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Synthesis of Some Quinolinyl Chalcone Analogues and Investigation of Their Anticancer and Synergistic Anticancer Effect with Doxorubicin[1]

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Abstract—Two derivatives of 2-(4-acetylanilino)quinolines (**IIIa**, **b**) were synthesized as scaffolds for synthesis of open chalcone analogues (**Va**–**f**) through Claisen–Schmidt condensation with a set of aromatic aldehydes (**IVa**–**d**). Derivatives (**Va**, **b**) were further manipulated into cyclic α,β-unsaturated ketones by Michael-addition of acetylacetone and ethylacetoacetate affording derivatives (**VI**–**VII**). Deethoxycarboxylation of derivatives (**VIIa**, **b**) afforded cyclohexenons (**VIIIa**, **b**) allowing formation of a mini library of α,β-unsaturated ketones for screening their anticancer and synergistic anticancer effect with doxorubicin using colon cancer cell line (Caco-2). Two open enones, (**Vb**) and (**Ve**), showed significant anticancer activity with IC_{50} of 5.0 and 2.5 μM respectively. Only one cyclic enone, (**VIa**) showed synergistic anticancer activity with doxorubicin at 10 μM.

Keywords: quinoline, chalcones, doxorubicin, antiproliferative effect, synergistic effect

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INTRODUCTION

Chalcones, i.e. 1,3-diaryl-2-propen-1-ones (Fig. 1) are important class of natural products belonging to the flavonoid and isoflavonoid families [1]. They are easily accessible via Claisen–Schmidt condensation of acetylated aryls with aldehydes, therefore, an endless number of chalcone derivatives is continually reported.

The wide diversity of the periphery around the enone core (Fig. 1) gave the chalcones the potency to have a reported broad spectrum of biological activities as anticancer [2], antioxidant [2], anti-inflammatory-analgesic-antipyretic [3], antimicrobial [4], antiparasitic [5], antihepatotoxic [3], antiallergic [6], antihyperglycemic [7], NO-synthase inhibitors [8], antifertility [9], antinociceptive [10], as immunomodulators [11], anticonvulsant [12], antiangial [3b], anticataracts [13] and as probes for in vivo imaging of β-amyloid plaques in Alzheimer's disease [14], In bioorganic chemistry, they served as tags for glycoconjugates involved as thiol-specific carbohydrate reagents utilized in affinity separation of protein-type toxins as well as posttranslation of free-cysteine containing proteins [15]. The enone core was reported to be responsible for eliciting the biological activity of chalcones through targeting specific host enzymes if the periphery of this core is optimum for the recognition event [1a, 16].

One of the emerging discoveries in the role of chalcones in cancer therapy, even if they have no own cyto-

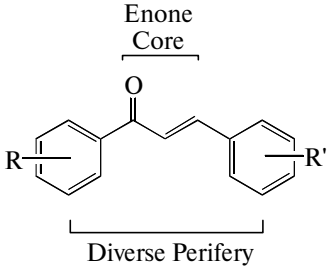

Fig. 1. Chemical structure of chalcones.

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toxicity, is their synergistic effect with the anticancer natural product doxorubicin. Thus, chalcones with specific basic periphery were reported to enhance the curative potency of this drug through inhibition of the drug-efflux protein Pgps characteristic for cancer cells and responsible for their drug resistance response [17].

Therefore, we initiated a program to trace this encouraging discovery, at this stage, to synthesize a set of quinoline-containg chalcone analogues to investigate their own anticancer activity as well as their synergistic anticancer potency with doxorubicin taking colon-cancer cell line (Caco-2) as cancer module. Quinoline was elected both for its basic nature and its known pharmacophore activities [18], besides, the designed series sustains a *p*-imino group relative to the enone moiety which is necessary for protonation under physiological pH to ensue the potential activity.

