and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each tested agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the panel of three cell lines over a 5-log dose range.

Acknowledgements

We thank the IIF-FF and CDCH-UCV (grants IIF: 04.2002, PG. 06-30-4590-2000), CONDES-LUZ grant CC0447-02 and CONICT (grant No. LAB-97000665) for financial support.

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Received on March 17, 2003