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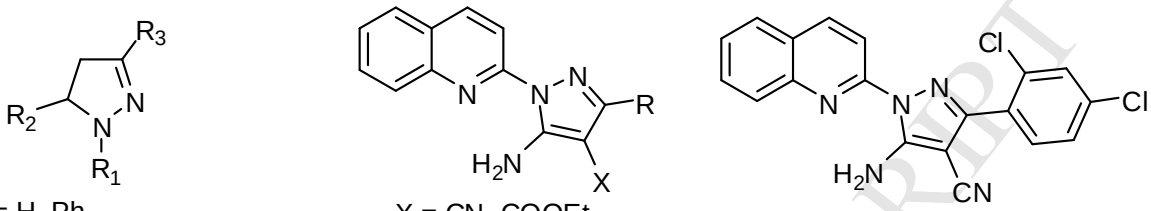
Graphical abstract:



R_1 = H, Ph,
R_2, R_3 = 2-phenoxyquinoline, 2-chlorophenyl, 4-methoxyphenyl

X = CN, COOEt
R = CH_3, SCH_3, 3-Cl-C_6H_4
2,4-Cl_2-C_6H_3

MIC values (0.12 - 0.98 µg/mL)

Antibacterial and antifungal activities

New three series of quinoline derivatives bearing a pyrazole moiety have been synthesized and evaluated for antibacterial and antifungal activity.

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Quinoline derivatives bearing pyrazole moiety: Synthesis and biological evaluation as possible antibacterial and antifungal agents

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Abstract: In an attempt for development of new antimicrobial agents, three series of quinoline derivatives bearing pyrazole moiety have been synthesized. The first series was synthesized through the synthesis of 4-(quinolin-2-yloxy)benzaldehyde and 4-(quinolin-2-yloxy)acetophenone and then treatment with ketone or aldehyde derivatives to afford the corresponding chalcones. Cyclization of the latter chalcones with hydrazine derivatives led to the formation of new pyrazoline derivatives. The second series was synthesized *via* the synthesis of 2-hydrazinylquinoline and then treatment with formylpyrazoles to afford the corresponding hydrazonyl pyrazole derivatives. The third series was synthesized through the treatment of 2-hydrazinylquinoline with ethoxyethylidene, dithioacetal and arylidene derivatives to afford the corresponding pyrazole derivatives. The synthesized compounds were evaluated for their expected antibacterial and antifungal activities; where, the majority of these compounds showed potent antibacterial and antifungal activities against the tested strains of bacteria and fungi. Pyrazole derivative **13b** showed better results when compared with the reference drugs as revealed from their MIC values (0.12 - 0.98 μg/mL). The pyrazole derivative **13b** showed fourfold potency of gentamycin in inhibiting the growth of *S. flexneri* (MIC 0.12 μg/mL). Also, compound **13b** showed fourfold potency of amphotericin B in inhibiting the growth of *A. clavatus* (MIC 0.49 μg/mL) and *C. albicans* (MIC 0.12 μg/mL), respectively. The same compound showed twofold potency of gentamycin in inhibiting the growth of *P. vulgaris* (MIC 0.98 μg/mL), equipotent to the ampicillin and amphotericin B in inhibiting the growth of *S. epidermidis* (MIC 0.49 μg/mL), *A. fumigatus* (MIC 0.98 μg/mL), respectively. Thus, these studies suggest that quinoline derivatives bearing pyrazole moiety are interesting scaffolds for the development of novel antibacterial and antifungal agents.

Keywords: Quinolines; Pyrazoles; Antibacterial and antifungal agents

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1. Introduction

Microbial infections are causing widespread and serious diseases. In order to prevent these serious medical problems, the discovery of new types of antibacterial and antifungal agents is a very important task. In recent years, the research has been focused on the development of new antimicrobial agents, which may act through structure design and novel targets, overcoming the problem of acquired resistance [1-4]. Quinoline derivatives possess very good antibacterial as well as antitubercular activities [5-7] with a variety of other different pharmacological activities such as anticancer [8], and antimalarial [9]. The prominent antibacterials are fluoroquin- olones like Ciprofloxacin, Sparfoxacin, Gatifloxacin. The antifungal-antiprozoal is Clioquinol, the antimalarials are Quinine, Quinidine, Chloroquine, Mefloquine, Amodia- quinine, Primaquine. The antiviral is Saquinavir. Ciprofloxacin and Moxifloxacin are showed significant antitubercular activity and are recommended by WHO as second line anti-TB drugs [10]. Furthermore, quinoline moiety is found in a large variety of naturally occurring compounds and also chemically useful synthons bearing diverse bioactivities [11]. In this perspective, we have chosen the quinoline skeleton for the design of new bioactive molecules. On the other hand, pyrazole derivatives possess a wide spectrum of chemotherapeutic activities including antituberculosis, [12] antiviral [13] and anti-inflammatory [14] activities. The majority of known quinoline drugs have a side chain on the 4- or 8-position of the quinoline building block. However, recently, it has been reported that moving a functionalized side chain around the quinoline core to the other position can result in retention of biological and providing new opportunities for the design of bioactive compounds. These observations gave us an additional motivation to combination of the quinoline scaffold with pyrazole derivatives at 2-position of the quinoline. Therefore, we initiated a program to synergies the antimicrobial activity of quinoline and pyrazole in an effort to discover potent antimicrobial agents.