RESULTS AND DISCUSSION

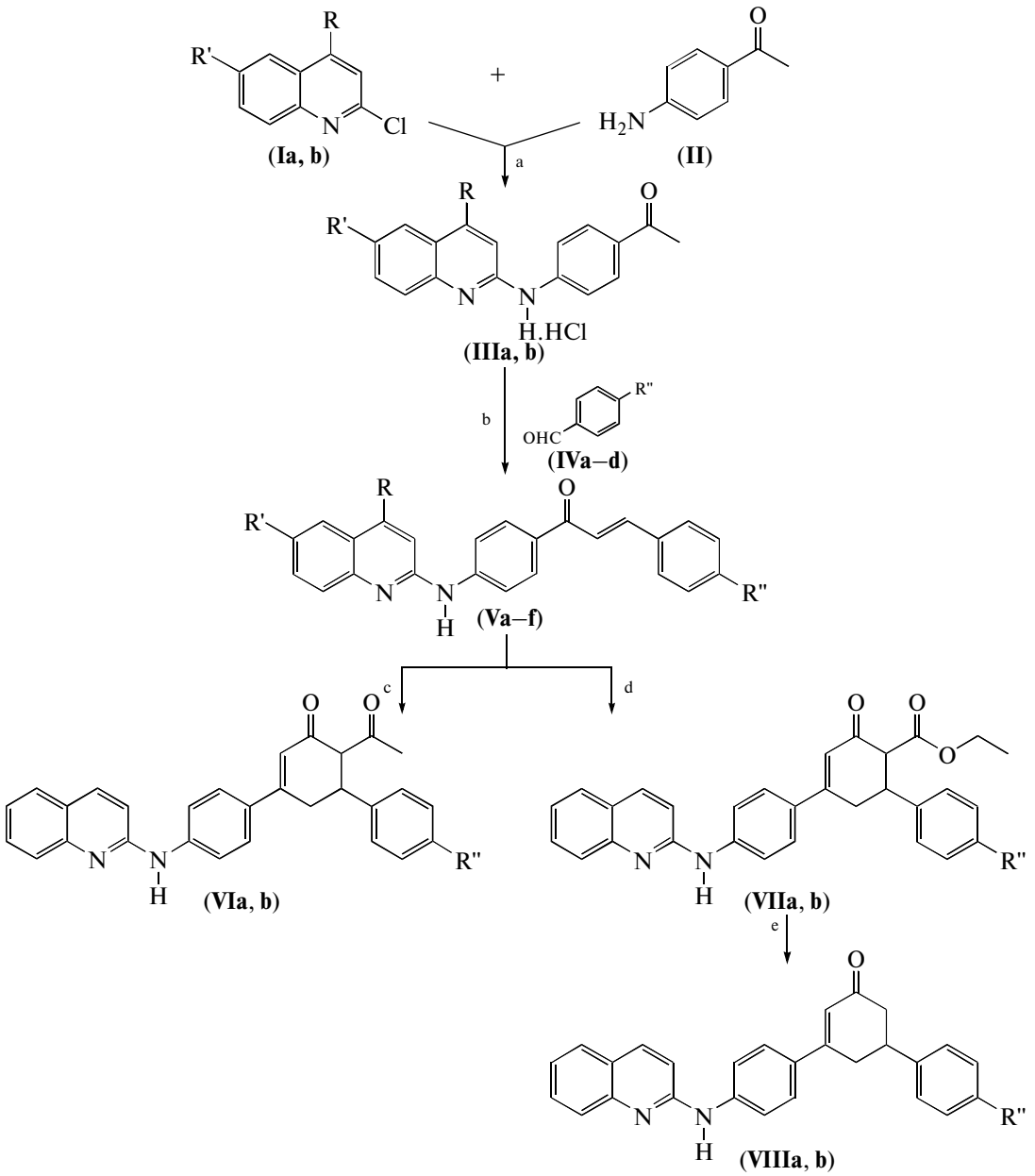
Chemistry

2-(*p*-Acetylanilino)quinolines (**IIIa, b**) were synthesized from the relevant 2-chloroquinolines (**Ia**, **b**) and *p*-acetylaniline (**II**) in refluxing EtOH containing drops of cone. HCl as described by Ashour et al. for (**IIIa**) [19]. Compound (**IIIb**) showed a broad band for the NH_2^+ at 3375 cm^{-1}, IR spectrum, as well as a bathochromic shifted band at 1660 cm^{-1} for the aromatic C=O group. In the ^1H NMR spectrum, the NH_2^+ signal was downfield shifted out of scale at δ 10.87 ppm due to the quaternary nature of the nitrogen atom. Claisen–Schmidt condensation of quinolines (**IIIa, b**) with a set of aldehydes (**IVa–d**) (scheme and table), afforded the required first set of cinnamoylquinolines (**Va–f**) in very good yields, most of the case. IR-spectra showed clearly the existence of the characteristic stretching vibration bands of the enone moiety at ν 1652–1642 cm^{-1} for the C=O and 1609–1599 cm^{-1} for the olefinic groups. The expected bathochromic-shift of the carbonyl groups refers to their conjugation with the olefinic double bond. The last group, i.e. the olefinic protons in the ^1H NMR spectra could not be assigned due to overlap of its protons with aromatic protons at δ ≈ 8.20–6.90 ppm while the NH signal appeared clearly downfield as broad singlet at δ ≈ 9.80 ppm. In all next derivatives, the N–H signal was observed at nearly the same value and it was D_2O exchangeable. In ^{13}C NMR, the C=O signal was observed in compound (**Ve**) at δ 186.67 ppm.

Chalcone analogues (**Va, b**) were further manipulated to be converted into cyclic enones with side chain diversity to have a mini library of the required class. To this endeavor, Michael-addition of acetylacetone and ethylacetoacetate with compounds (**Va, b**) in refluxing EtOH containing NaOMe afforded cyclic enones with acetyl, (**VIa, b**) and ethyloxycarbonyl, (**VIIa, b**) side chains in good yields. In the IR-spectra of these compounds, clear NH stretching vibration bands appeared at ≈3333 cm^{-1} as for derivatives (**Va–f**). Furthermore, a second C=O band arose at the ordinary frequency ≈1720 cm^{-1} due to the acetyl and ester side chains besides the enone's C=O stretching vibration band at ≈1650 cm^{-1}. In the ^1H NMR spectra of compounds (**VIa, b**) a signal at δ 6.48 ppm was observed in both compounds which might refer to the single olefinic proton of the cyclohexene ring. This proton appeared as doublet with coupling constant less than 1.0 Hz in enone (**VIa**) and 1.8 Hz for enone (**VIb**). This coupling is due to the allylic-like coupling with H4 protons. For the cyclohexenone protons at C4, C5 and C6 atoms, the ^1H NMR of compound (**VIb**) was much clear than for compound (**VIa**), thus, the common coupling of 1.8 Hz for the four-doublet signal at δ 2.94 ppm assigned it to be one of the diastereotopic protons of C4 methylene protons. Other common coupling of 17.4 Hz in the previous signal with the doublet-of-doublet at δ 3.07 ppm assigned the later to be the other diastereotopic proton of the same methylene group. The multiplet at δ 3.44 ppm, which was deoverlaped from the H_2O signal of DMSO by D_2O labeling, was assigned for the cyclohexenone's H5 while the doublet-of-doublet at δ 2.76 is for H6 of the same ring. A multiplicity of doublet-of-doublet rather than a doublet for H6 might be attributed to the racemic nature of this compound. Both compounds showed one signal for both carbonyl groups at δ ≈ 198.4 ppm in the ^{13}C NMR.

