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# Utilization of 2-substituted 3, 1-benzoxazin-4-ones in synthesis of some quinazoline annulated derivatives

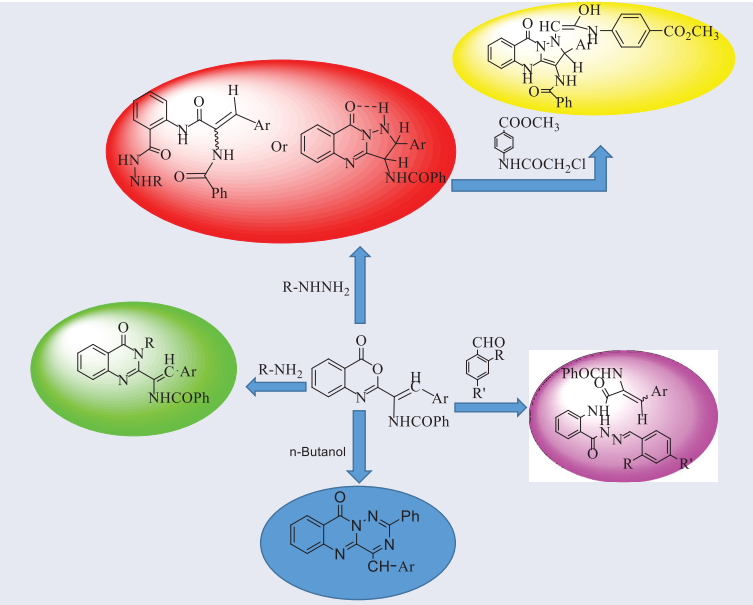
Asmaa M. Abd-Alaziz, Hanan A. Sallam, Ahmed S. A. Youssef, and Abeer M. El-Naggar

Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

**ABSTRACT**

In this study, a new series of quinazoline and their annulated derivatives have been synthesized. A number of quiazolinone derivatives substituted at position-3 were prepared from 3, 1-benzoxazinone by the treatment of 3,1-benzoxazinone with different nitrogen nucleophiles such as, hydrazine hydrate, phenylhydrazine, ethanolamine, and cyano acetohydrazide afforded the quinazolinone derivatives **7–11**. The reaction of hydrazide derivative **5** with aromatic aldehydes gave the Schiff's bases derivative **16a–c**. Some of the synthesized compounds were tested against the breast cancer cell line (MCF-7). The structures of all the newly synthesized compounds were established based on IR, $^{1}H$, $^{13}C$ NMR, mass spectral data, and elemental analyses.

**GRAPHICAL ABSTRACT**

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**KEYWORDS**
3,1-Benzoxazinone; pyrazoloquinzolinone; triazinoquinazolinone; Schiff's bases

**CONTACT** Abeer M. El-Naggar elsayedam@sci.asu.edu.eg Chemistry Department, Faculty of Science, Ain Shams University, Abassia 11566, Cairo, Egypt.

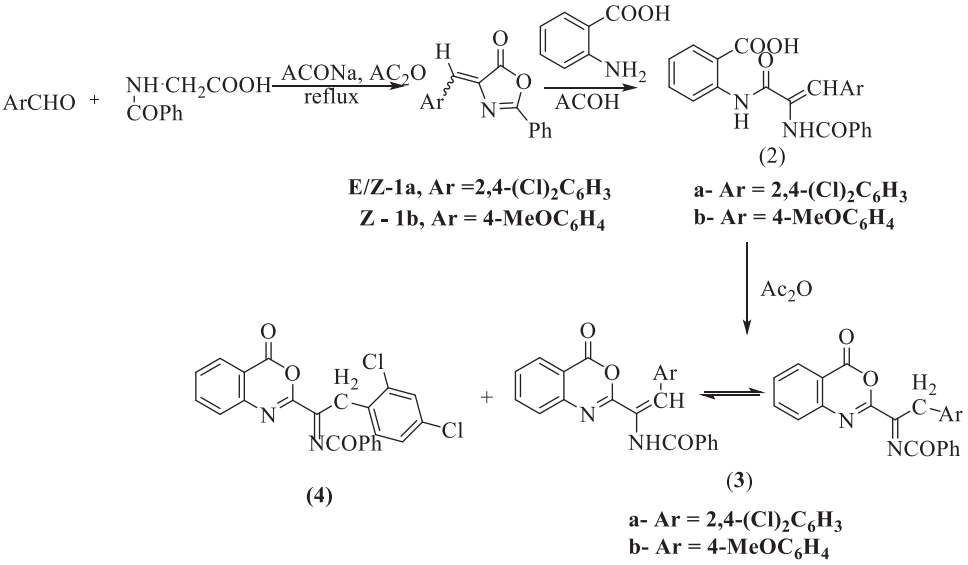
Supplemental data for this article can be accessed on the publisher's website.

## Introduction

Benzoxazinone derivatives are considered important in chemical syntheses of various physiologically and pharmaceutically important compounds. They have a kind of biological effects including antitubercular,[1] antifungal,[2–5] antimalarial, anticancer, anti-HIV,[3,6] antiviral, and antibacterial activities.[4,7,8] 3H-Quinazolin-4-ones and their derivatives have been described to have a significant activity as antihypertensive,[9] antifibrillatory, choleretic, antiphlogistic,[10] antimitotic anticancer,[11] antifungal,[12,13] and anticonvulsant agents.[14] They have also been successfully tested as central nervous system depressants,[15] muscle relaxants[16] and for their antineoplastic activity.[17] In continuation of our previous work on quinazolinone molecular system by incorporating different substituted amines as cyanoacetamide, thiazolidinone, thiazolidinthione, azet-2-thione, azet-2-one, thiadiazole, and pyrazolone on third position of quinazolinone[18–20] and fused annulated pyrazolo, imidazole, and tetrazinoquinazolinone[21] and studies of their antimicrobial activities, we have tried in this investigation to synthesize a new series of N-3-substituted-quinazoline-4(3*H*)-one derivatives incorporating *N* and *O* heterocyclic derivatives.