2. Results and discussion

2.1. Chemistry

4-(Quinolin-2-yloxy)benzaldehyde **1** was obtained by the reaction of 2-choloroquinoline and 4-hydroxybenzaldehyde in DMF in the presence of anhydrous potassium carbonate (Scheme 1). Claisen-Schmidt condensation of 4-(quinolin-2-yloxy)benzaldehyde **1** with 4-methoxyacetophenone in ethanolic sodium hydroxide furnished the chalcone derivative **2**. The structures of the latter products were derived from their spectroscopic as well as elemental analyses. IR spectrum of compound **2** showed the characteristic bands for CH-aliphatic, conjugated C=O and olefin C=C at 2927, 1658 and 1602 cm^{-1}, respectively. Its ^{1}H NMR spectrum displayed a pair of a doublet signal at: δ = 7.78 ppm, with a coupling constant indicating *trans*-olefinic protons. Cyclocondensation of chalcone **2** with hydrazine hydrate in boiling ethanol furnished the respective pyrazoline **3**. Also, cyclocondensation of chalcone **2** with phenylhydrazine in boiling ethanol furnished the corresponding pyrazoline **4**. The structures of the pyrazolines **3** and **4** were derived from spectral data and elemental analyses. IR spectra showed the disappearance of the C=O band of chalcones, and appearance

of a strong band appeared at 1593-1599 cm^{-1} corresponding to C=N of the pyrazoline ring. Pyrazoline **3** showed an additional sharp band at 3341 cm^{-1} due to their NH stretching vibration. ^1H NMR spectra of pyrazolines **3** and **4** displayed ABX spin system for protons attached to the C-4 and C-5 carbon atoms of the pyrazoline ring. Chemical shifts and the coupling constant values unequivocally prove the pyrazoline structures. ^1H NMR spectra revealed the signals of CH_2 protons of the pyrazoline ring in the region 2.89-3.08 and 3.84-3.87 ppm as a pair of doublets. The CH proton appeared as doublet of doublets at: δ = 4.87 & 5.32 ppm. Initially, the acetyl derivative **5** was obtained via coupling of 2-choloroquinoline with 4-hydroxyacetophenone in DMF in the presence of anhydrous potassium carbonate (Scheme 1). The presence of the acetyl group in compound **5** makes it a versatile precursor for the synthesis of chalcones and pyrazoline derivatives. Thus, the Claisen-Schmidt condensation of **5** with aldehyde derivatives (namely; 2-chlorobebalehyde and 4-methoxybenzaldehyde) in ethanolic sodium hydroxide furnished the chalcone derivative **6a,b**. Structure of the latter products was derived from their spectroscopic as well as elemental analytical data. For example, IR spectrum of compound **6a** showed the characteristic band for conjugated C=O at 1657 cm^{-1} and its ^1H NMR spectrum displayed a pair of doublets at δ = 7.57 and 8.22 ppm, with a coupling constant indicating *trans*-olefinic protons. Cyclocondensation of chalcones **6a,b** with hydrazine hydrate in boiling ethanol furnished the respective pyrazolines **7a,b**. Similarly, cyclocondensation of chalcones **6a,b** with phenylhydrazine in boiling ethanol furnished the corresponding pyrazolines **8a,b**. The structures of the pyrazolines **7a,b** and **8a,b** were derived from spectral data and elemental analyses. IR spectra showed the disappearance of the C=O band of chalcones. Pyrazolines **7a,b** showed an additional sharp band in the region 3330-3317 cm^{-1} due to their NH stretching vibration. ^1H NMR spectra revealed the signals of CH_2 protons of the pyrazoline ring in the regions 2.89-2.92 and 3.80-3.82 ppm. CH proton appeared at: δ = 4.81-4.87 ppm.