Side chain variations and yields of compounds (**I**)–(**VIII**)

Compd	R	R'	R"	Yield (%)
(**Ia**)	H	H	–	–
(**Ib**)	CH_3	CH_3	–	–
(**IIIa**)	H	H	–	83
(**IIIb**)	CH_3	CH_3	–	98
(**IVa**)	–	–	H	–
(**IVb**)	–	–	OMe	–
(**IVc**)	–	–	NMe_2	–
(**IVd**)	–	–	Cl	–
(**Va**)	H	H	H	85
(**Vb**)	H	H	Cl	85
(**Vc**)	CH_3	CH_3	H	86
(**Vd**)	CH_3	CH_3	OMe	80
(**Ve**)	CH_3	CH_3	NMe_2	77
(**Vf**)	CH_3	CH_3	Cl	47
(**VIa**)	H	H	H	65
(**VIb**)	H	H	Cl	66
(**VIIa**)	H	H	H	75
(**VIIb**)	H	H	Cl	70
(**VIIIa**)	H	H	H	50
(**VIIIb**)	H	H	Cl	96



Scheme. Reagents and conditions: (a) EtOH, HCl, rfx; (b) NaOH, EtOH–H_2O, rt; (c) (**Va**) or (**Vb**), acetylacetone, NaOMe, MeOH, rfx; (d) (**Va**) or (**Vb**), ethylacetoacetate, NaOMe, MeOH, rfx; (e) NaOH, aq. EtOH, rfx.

In the ^{1}H NMR spectra of compounds (**VIIa, b**) the ethyl group's signals appeared normally as triplet for the $-CH_3$ and a quartet for the $-CH_2-$ groups at $\delta \approx 0.90$ and 3.90 ppm respectively. The cyclohexenone ring showed the diastereotopic C6 methylene protons as doublet-of-doublet for each one at $\delta \approx 4.11$ and 2.78 ppm. Proton H5 appeared as multiplet at $\delta \approx 3.60$ ppm, while, the methine proton, i.e. H4, appeared as doublet at $\delta \approx 3.00$ ppm.

The labile ethyloxycarbonyl side chains of derivatives (**VIIa, b**) could be easily removed by treatment with NaOH in refluxing aqueous EtOH to afford a third variety of cyclic enones (**VIIIa, b**). The IR spectra revealed disappearance of the C=O band at $\approx$1720 cm^{-1}, while, disappearance of the ethyl protons signals in the ^{1}H NMR spectra were good evidences for this elimination. Compound (**VIIIb**) showed a smooth spectrum as compound (**VIb**). Mass-spectra as well as elemental analysis were in good agreement with the molecular formulas of all compounds.

The anti-proliferative effect of 10 of the synthesized enones (Fig. 2) was examined using Caco-2 colon cancer cells and MTT assay [20]. Moreover the synergetic effects of these derivatives in combination with the drug Doxorubicin were also examined. The results revealed that highly significant, $p < 0.001$, anti-proliferative effect were observed with compounds

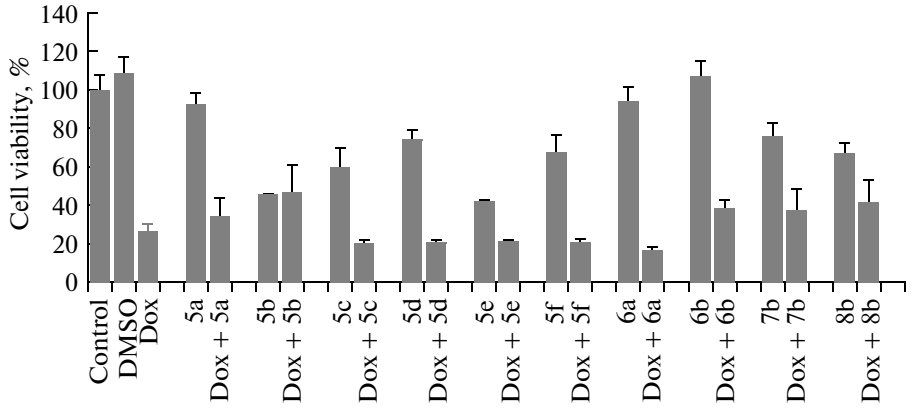


Fig. 2. Antiproliferative response of Caco-2 Colon cancer cell line to compounds (**Va–e**), (**VIa**, **b**), (**VIIb**) and (**VIIIb**) at 10 μM with and without doxorubicin (Dox), 10 μM, for 48 h.

having open enone moieties including compounds (**Vb**) and (**Ve**) with inhibition potency of 54% and 58% and IC_{50} at 5.0 μM and 2.5 μM respectively. These results, might roughly, exhibiting the relevance of the *p*-chloro substitution on ring B in nonmethylated quinolyl derivatives and the basic *p*-NMe_2 group on ring B in the methylated analogous. Although, compounds (**Va, Vc, Vd, Vf, VIa, VIIb** and **VIIId**) showed variable antiproliferative activities, their effects were statistically nonsignificant. On the other hand, i.e. the synergistic effect with doxorubicin, derivatives (**Vc–f**) showed a statistically nonsignificant synergistic effect and only compound (**VIa**) having a cyclic enone moiety with an acetyl side chain among the tested related cyclic derivatives showed a significant synergistic effect. Thus, the antiproliferative effect of doxorubicin alone, 74%, was elevated to 84% inhibition upon mixing with (**VIb**) which corresponds to an increased potency of 10%. The non significant anticancer activity of this compound along with its statistically significant effect on the antiproliferative efficacy of doxorubicin encourages that this compound is synergistic and its activity might be referred to its probable inhibition of cancer cells drug efflux proteins Pgp due to the similarities of the active core with the series reported by Go et al. [17].