## Results and discussion

Hippuric acid reacted with 2,4-dichloro- and 4-methoxybenzaldehydes in acetic anhydride in the presence of fused sodium acetate as a base to give 4-arylideneoxazolone derivatives **1a, b**. The IR spectra of compounds **1a, b** exhibited bands for CO, C=N, and C=C groups. Further support for their assigned structures was gained from their $^1$H NMR spectra, which revealed two singlet signals for the olefinic proton of compound **1a** at $\delta$ 7.57, 8.19 ppm that suggested its existence as a mixture of *E*/*Z* stereoisomers. However, one singlet signal was observed for the olefinic proton of **1b** at $\delta$ 7.33 ppm, which was in accordance with its existence as the Z-configurated isomer. Moorkoth et al.[22] prepared the other isomer but they did not mention its configuration, thus according to its *J* value for the olefinic proton ($J = 6.91$ Hz) must have the *E*-configuration. The higher $\delta$ value for the olefinic proton of the *Z*-isomer as compared to that of the *E*-counterpart may be due to its existence in the deshielding regions of the aryl and oxazolone carbonyl. The integration values for olefinic proton of compound **1a** showed that the *E*/*Z* mixture exist in ratio of 3:2. The high percentage of the *E*-isomer may be due to the extension of conjugation because of coplanarity of the aryl group with oxazolone ring. Heating of the oxazolones **1a, b** with anthranilic acid in acetic acid afforded the open chain adducts **2a, b**. Their spectral data were in accordance with the proposed structure (cf. Experimental). Refluxing the benzoic acid derivatives **2a, b** with acetic anhydride yielded mixtures of the benzoxazinone derivatives **3, b** as equilibrium mixtures in the ratios of 79:21 and 31: 69, respectively, as well as compound **4** in case of compound **2b** (Scheme 1). The structures of compounds **3** and **4** were evidenced by studying their spectral data. Their IR spectra exhibited bands corresponding to NH and CO groups. Inspection of the $^1$HNMR spectra of compounds **3a, b** revealed the existence of one singlet signal in the upfield region for protons of $CH_2$ group as well as two singlet signals in the downfield region, one for the olefinic proton and the other for NH proton which was exchanged upon shaking with $D_2O$. This suggested that they are existing as mixtures of enamine–imine tautomers in the

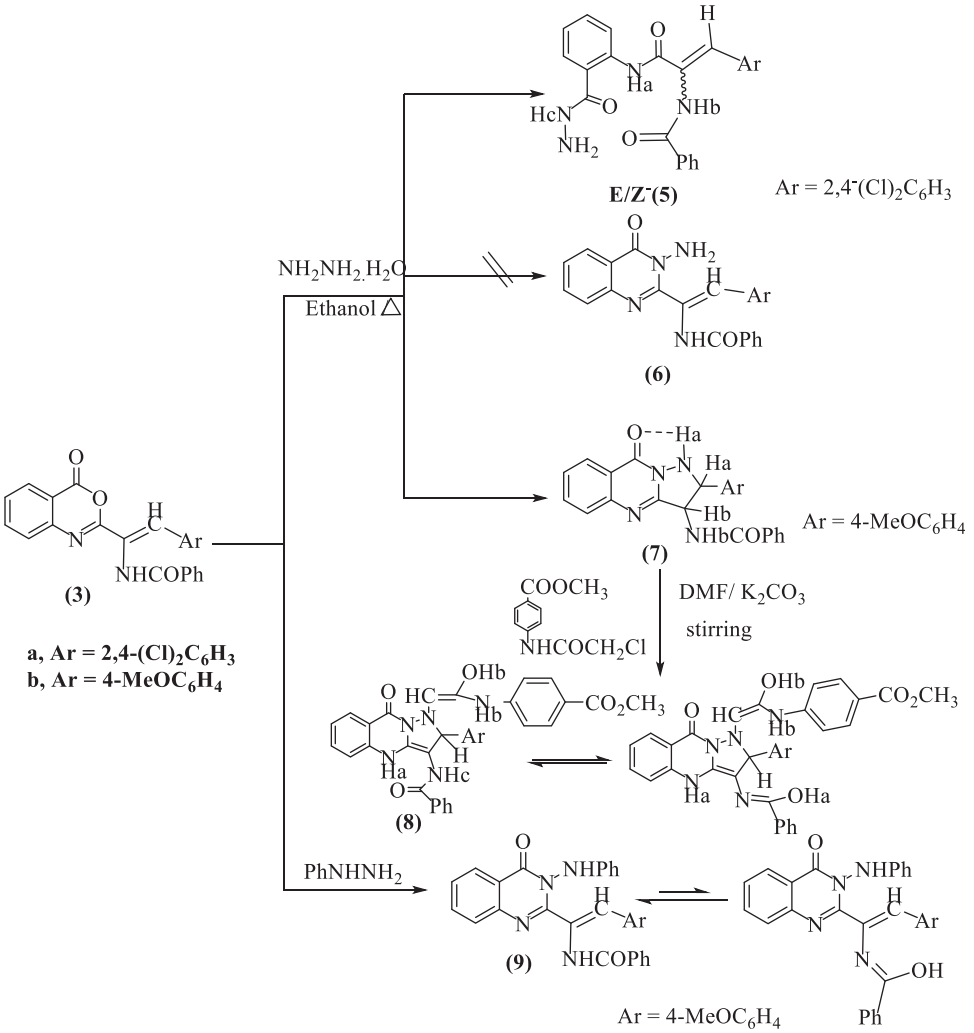



**Scheme 1.** Synthesis of compounds **1–4**.

ratios 80:20 and 31:69, respectively. However, the $^1$HNMR spectrum of **4** exhibited a singlet signal for methylene protons and was devoid of any signals for the olefinic and NH protons. The EIMS spectra of compounds **3a**, **b** and **4** supported their structures as they showed their molecular ion peaks.

The aim of the work is to utilize 4*H*-3, 1-benzoxazin-4-one derivatives **3a, 3b** for the synthesis of 3-aminoquinazolin-4(3*H*)-one derivatives which are promising intermediates for diverse organic synthesis. Thus, hydrazinolysis of **3a** using hydrazine hydrate in boiling ethanol afforded the open chain hydrazide **5** instead of the 3-amino quinazolinone derivative **6** (Scheme 2). The structure of the hydrazide **5** was substantiated from its analytical and spectral data. Thus, its IR spectrum exhibited bands for NH and CO groups. The appearance of two singlet signals for the olefinic proton as well as extra exchangeable broad singlet signals for NH protons in the down field region of its $^1$HNMR spectrum is in accordance with its existence as a mixture of *E*/*Z* stereoisomers in the ratio of 54:46. The higher $\delta$ value for the signal of the olefinic proton of the *Z*-isomer as compared with that of the *E*-counterpart may be due to its existence in the deshielding regions of dichlorophenyl and the carbonyl groups.

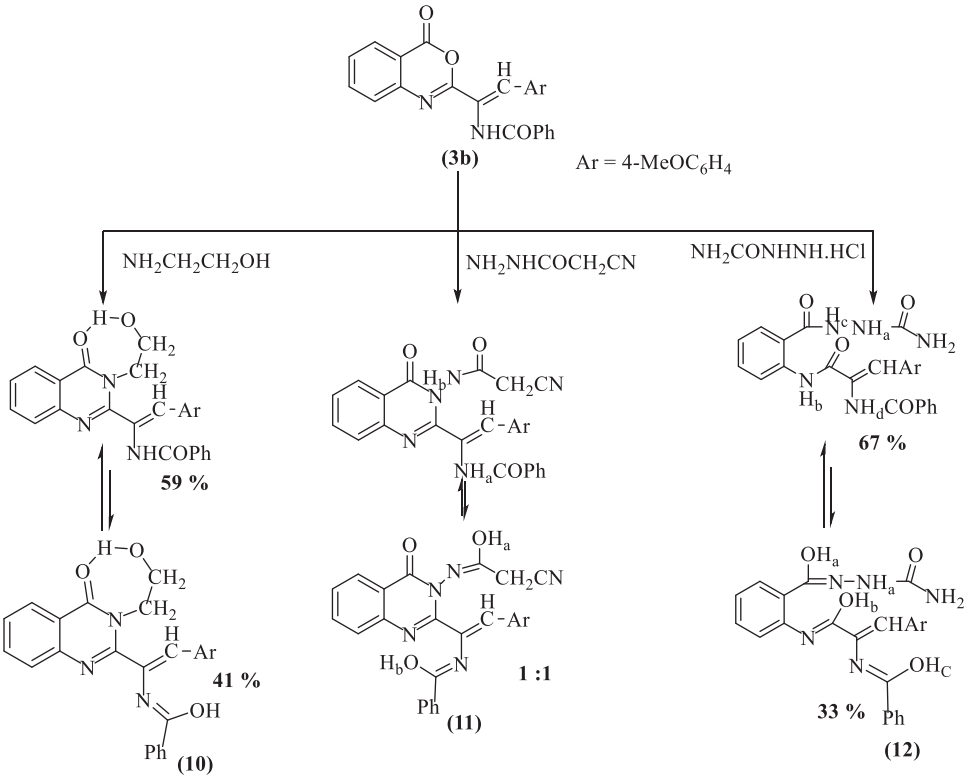
However, hydrazinolysis of **3b** with hydrazine hydrate afforded the pyrazoloquinazolinone derivative **7**. IR spectrum of compound **7** showed bands for NH and CO groups. Further support for the proposed structure of **7** was gained from its $^1$HNMR spectrum that revealed the coupling pattern between protons of CHb–CHa–NHa moiety. Proton Ha appeared as doublet–doublet signals at $\delta$ 4.97 ppm with $J = 11.2$, 11.6 Hz due to coupling with CHb and NHa protons. The reason for coupling with NHa proton may be due to chelation with C=O group. Proton Hb appeared as triplet signal at $\delta$ 4.48 ppm with $J = 11.2$, 11.6 Hz, also proton NHa appeared as doublet signal at $\delta$ 5.33 ppm with $J = 11.2$ Hz which was exchanged upon shaking with $D_2O$. Reaction of **7** with methyl 4-(2-chloroacetamido) benzoate afforded compound **8**. The structure of **8** was evidenced by studying its spectral data. Its IR spectrum showed bands characteristic for NH and




**Scheme 2.** Synthesis of compounds **5**, **7–9**.

CO groups. The $^1$HNMR spectrum of compound **8** revealed the existence of extra broad singlet signals in the down field region; this suggests its existence as an equilibrium mixture of lactam–lactim tautomers in a ratio of 1:1. Heating an alcoholic solution of **3 b** with phenylhydrazine yielded the quinazolinone derivative **9**. Its IR spectrum exhibited bands for C=O and NH groups. Furthermore, the $^1$HNMR spectrum supported its structure as it showed signals for protons of $OCH_3$, NH and aromatic groups as well as a broad singlet signal in the down field region at $\delta$ 11.84 ppm corresponding to proton of OH group. This suggests the existence of compound **9** as an equilibrium mixture of lactam–lactim tautomers in the ratio 72:28.