EXPERIMENTAL

All chemicals and solvents were purchased from the local Egyptian productions and Merck chemical industries and were directly used without purification. Melting points were uncorrected and measured on Mel-TempII laboratory devices USA using open capillary tubes. TLC Monitoring tests were carried out using plastic sheets precoated with silica gel 60F245 (layer thickness 0.2 mm) purchased from Merck. Mixture benzene–MeOH, 9 : 1 was commonly used as mobile phase. Spots were visualized by their fluorescence under UV-lamp (λ 245 and 366 nm) and staining with iodine. IR spectra were recorded on USA Perkin-Elmer 1430 spectrophotometer using KBr disks technique in the toxicology Center, Faculty of Science, Suez Canal University, Egypt. ^{1}H NMR and ^{13}C spectra were recorded on Varian Gemini 200 MHz spectrometer in the micro analytical center of the Faculty of Science, Cairo University and Bruker 600 MHz spectrometer, central laboratory, King Abd El Aziz University, Gedah, KSA, DMSO-d_6 was used as solvent using Trimethylsilane (TMS) as internal standard and D_2O was used to locate the N–H signal. Mass spectra were recorded on Shimadzu Qp-2010 Puls spectrometer (Japan) in the micro analytical Center of the Faculty of Science, Cairo University. Elemental analysis was performed in the micro analytical Center, Faculty of Science, Cairo University.

General Procedure for the Synthesis of (IIIa, b)

A mixture of chloroquinoline (**Ia, b**) (0.1 mol) and *p*-acetylaniline (**II**) (0.1 mol) in absolute EtOH (40 mL) containing cone HCl (50 μL) was refluxed for 8 h then left to reach ambient temperature. The crystalline precipitate formed was filtered and dried at the pump.

1-[4-(4,6-Dimethylquinolin-2-ylamino)phenyl]ethanone hydrochloride (IIIb). Lemon yellow crystals in 98% yield from EtOH–H_2O. Mp 174–177°C. IR (KBr): 3375 (N–H_{str}), 1660 (C=O_{str}). Found, %: C 69.70, H 6.00, N 8.90. $C_{19}H_{19}ClN_2O$. Calcd., %: C 69.83, H 5.86, N 8.57. ^{1}H NMR (200 MHz, DMSO): δ 10.89 (2 H, br.s, $-NH_2^+$), 8.06–7.19 (8 H, m, Ar), 2.64 (3 H, s, $-COCH_3$), 2.58, 2.48 (6 H, 2s, $2CH_3$).

General Procedure for the Synthesis of Chalcones (Va–f)

A mixture of the acetyl derivative (**IIIa, b**) (3.0 mmol), the appropriate aldehyde (**IVa–f**) (7.0 mmol) and NaOH (7.0 mmol) in EtOH–H_2O (10.0 : 0.5 mL) was stirred overnight. The precipitate formed was filtered at the pump, washed with a little cold EtOH, dried well and recrystallized from the relevant solvent.

(*E*)-3-Phenyl-1-[4-(quinolin-2-ylamino)phenyl]prop-2-en-1-one (Va). Yellow crystals in 85% yield from MeOH. Mp 203–204°C. IR (KBr): 3318 (N–H_{str}), 1649 (C=O_{str}), 1606 (C=C_{str}). Found, %: C 82.10, H 5.30, N 7.90. $C_{24}H_{18}N_2O$. Calcd., %: C 82.26, H 5.18, N 7.99. ^{1}H NMR (600 MHz, DMSO): δ 9.98 (1 H, s, $NH_{D2O\ exch.}$), 8.20 (4 H, m, Ar), 8.15 (1 H, d, $J_{A,B}$ 9.0, Ar), 8.00 (1 H, d, *J* 15.6, Ar), 7.90 (2 H, m, Ar), 7.79 (2 H, m, Ar), 7.71 (1 H, d, $J_{A,B}$ 15.6, Ar), 7.64 (1 H, m, Ar), 7.46 (3 H, m, Ar), 7.36 (1 H, m, Ar), 7.14 (1 H, d, $J_{A,B}$ 9.0, Ar). EI MS, *m/z* (%): 350 (100, M^+), 333 (9.0), 247 (29.9), 218 (43.3), 174 (32.8), 128 (61.2).

(*E*)-3-(4-Chlorophenyl)-1-[4-(quinolin-2-ylamino) phenyl]prop-2-en-1-one (Vb). Yellow crystals in 85% yield from EtOH. Mp 221–223°C. IR (KBr): 3314 (N–H_{str}), 1652 (C=O_{str}), 1604 (C=C_{str}), 809 (C–Cl_{str}). Found, %: C 74.60, H 4.30, N 7.60. $C_{24}H_{17}ClN_2O$. Calcd., %: C 74.90, H 4.45, N 7.28. ^{1}H NMR (200 MHz, DMSO): δ 9.98 (1 H, s, NH), 8.21–7.11 (15 H, m, Ar, –CO–CH=CH–), 7.15 (1 H, d, $J_{A,B}$ 9.0, Ar). EI MS, *m/z* (%): 384 (100, M^+), 355 (26.6), 247 (21.2), 218 (46.4), 174 (36.9).