Reactions of compound **3b** with ethanolamine and cyano acetohydrized gave the benzoxazinone derivatives **10** and **11**, respectively. However, its reaction with semicarbazide hydrochloride yielded the open chain semicarbazide derivative **12**. The IR spectra of compounds **10–12** showed bands for NH and CO groups as well as a

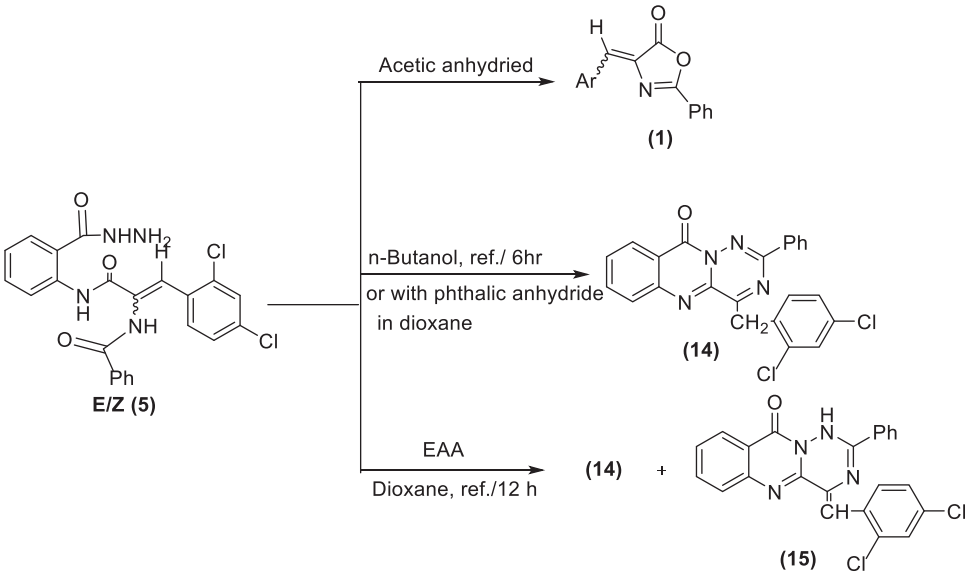



**Scheme 3.** Synthesis of compounds **10–12**.

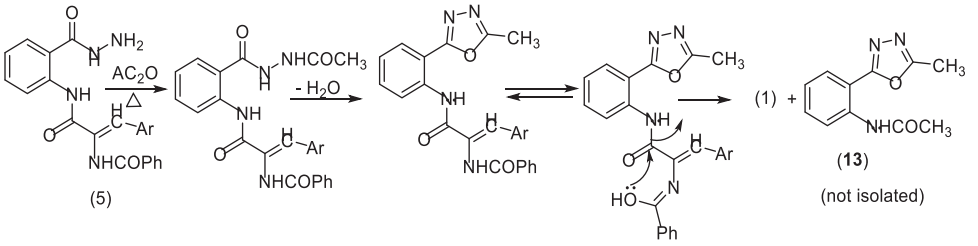
band for OH group in case of compound **10** and a band for CN group in case of compound **11**. The $^{1}$HNMR spectra shed further light on the proposed structures of compounds **10–12** as they revealed signals for protons of OH, NH, multiplet signals for aromatic protons beside multiplet signals for protons of $-CH_2-CH_2-$ group of compound **10** and a singlet signal for methylene protons of compound **11**. The appearance of two signals for protons of $OCH_3$ group as well as two exchangeable broad singlet signals in the down field region, one for NH proton and the other for OH proton, suggests the existence of compound **10** as an equilibrium mixture of lactam–lactim tautomers in the ratio of 56:41. In addition, the exchangeable triplet signal at $\delta$ 4.62 ppm for OH proton of $HOCH_2CH_2$ group suggests the chelation shown in Scheme 3. Investigation of the $^{1}$HNMR spectra of compounds **11**, **12** showed extra broad singlet signals in the down field region; that was in accordance with the existence of **11**, **12** as equilibrium mixtures of lactam–lactim tautomers of 1:1 and 67:33 ratios, respectively. Further evidence for the structure of **11** was gained from its $^{13}$CNMR spectrum (cf. Experimental).

Heating the hydrazide derivative **5** with acetic anhydride to get the corresponding amino quinazolinone derivative led to a compound that was identical in all respects m.p and TLC with the oxazolone **1** (Scheme 4).