(*E*)-1-[4-(4,6-Dimethylquinolin-2-ylamino)phenyl]-3-phenylprop-2-en-1-one (Vc). Yellow crystals in 86% yield from EtOH/H_2O. Mp 193–195°C. IR (KBr): 3342 (N–H_{str}), 1652 (C=O_{str}), 1609 (C=C_{str}). Found, %: C 82.30, H 5.60, N 7.40. $C_{26}H_{22}N_2O$. Calcd., %: C 82.51, H 5.86, N 7.40. ^{1}H NMR (200 MHz, DMSO): δ 9.82 (1 H, s, NH), 8.19–7.00 (14 H, m, Ar, –CO–CH=CH–), 6.98 (1 H, s, –CO–CH=CH–) 2.60, 2.49 (6 H, 2 s, $2CH_3$). EI MS, *m/z* (%): 378 (87.0, M^+), 377 (100), 349 (28), 24.7 (25).

(*E*)-1-[4-(4,6-Dimethylquinolin-2-ylamino)phenyl]-3-(4-methoxyphenyl)prop-2-en-1-one (Vd). Orange crystals in 80% yield from benzene: petroleum ether. Mp 224–226°C. IR (KBr): 3345 (N–H_{str}), 1648 (C=O_{str}), 1599 (C=C_{str}), 1257, 1226 (C–O–C_{aryl}), 1034 (C–O–C_{alkyl}). Found, %: C 79.50, H 6.10, N 7.10. $C_{27}H_{24}N_2O_2$. Calcd., %: C 79.39, H 5.92, N 6.86.^{1}H NMR (200 MHz, DMSO): δ 9.78 (1 H, s, –NH), 8.18–6.98 (14 H, m, 4Ar, –CH=CH–), 3.84 (3 H, s, $-OCH_3$), 2.60, 2.48 (6 H, 2s, 2 $-CH_3$). EI MS, *m/z* (%): 408 (100, M^+), 407 (80), 393 (53.3), 392 (46.7), 391 (40), 317 (33.3), 309 (40), 308 (40), 307 (26.7), 279 (40), 278 (40), 246 (66.7).

(*E*)-3-[4-(Dimethylaminophenyl)]-1-[4-(quinolin-2-ylamino)phenyl]prop-2-en-1-one (Ve). Red crystals in 77% yield from benzene : EtOH. Mp 248–250°C. IR (KBr): 3340 (N–H_{str}), 1642 (C=O_{str}), 1599 (C=C_{str}). Found, %: C 79.60, H 6.60, N 10.10. $C_{28}H_{27}N_3O$. Calcd., %: C 79.78, H 6.46, N 9.97. ^{1}H NMR (600 MHz, DMSO): δ 9.47 (1 H, s, $-NH_{D2O\ exch}$), 8.13 (2 H, m, Ar), 7.70 (2 H, d, $J_{A,B}$ 9.0, Ar), 7.68–7.62 (6 H, m, Ar), 7.46 (1 H, dd, *J* 1.2, 8.4, Ar), 6.94 (1 H, s, –CO–CH=CH–), 6.74 (2 H, d, $J_{A,B}$ 9.0, Ar), 3.00 (6 H, s, $-NMe_2$), 2.60, 2.46 (6 H, 2 s, $2(CH_3)_{quin}$). ^{13}C NMR (150 MHz, DMSO): δ 181.67 (C=O), 152.96, 151.81, 145.89, 144.42, 143.81, 132.38, 131.34, 130.58, 130.46, 129.92, 129.77, 129.71, 127.00, 124.08, 123.16, 122.34, 122.34, 117.14, 116.27, 114.08, 114.01, 112.00, 111.82 (C_{Ar}), 39.78 ($-NMe_2$), 21.17, 18.50 [2 $(CH_3)_{quin}$]. EI MS, *m/z* (%): 421 (100, M^+), 406 (85.0), 289 (23.8), 195 (36.7).

(*E*)-3-(4-Chlorophenyl)-1-[4-(4,6-dimethylquinolin-2-ylamino)phenyl]prop-2-en-1-one (Vf). Yellow crystals in 47% yield from benzene: EtOH. Mp 260–262°C. IR (KBr): 3333 (N–H_{str}), 1652 (C=O_{str}), 1599 (C=C_{str}), 793 (C–Cl_{str}). Found, %: C 75.30, H 4.90, N 6.80. $C_{26}H_{21}ClN_2O$. Calcd., %: C 75.63, H 5.13, N 6.78. ^{1}H NMR (600 MHz, DMSO): δ 9.47 (1 H, s, $-NH_{D2O\ exch}$), 8.17 (4 H, m, Ar), 8.02 (1 H, d, $J_{A,B}$ 15.6, Ar), 7.94 (2 H, m, Ar), 7.70–7.66 (3 H, m, Ar), 7.53–7.46 (3 H, m, Ar), 6.95 (1 H, m, –CO–CH=CH–), 2.58, 2.46 (6 H, 2s, $(CH_3)_{quin}$). EI MS, *m/z* (%): 414 (24), 412 (70.7, M^+), 384 (38.7), 247 (48).

*General Procedure for Synthesis of Compounds (**VIa, b**)*

A mixture of acetylacetone (3.0 mL, 29.0 mmol) and NaOMe (1%, 10 mL) was kept for an hour at ambient temperature then added to the appropriate chalcone derivative (**Va, b**) (2.0 mmol) and the mixture was refluxed for 15 h then left to reach ambient temperature. The precipitate formed was filtered at the pump, dried well then recrystallized from the proper solvent.

6-Acetyl-5-phenyl-3-[4-(quinolin-2-ylamino)phenyl] cyclohex-2-en-1-one (VIa). Yellow crystals from EtOH : benzene in 65% yield. Mp 248–251°C. IR (KBr): 3333 (N–H_{str}), 1718 (C=O_{acetyl}), 1641 (C=O_{enone}), 1586 (C=C_{str}). Found, %: C 80.50, H 5.30. $C_{29}H_{24}N_2O_2$. Calcd., %: C 80.53, H 5.59. ^{1}H NMR (600 MHz, DMSO): δ 9.82 (1 H, s, $-NH_{D2O\ exch}$), 8.08–7.09 (15 H, 3m, Ar), 6.48 (1 H, d, $J_{allylic}$ < 1.0, $H2_{cyclohex}$), 3.45 (1 H, d, $H5_{cyclohex,\ overlapped}$), 3.50–2.77 (3 H, m, H4, H4', $H6_{cyclohex}$), 2.49 (3 H s, $COCH_3$). ^{13}C NMR (150 MHz, DMSO): δ 198.43 (2C=O), 158.24, 153.90, 146.83, 144.06, 143.65, 137.31, 129.77, 129.57, 129.12, 128.65, 127.70, 127.40, 127.24, 126.77, 126.58, 123.87, 123.27, 121.78, 118.04, 114.45 ($C_{Ar,}$ $C23_{olef}$), 43.72–34.97 ($C4_{aliph}$). EI MS, *m/z* (%): 432 (20.5, M^+), 390 (100), 285 (74.8), 257 (33.1), 218 (20.5).

6-Acetyl-5-(4-chlorophenyl)-3-[4-(quinoIin-2-ylamino) phenyl]cyclohex-2-en-1-one (VIb). Yellow crystals from EtOH : benzene in 66% yield. Mp 252–254°C. IR (KBr): 3338 (N–H_{str}), 1646 (C=O_{str}), 1585 (C=C_{str}), 819 (C–Cl_{str}). Found, %: C 75.00, H 5.10, N 5.80. $C_{29}H_{23}ClN_2O_2$. Calcd., %: C 74.59, H 4.96, N 5.99. ^{1}H NMR (600 MHz, DMSO): δ 9.76 (1 H, s, $NH_{D2O\ exch}$), 8.10 (5 H, m, Ar), 7.76–7.70 (6 H, m, Ar), 7.61–7.59 (2 H, m, Ar), 7.08 (1 H, d, *J* 9.0, Ar), 6.48 (1 H, d, $J_{allylic}$1.8, $H2_{cyclohex}$), 3.44 (1 H, m, $H5_{cyclohex}$), 3.07 (1 H, dd, *J* 4.2, J_{gem} 17.4, $H4_{cyclohex}$),

2.94 (1 H, 4d, $J_{allylic}$ 1.8, $J_{4,5}$10.8, J_{gem} 17.4, $H4'_{cyclohex}$), 2.76 (1 H, dd, J 13.2, 16.2, $H6_{cyclohex}$), 2.50 (3 H, s, $COCH_3$). ^{13}C NMR (150 MHz, DMSO): δ 198.03 (2C=O), 157.98, 153.76, 146.76, 143.59, 143.48, 143.02, 137.26, 131.21, 129.70, 129.43, 129.15, 128.45, 128.39, 127.65, 127.36, 126.51, 123.79, 123.21, 121.68, 117.94, 117.87, 114.36, 114.31 ($C_{Ar, olef}$), 43.42 ($C6_{cyclohex}$), 39.64, 34.64 ($3C_{aliph}$). EI MS, *m/z* (%): 424 (100, M^+), 285 (63), 257 (37.5), 218 (12.5).

*General Procedure for Synthesis of Compounds (**VIIa, b**)*

Ethylacetoacetate (3.09 g, 0.02 mol) was stirred with NaOMe solution (1%, 10 mL) at rt for 1 h then refluxed for 10 h with the appropriate chalcone derivative (**Va, b**) (1.0 g, 0.002 mol) and left to reach ambient temperature. The precipitate formed was filtered at the pump, dried then recrystallized from the relevant solvent.

Ethyl 2-oxo-6-phenyl-4-[4-(quinolin-2-ylamino)phenyl]cyclohex-3-enecarboxylate (VIIa). Yellow crystals in 75% yield from benzene : petroleum ether. Mp 232°C. IR (KBr): 3335 ($N-H_{str}$), 1744 ($C=O_{ester}$), 1645 ($C=O_{enone}$), 1586 ($C=C_{str}$). Found, %: C 77.70, H 5.60, N 6.20. $C_{30}H_{26}N_2O_3$. Calcd., %: C 77.90, H 5.67, N 6.06. ^1H NMR (200 MHz, DMSO): δ 9.78 (1 H, br. s, NH), 8.11 (1 H, d, $J_{A,B}$ 8.4, Ar), 7.78–7.26 (13 H, m, Ar), 7.11 (1 H, d, $J_{A,B}$ 9.1, Ar), 6.53 (1 H, d, J 16.4, $H3_{cyclohex}$), 4.11 (1 H, dd, $H6_{cyclohex}$), 3.91 (2 H, q, J 7.08, $CH_{2\ ethyl}$), 3.65 (1 H, m, $H5_{cyclohex}$), 3.07 (1 H, d, J 7.26, $H5'_{cyclohex}$), 2.78 (1 H, dd, $H1_{cyclohex}$), 0.92 (3 H, t, J 7.08, $-CH_3$).