However, its heating in *n*-butanol or with phthalic anhydride in dioxane afforded the triazinoquinazoline derivative **14**. On the other hand, its treatment with ethyl acetoacetate in refluxing dioxane yielded compound **15** which was a mixture of compound 14



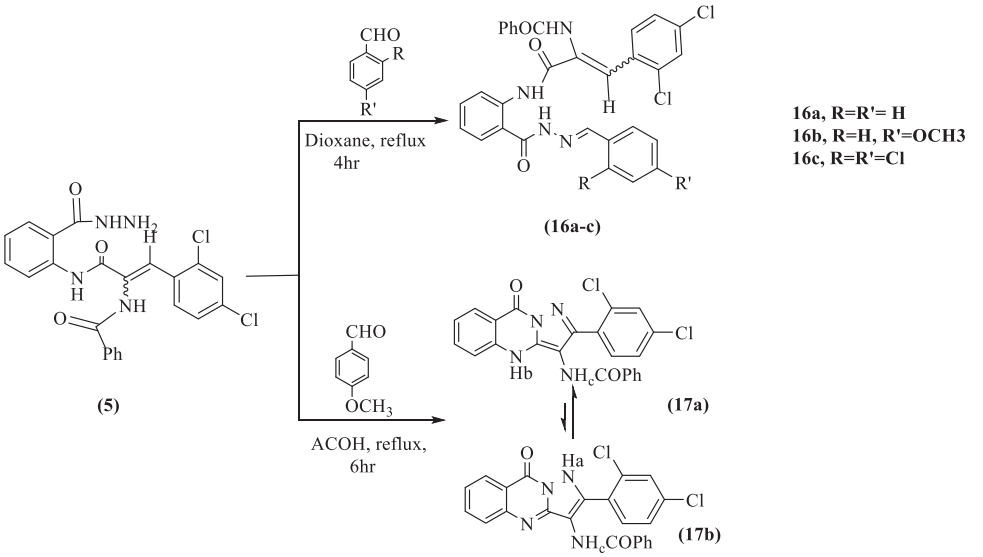

**Scheme 4.** Synthesis of compounds **14**, **15**.




**Scheme 5.** Suggested mechanism for reaction of compound **5** with $AC_2O$.

and its amino tautomer. The structures of compounds **14** and **15** were evidenced from their analytical and spectral data (Scheme 4). Their IR spectra showed absorption bands for C=O group as well as NH group for compound **15**. Furthermore, their $^1$HNMR spectra shed further lights on the proposed structures as they exhibited signals for $CH_2$, NH, and aromatic protons. The $^1$HNMR spectrum of compound **15** showed the existence of singlet signals for methylene and olefinic protons as well as an exchangeable broad singlet signal for NH proton. This suggests its existence as a mixture of **14** and its amino tautomer in the ratio of 14:86 (Scheme 4). The reason for the absence of any role for phthalic anhydride or ethyl acetoacetate in these reactions may be due to the facile cyclization of hydrazide **5** just on heating in high boiling solvents. The formation of oxazolone **1** is depicted in (Scheme 5).

Schiff's bases have biological importance and possess a variety of activities viz. Anticonvulsant, antimicrobial, antimycobacterial, anticancer, antitumor, antimalarial, and antitubercular activities.[23–29] This encouraged us to synthesize a number of Schiff's bases by reacting hydrazide **5** with different aromatic aldehydes and to study the effect of different substituents on the biological activity of the formed Schiff's bases. Thus heating of the hydrazide **5** with aromatic aldehydes namely, benzaldehyde, 4-

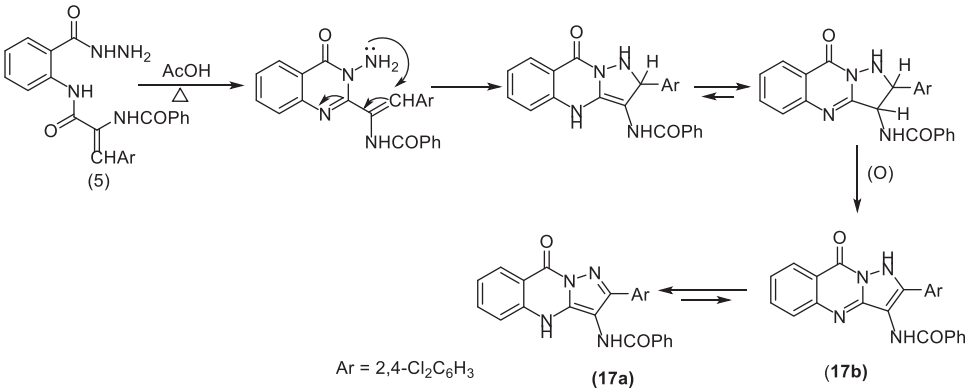



**Scheme 6.** Synthesis of compounds **16 (a–c)** and **17**.

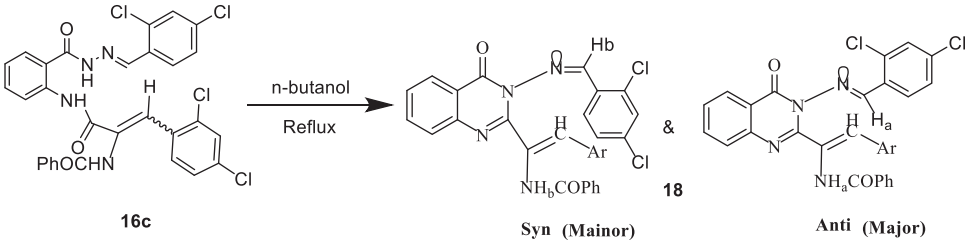
methoxybenzaldehyde, and 2, 4-dichlorobenzaldehyde in dioxane afforded the Schiff's bases **16a–c**. However, refluxing the hydrazide **5** with 4-methoxybenzaldehyde or cyclopentanone in acetic acid yielded the pyrazoloquinazoline derivative **17** (cf. Scheme 6). It is observed that 4-methoxybenzaldehyde or cyclopentanone have no role in the course of the reaction.

The structures of compounds **16 a–c** and **17a, b** were deduced from their analytical as well as spectral data. Their IR spectra showed bands correlated with NH and C=O groups. Further evidence was gained from their $^{1}$HNMR spectra that showed broad singlet signals for NH protons as well as singlet signals for olefinic and imino methine protons for compounds **16a–c**. The appearance of extra exchangeable broad singlet signals in the down field region of the $^{1}$HNMR spectrum of compound **16 b** suggests its existence as a mixture of ***E*/*Z*** stereoisomers in an equal ratio. In addition, it was observed that the signals for the NH proton of NHCOPh group as well as that of CH = proton of the two isomers appear as doublet signals with the same *J* values; rather than singlets. This may be due to the dipolar coupling in space between these protons (nuclear Overhouser effect). Accurate examination of the $^{1}$HNMR spectrum of compounds **17a, b** revealed the existence of extra broad singlet signals in the down field region, which is in accordance with its existence as an equilibrium mixture of the tautomers **17a** and **17 b** in the ratio of 3:2 (cf. Scheme 6). The formation of compound **17a, b** is depicted in Scheme 7.

Heating the Schiff base **16c** with n-butanol afforded the quiazolinone derivative **18.** Its structure was evidenced from its spectral as well as analytical data. The IR spectrum exhibited bands for NH and CO groups. Inspection of the $^{1}$HNMR spectrum revealed the appearance of extra signals for imino methine and NH protons that were in accordance with its existence as a mixture of syn (minor) and anti (major) isomers (Scheme 8).




Scheme 7. Suggested mechanism for formation of compound **17a, b.**




Scheme 8. Synthesis of compound **18.**

## Biological activity

### *In vitro cytotoxic activity*

The *in vitro* anticancer activity of the 13 newly synthesized compounds was evaluated against the breast cancer cell line (MCF-7). Doxorubicin was used as reference standard and showed IC50 values of $4.17 \pm 0.2\,\mu M$ for the (MCF-7) cell lines. The results were expressed as $IC_{50}$ values and listed in (Table 1; Figure 1). The obtained results indicated that the tested compounds exhibited good, moderate, or weak antiproliferative activities against the tested cell line. Firstly, compounds **5** and **7** were found to be the most potent derivatives against MCF-7 cell line. Compound **7** showed a very strong activity against MCF-7 cell line ($IC_{50}$ = **6.18 ± 0.4** μM). Compound **5** also exhibited strong activity toward MCF-7 ($IC_{50}$ = **9.73 ± 0.8** μM). Compounds **9, 10, 12,** and **16a** show a strong activity toward against MCF-7 ($IC_{50}$ = **11.29 ± 1.0** μM), ($IC_{50}$ = **15.17 ± 1.3** μM), ($IC_{50}$ = **11.29 ± 1.0** μM), ($IC_{50}$ = **13.02 ± 1.2** μM) and ($IC_{50}$ = **18.24 ± 1.5** μM), respectively. However, Compounds **11, 3a, 8, 3 b, 17** possessed moderate antiproliferative activities against MCF-7 ($IC_{50}$ = 28.81 ± 2.3 μM). Finally, compound **16b** showed weak antiproliferative activities against the tested cell line.

## Conclusion

During this investigation, N-3-substituted-quinazoline-4(3*H*)-one derivatives incorporated *Nitrogen* and *oxygen* heterocyclic derivatives. All the synthesized derivatives were characterized by spectral data. In addition, some of the synthesized products were

**Table 1.** *In vitro* anti-proliferative activities toward MCF-7 cell lines.

| Compounds | *In vitro* cytotoxicity $IC_{50}$ (μ molar/ml)[a] MCF-7 |
|---|---|
| **3a** | 49.23 ± 3.3 |
| **3b** | 43.17 ± 2.9 |
| **5** | 9.73 ± 0.8 |
| **7** | 6.18 ± 0.4 |
| **8** | 35.49 ± 2.3 |
| **9** | 11.29 ± 1.0 |
| **10** | 15.17 ± 1.3 |
| **11** | 26.86 ± 2.1 |
| **12** | 13.02 ± 1.2 |
| **16a** | 18.24 ± 1.5 |
| **16b** | 74.11 ± 4.1 |
| **16c** | 54.60 ± 3.8 |
| **17** | 21.54 ± 1.7 |
| **DOX** | 4.17 ± 0.2 |

DOX: doxorubicin.
[a]IC50 (μM): 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic).

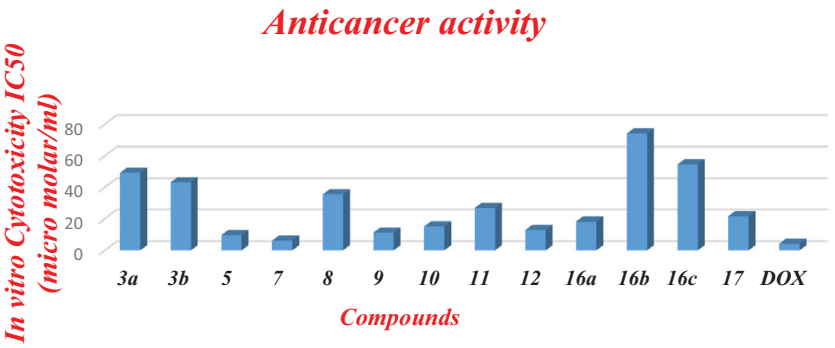



**Figure 1.** *Anticancer activity of the synthesized compounds.*

screened for their *in vitro* anticancer activity. The majority of the tested compounds exhibited significant anticancer activity.