Ethyl 6-(4-chlorophenyl)-2-oxo-4-[4-(quinolin-2-ylamino)phenyl]cyclohex-3-enecarboxylate (VIIb). Yellow crystals in 70% yield from EtOH : H_2O : ether. Mp 229–230°C. IR (KBr): 3334 ($N-H_{str}$), 1744 ($C=O_{ester}$), 1645 ($C=O_{enone}$), 1587 ($C=C_{str}$), 784 ($C-Cl_{str}$). Found, %: C 72.30, H 4.80, N 5.50. $C_{30}H_{25}ClN_2O_3$. Calcd., %: C 72.50, H 5.07, N 5.64. ^1H NMR (200 MHz, DMSO): δ 9.79 (1 H, br. s, NH), 8.11 (1 H, d, $J_{A,B}$ 7.6, Ar), 7.78–7.43 (12 H, m, Ar), 7.10 (1 H, d, $J_{A,B}$ 9.0, Ar), 6.53 (1 H, d, $J \approx 16.0$, $H3_{cyclohex}$), 4.12 (1 H, dd, J 12.0, $H6_{cyclohex}$), 3.93 (2 H, q, J 7.08, $-CH_{2\ ethyl}-$), 3.63 (1 H, m, $H5_{cyclohex}$), 3.06 (1 H, d, J 8.34, $H5'_{cyclohex}$), 2.57 (1 H, m, $H1_{cyclohex}$), 0.95 (3 H, t, J 7.08, $-CH_3$). EI MS, *m/z* (%): 496 (5.1, M^+), 467 (6.1), 449 (32.7), 423 (41.8), 395 (21.4), 339 (17.3), 322 (50.0), 285 (56.1).

*General Procedure for the Synthesis of Compounds (**VIIIa, b**)*

A mixture of the appropriate ethylester (**VIIa, b**) (0.002 mol) and NaOH (1.5 g, 0.037 mol) in EtOH : H_2O, 1.5 : 1 (25 mL) was refluxed for 6 h then left to reach ambient temperature. The precipitate formed was filtered in vacuo and recrystallized from the relevant solvent(s).

5-Phenyl-3-[4-(quinolin-2-ylamino)phenyl]cyclohex-2-en-1-one (VIIIa). Yellow crystals from benzene: EtOH in 50% yield. Mp 258°C. IR (KBr): 3332 ($N-H_{str}$), 1650 ($C=O_{str}$), 1585 ($C=C_{str}$). Found, %: C 82.80, H 5.90, N 7.30. $C_{27}H_{22}N_2O$. Calcd., %: C 83.05, H 5.68, N 7.17. ^1H NMR (200 MHz, DMSO): δ 9.73 (1 H, br. s, –NH), 8.12–7.07 (15 H, m, Ar), 6.49 (1 H, s, –CO–CH=CH), 3.20–2.53 (5 H, m, $H6_{cyclohex}$, $H6'_{cyclohex}$, $H5_{cyclohex}$, $H4_{cyclohex}$, $H4'_{cyclohex}$). EMS, *m/z* (%): 390 (100, M^+), 361 (12.8), 285 (84.6), 257 (43.6), 218 (38.5).

5-(4-Chlorophenyl)-3-[4-(quinolin-2-ylamino)phenyl]cyclohex-2-en-1-one (VIIIb). Yellow crystals from benzene : EtOH in 96% yield. Mp 242–245°C. IR (KBr): 3338 ($N-H_{str}$), 1642 ($C=O_{str}$), 1584 ($C=C_{str}$), 820 ($C-Cl_{str}$). Found, %: C 76.20, H 5.10, N 6.70. $C_{27}H_{21}ClN_2O$. Calcd., %: C 67.32, H 4.98, N 6.59. ^1H NMR (600 MHz, DMSO): δ 9.77 (1 H, s, $-NH_{D2O\ exch}$), 8.10–8.08 (4 H, m, Ar), 7.73 (3 H, m, Ar), 7.60 (1 H, m, Ar), 7.46 (2 H, m, Ar), 7.40 (2 H, m, Ar), 7.32 (1 H, m, Ar), 7.08 (1 H, d, $J_{A,B}$ 9.0, Ar), 6.48 (1 H, d, $J_{allylic}$ 1.8, $H2_{cyclohex}$), 3.40 (1 H, m, $H5_{cyclohex}$), 3.05 (1 H, dd, J 4.2, 17.4, $H4_{cyclohex}$), 2.94 (1 H, 4 d, $J_{allylic}$ 1.8, J_{gem} 17.4, J 10.8, $H4'_{cyclohex}$), 2.76 (1 H, dd, J 13.2, 16.2, $H6_{cyclohex}$), 2.52 (1 H, m, $H6'_{cyclohex}$). ^{13}C NMR (150 MHz, DMSO): δ 198.10 (C=O), 158.04, 153.83, 146.78, 143.62, 143.03, 137.29, 131.23, 129.73, 129.46, 129.18, 128.47, 128.42, 127.67, 127.38, 126.53, 123.82, 123.24, 121.71, 117.98, 114.38 ($23C_{Ar, olef}$), 43.44, 39.50, 34.66 ($3C_{aliph}$).

Cell Proliferation Studies

The proliferation inhibition ability of 10 compounds of the prepared α,β-unsaturated ketones was determined by MTT assay method described by Mosmann, 1983 using colon cancer cell line (Caco-2) obtained from VACSERA, the holding company for biological products and vaccines, Cairo, Egypt. Briefly, Colon cancer cell line (Caco-2) was seeded at 20000 cells/well in 96 well tissue culture plates, volume 100 μL/well and incubated for 24 h at 37°C and 5% CO_2. The cells, except the control, were treated with DMSO, Doxorubicin at 10 μM, derivatives **Va–f, VIa, b, VIIb** and **VIIIb**) at 10 μM, combination between these derivatives at 10 μM and Doxorubicin at 10 μM. After incubation for 48 h, 25 μL of (0.5 mg/mL) MTT stain was added to each well and the plates were incubated at 37°C for 4 h, then, a stop solution was added to each well and mixed thoroughly by repeated pipetting with a multichannel pipette. The

plates were then shacked at room temperature for an hour. The developed color was measured at 570 nm using ELIZA micro plate reader. Cell viability was calculated according to the following equation

Viability % = (test reading/control reading) × 100.