## Experimental

All commercially available solvents and reagents of analytical grade were used without further purification. All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. IR spectra were recorded on a Pye-Unicam SP-3-300 infrared spectrophotometer (KBr dicks) and expressed in wave number ($cm^{-1}$). $^{1}H$ NMR spectra were run at 400 MHz on a Varian Mercury VX-300 and BrukerAvance III NMR spectrometer at the main defense chemical laboratory. While $^{13}C$ NMR spectra were run at Bruker DPX 600 at 150 MHz using tetramethylsilane (TMS) as an internal standard in deuterated dimethylsulphoxide (DMSO-$d_6$) at the central laboratory faculty of science of Kuwait university. Chemical shifts ($\delta$) are quoted in ppm. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; $t_d$, triplet doublets. All coupling constant ($J$) values are given in hertz. The mass spectra were recorded on

Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were performed at the central laboratory of faculty of science, Cairo University on CHN analyzer and all compounds were within ± 0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. All reagents and solvents were purified and dried by standard techniques. All the newly synthesized compounds gave a satisfactory elemental analysis.

## General procedure

A mixture of 2, 4-dichlorobenzaldehyde (1.75 g, 0.01 mol) or 4-methoxybenzaldehyde (1.35 g, 0.01 mol) and hippuric acid (1.78 g, 0.01 mol) in acetic anhydride (10 mL) and fused sodium acetate (0.83 g, 0.01 mol) was refluxed for 1 h, cooled, then poured on ice water. The formed precipitate was filtered off, dried, and recrystallized from suitable solvents to give (**1a, b**).

### 4-(2, 4-Dichlorobenzylidene)-2-phenyl-1, 3-oxazol-5-one (1a)

Yield: 60%; orange crystals; *m.p.* 190–192 °C (pet (60–80)-benzene); IR (KBr) ($\upsilon$, $cm^{-1}$): 3090, 3059 ($CH_{aryl}$), 1798, 1765 (C=O), 694, 756 ($\delta_{5H}$); $^1$HNMR ($CDCl_3$) $\delta$ 7.38–8.92 (m, 8 H, Ar-H); ***for Z-isomer:*** $\delta$ 8.19 (s, 1 H, CH=); ***for E-isomer:*** $\delta$ 7.57(s, 1 H, CH=). MS (*m/z*): 322 ($M^{+\cdot}$ + 4, 1), 320 ($M^{+\cdot}$ +2, 6.3), 318 ($M^{+\cdot}$, 9.2), 317 (55.2), 186 (46), 184 (71.8), 157 (45), 114 (68.4), 106 (63.7), 105 (PhCO, 99.9), 99 (17.8), 88 (21.4), 77 (Ph, 99.9), 51 (99.9), 50 (57.1); Anal. Calcd for: $C_{16}H_9Cl_2NO_2$ (318.2): C, 60.40; H, 2.85; N, 4.40; Found: C, 60.23; H, 2.76; N, 4.22%.

### 4-(Methoxybenzylidene)-2-phenyl-1, 3-oxazol-5-one (1b)

Yield: 60%; yellow crystals; *m.p.* 180–182 °C (pet (60–80)-benzene); IR (KBr)($\upsilon$, $cm^{-1}$): 3081, 3015 ($CH_{aryl}$), 2974, 2935, 2843 ($CH_{alkyl}$), 1789, 1770 (C=O), 696, 773 ($\delta_{5H}$), 831($\delta_{2H}$). $^1$HNMR (DMSO $d_6$) $\delta$ (ppm): 3.84 (s, 3 H, $OCH_3$), 7.09 (d, 2 H, Ar-H, $J$ = 9.2 Hz), 7.33 (s, 1 H, CH=), 7.62 (t, 2 H, Ar-H, $J$ = 7.2, 8 Hz), 7.69 (t, 1 H, Ar-H, $J$ = 7.2, 8.8 Hz), 8.1 (d, 2 H, Ar-H, $J$ = 8.8 Hz), 8.29 (d, 2 H, Ar-H, $J$ = 8.8 Hz); Anal. Calcd for: $C_{17}H_{13}NO_3$ (279.30): C, 73.11; H, 4.69; N, 5.02; Found: C, 73.23; H, 4.76; N, 4.88%.

## Reactions of 1a, b with anthranilic acid

A mixture of **1a** (3.2 g, 0.01 mol) or **1b** (2.7 g, 0.01 mol) and anthranilic acid (1.37 g, 0.01 mol) was refluxed in acetic acid (20 mL) for 2 h. The formed precipitate was filtered, dried, and recrystallized from suitable solvents to give (**2a**, **2b**).