The obtained data were statically analyzed using STATE view using post-Hock ANOVA.

CONCLUSIONS

A set of quinoline derivatives having a enone moiety can be easily synthesized via Claisen–Schmidt condensation and Michael-addition reactions affording a series of α,β-unsaturated ketones bearing the basic quinolyl grafts for investigation of their anticancer and synergistic anticancer potency with doxorubicin. Both anticancer and synergistic anticancer activities were observed which supports synthesis of more derivatives of this series.

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REFERENCES

1. (a) Sharma, A., Chakravarti, B., Gupt, M.P., Siddiqui, J.A., Konwar, R., and Tripathi, R.P., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 4711–4720; (b) Kong, Y., Wang, K., Edler, M.C., Hamel, E., Mooberry, S.L., Paige, M.A., and Brown, M.L., *Bioorg. Med Chem.*, 2010, vol. 18, pp. 971–977.
2. Go, M.L., Wu, X., and Liu, X.L., *Curr. Med. Chem.*, 2005, vol. 12, pp. 483–499.
3. (a) Kim, H.K., Namgoong, S.Y., and Kim, H.P., *Arch. Pharm. Res.*, 1993, vol. 16, pp. 18–24; (b) Bandgar, B.P., Gawande, S.S., Bodade, R.G., Totre, J.V., and Khobragade, C.N., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 1364–1370.
4. (a) Lin, Y.M., Zhou, Y., Flavin, M.T., Zhou, L.M., Nie, W., and Chen, F.C., *Bioorg. Med. Chem.*, 2002, vol. 10, pp. 2795–2802; (b) López, S.N., Castelli, M.V., Zacchino, S.A., Dominguez, J.N., Lobo, G., Jaime, C.C., Cortes, J.C.G., Ribas, J.C., Devia, C., Ana, M.R., and Ricardo, D.E., *Bioorg. Med. Chem,.* 2001, vol. 9, pp. 1999–2013; (c) Ninomiya, Y., Shimma, N., and Ishitsuka, H., *Antiviral Res.*, 1990, vol. 13, pp. 61–74.
5. (a) Nielsen, S.F., Christensen, S.B., Cruciani, G., Kharazmi, A., and Liljefors, T., *J. Med. Chem.*, 1998, vol. 41, pp. 4819–4832; (b) Liu, M., Wilairat, P., and Go, M.L., *J. Med. Chem.*, 2001, vol. 44, pp. 4443–4452.
6. (a) Claesson, H.E. and Dahlen, S.E., *J. Int. Med.*, 1999, vol. 245, p. 205; (b) Lieberman, P., *Allergy Asthma Proc.*, 2009, vol. 30, pp. 345–348.
7. Raju, B.C., Yiwari, A.K., Kumar, J.A., Ali, A.Z., Agwane, S.B., Saidachary, G., and Madhusudana, K., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 358–365.
8. Carrion, M.D., Luisa, C., Lopez, L.C., Camacho, M.E., Tapias, V.G., Escames Castroviejo, D.A., Espinosa, A., Gallo, M.A., and Entrena, A., *Eur. J. Med. Chem.*, 2008, vol. 43, pp. 2579–2591.
9. Moersch, G.W., Morrow, D.F., and Neuklis, W.A., *J. Med. Chem.*, 1967, vol. 10, pp. 154–158.
10. Milano, J., Oliveira, S.M., Rossato, M.F., Sauzem, P.D., Machado, P., Beck, P., Zanatta, N., Martins, M.A.P., Mello, C.F., Rubin, M., Ferreira, J., and Bonacorso, H.G., *Eur. J. Pharmacol.*, 2008, vol. 581, pp. 86–96.
11. Pergola, C. and Werz, O., *Expert Opin. Ther. Patents,* 2010, vol. 20, pp. 355–375.
12. Ozdemir, Z., Kandilci, H.B., Gumusel, B.H., Calıs, U., and Bilgin, A.A., *Eur. J. Med. Chem.*, 2007, vol. 42, pp. 373–379.
13. Varma, S.D., Mikuni, I., and Kinoshita, J.H., *Science*, 1975, vol. 188, pp. 1215–1216.
14. Ono, M., Haratake, M., Mori, H., and Nakayama, M., *Bioorg. Med. Chem.*, 2007, vol. 15, pp. 6802–6809.
15. Aly, M.R.E., Rochaix, P., Amessou, M., Johannes, L., and Florent, J.-C., *Carbohydr. Res.*, 2006, vol. 341, pp. 2026–2036.
16. Dong, X., Wang, L., Huang, X., Liu, T., Wie, E., Du, L., Yang, B., and Hu, Y., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 5519–5527.
17. Liu, X.-L., Tee, H.-W., and Go, M.-L., *Bioorg. Med. Chem.*, 2008, vol. 16, pp. 171–180.
18. Lubenets, V.I., Stadnitskaya, N.E., and Novikov, V.P., *Russ. J. Org. Chem.*, 2000, vol. 36, pp. 851–853.
19. Kamel, M., Nabih, I., Gadalla, K.Z., and Ashour, M., *Pharmazie*, 1986, vol. 41, pp. 55–56.
20. Mosmann, T., *J. Immunol. Meth.*, 1983, vol. 65, pp. 55–63.