### 2-(2-Benzamido-3-(2, 4-dichlorophenyl) acrylamido) benzoic acid (2a)

Yield: 70%; white crystal; *m.p.* 238–240 °C (EtOH/Dioxane); IR ($\nu$, $cm^{-1}$): 3442 (OH), 3309, 3225 (NH), 3057 ($CH_{aryl}$), 1710, 1671, 1651 (CO), 699,758 ($\delta_{5H}$). $^1$H-NMR

(DMSO-$d_6$) $\delta$ (ppm): 7.17 (t, 1 H, Ar-H, $J_o$ = 8 Hz, $J_m$ = 1.2 Hz), 7.4 (dd, 2 H, Ar-H, $J_o$ = 8 Hz, $J_m$ = 2 Hz), 7.47 (s, 1 H, CH=), 7.51 (t, 2 H, Ar-H, $J_o$ = 8.4, 6.8 Hz), 7.58 (t, 2 H, Ar-H, $J_o$ = 6.8, 7.6 Hz), 7.65 (t, 1 H, Ar-H, $J_o$ = 8.8 Hz, $J_m$ = 2 Hz), 7.72 (d, 1 H, Ar-H, $J_m$ = 2 Hz), 7.92 (d, 1 H, Ar-H, $J_o$ = 7.6 Hz), 7.98 (dd, 1 H, Ar-H, $J_o$ = 7.8 Hz, $J_m$ = 2 Hz), 8.78 (d, 1 H, Ar-H, $J_o$ = 7.6 Hz), 10.34 (br.s, 1 H, NHCO, exchangeable), 12.16 (br.s, 1 H, NHCOPh, exchangeable), 13.48 (br.s, 1 H, OH, exchangeable). MS (*m/z*): 457($M^{+\cdot}$ +2, 4.8), 456 ($M^{+\cdot}$ +1, 11.8), 455 ($M^{+\cdot}$, 5.5), 439 (20.6), 438 (63), 437 (37.9), 436 (99.8), 403 (44), 401 (100), 105 (PhCO, 90.6), 104 (24.7), 77 (Ph, 70.9); Anal. Calcd for: $C_{23}H_{16}Cl_2N_2O_4$ (455.29): C, 60.68; H, 3.54; N, 6.15; Found: C, 60.43; H, 3.66; N, 6.41%

### 2-(2-Benzamido-3-(4-methoxyphenyl) acrylamido) benzoic acid (2b)

Yield 75%; white crystal; *m.p.* 250–252 °C (EtOH/Dioxane); IR ($cm^{-1}$) $\nu$: 3428 (OH), 3313, 3242 (NH), 3039, 3000 ($CH_{aryl}$), 2924, 2931, 2836 ($CH_{alkyl}$), 1687, 1670, 1650 (CO), 1602, 1580, 1526(C=C), 823 ($\delta_{2H}$), 695,757 ($\delta_{5H}$). $^1$H-NMR (DMSO-$d_6$) $\delta$ (ppm): 3.74 (s, 3 H, $OCH_3$), 6.92 (d, 2 H, Ar-H, $J_o$ = 8.8 Hz), 7.12–7.15 (m, 1 H, Ar-H, $J$ = 8.4,7.2 Hz), 7.51–7.64 (m, 7 H,Ar-H), 7.96 (s, 1 H, CH=), 8.06 (d, 2 H, Ar-H, $J$ = 8.4 Hz), 8.82 (d, 1 H, Ar-H, $J$ = 8.8 Hz)), 10.30 (br.s, 1 H, NHCO, exchangeable), 12.08 (br.s, 1 H, NHCOPh, exchangeable), 13.52 (br.s, 1 H, OH, exchangeable; Anal.Calcd for: $C_{24}H_{20}N_2O_5$ (398.4): C, 69.22; H, 4.84; N, 6.73; Found: C, 69.05; H, 4.49; N, 6.89%.

## Biological evaluation

### In vitro anticancer activity

Three human cancer cell lines, namely hepatocellular carcinoma (HePG-2), mammary gland (MCF-7), and colorectal carcinoma (HCT-116) are used to determine *in vitro* the anticancer activity of the synthesized compounds. The tested cell lines were supplied from the US National Cancer Institute. The reported standard procedure[30] was utilized as follows. The tested cells were plated in 96-well Microplates; the total volume per well was adjusted at 100 $\mu$L. Then, incubation of cells was performed at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity for 24 h before addition of synthesized compounds. After 24 h, only two plates of each cell line were selected and fixed *in situ* with TCA, in order to exemplify a measurement of the cell population for each cell line during drug application. The title compounds and fluorouracil, the reference drug, were dissolved in DMSO at 400-fold the desired final maximum test concentration and stored at freezing point prior to use. During addition of drug, the frozen concentrate was dissolved and diluted to twice the desired final maximum test concentration with gentamicin solution (50 mg/mL). To reach the desired final drug concentrations, different tested compound dilutions (100 mL) were added to the appropriate microtiter wells containing 100 mL of medium. The tested compounds as well as 5-fluorouracil as reference drug were added. Then, the plates were incubated for an additional 48 h at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity. The assay was terminated by the addition of cold TCA for adherent cells followed by incubation for 60 min at 4 °C. The supernatant was removed, and the plates were washed five times with excessive water and dried. A solution of

0.4% (w/v) sulforhodamine B (100 mL) in 1% acetic acid was added to each well, followed by incubation at room temperature for 10 min. After staining, the plates were washed with 1% acetic acid to remove unbound dye and air-dried. Bound stain was dissolved with 10 mM Trizma base. Spectrophotometric assay of the optical density (OD) of each well was determined at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). Boltzmann sigmoidal concentration–response curve was used to calculate the $IC_{50}$ values through the nonlinear regression fitting models (GraphPad Prism, version 5). The means of three separate experiments were reported as final result. ANOVA test was used to analyze the statistical differences, wherein the differences were considered to be significant at $p < 0.05$.

## Disclosure statement

The authors declare no conflict of interest, financial or otherwise.

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