Scheme 1:

2-Hydrazinylquinoline **9** [15] was prepared by the reaction of 2-choloroquinoline with hydrazine hydrate in boiling ethanol (Scheme 2). Condensation of the 2-hydrazinylquinoline **9** with formylpyrazoles in ethanol under reflux afforded the respective hydrazone derivatives **10a-c**. The structure of the hydrozonyl pyrazolines **10a-c** was established on the basis of their spectral data and elemental analyses. IR spectra showed bands in the region 3162 - 3183 cm^{-1} due to their NH stretching vibration. Beside the aromatic signals, singlet signal about 9.00 ppm are assigned for the azomethine proton. All ^1H NMR spectra of pyrazolines **10a-c** exhibited broad exchangeable signal for the NH proton about 11.12, 11.17 ppm. Moreover, ^{13}C NMR spectrum supported these assignments and added a strong evidence for these interpretations.

Scheme 2:

2-Hydrazinylquinoline **9** is considered as starting material for the synthesis of many substituted pyrazoline derivatives. Thus, treatment equimolar quantities of 2-hydrazinylquinoline **9** and 2-(1-ethoxyethylidene)malononitrile in ethanol when heated under

reflux gave a sole product, which was identified as 5-amino-4-cyano-3-methyl-pyrazole derivative **11a** as depicted in Scheme 3. By similar way, treatment equimolar quantities of 2-hydrazinylquinoline **9** and ethyl 2-cyano-3-ethoxybut-2-enoate in ethanol under reflux gave only a sole product, which was identified as 5-amino-4-ethylcarboxylate-3-methylpyrazole derivative **11b**. Interaction of 2-hydrazinylquinoline **9** with ketene dithioacetal derivative (2-(*bis*(methylthio)methylene)malononitrile) in ethanol under reflux afforded 5-amino-4-cyano-3-methylthio-pyrazole derivative **12a**. 5-Amino-4-ethyl-carboxylate-3-methylthio-pyrazole derivative **12b** was prepared through the reaction of 2-hydrazinylquinoline with ketene dithioacetal derivative [ethyl 2-cyano-3,3-bis(methylthio)acrylate] in ethanol under reflux. Finally, 5-amino-4-cyano-3-aryl-pyrazole derivatives **13a,b** were prepared through the reaction of 2-hydrazinylquinoline with arylidene malononitrle derivatives in ethanol under reflux. The structures of products were deduced from elemental analysis and spectroscopic data.

Scheme 3:

2.2. Biological activates

2.2.1. Animal toxicity study

The acute toxicity is usually measured by LD_{50} (the median lethal dose) which is the dose that kills 50% of the experimental animals under specified conditions. The LD_{50} will vary according to many factors, e.g. animal strain, room temperature, route of administration, season of the year, etc. Acute toxicity studies have to be carried out on several animal species generally on mice and rats. The basic principle of the determination of the LD_{50} depends on the determination of the highest dose that fails to kill any animal and this refers as the threshold dose or the maximal tolerated dose and determination of the minimal dose that kills all the animals, the former dose is referred to as LD_0; while the latter dose is referred to as LD_{100}. In between these two doses, several doses are chosen which produce different percentage of mortality. The methods of determination of the LD_{50} differ in the design of the experiment and the method of calculation of LD_{50}. The acute LD_{50} of the screened compounds were determined by Spearmane Karber method [16]. All the newly synthesized compounds were well tolerated up to the doses of 1200 mg/kg without any toxic manifestations. The results suggest that the tested compounds have very low toxic effects

2.2.2. Antibacterial and antifungal activities

The synthesized compounds were tested *in vitro* for antibacterial and antifungal activities against the following human pathogens strains: three Gram-positive bacteria, *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024) and *Bacillis subtilis* RCMB 010063; three Gram-negative bacteria, *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumonia* (RCMB 010093), and *Shigella flexneri* (RCMB 0100542), and three Fungi, *Aspergillus fumigatus* (RCMB 02564), *Aspergillus clavatus* (RCMB 02593) and *Candida albicans* (RCMB 05035). Agar-diffusion method [17] was used for the determination of the preliminary screening of antibacterial and antifungal activities. The antifungal agents were

evaluated against clinical isolates of standard strains of fungi by the broth dilution methods according to NCCLs [18]. Antimicrobial tests were carried out using 100 μL of tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 mL of dimethyl sulfoxide (DMSO). Ampicillin, Gentamycin and Amphotericin B (1 mg/mL) were used as standard references for Gram-positive bacteria, Gram-negative bacteria and antifungal activity, respectively. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial or fungal growth around the discs in mm. The inhibition zone diameters, attributed to the tested original concentration (5 mg/mL) as a preliminary test, are shown in Table 1.

Table 1:

From the tabulated data, it was found that: the mean values of the inhibition zone diameter obtained for these compounds suggest that all of the pyrazole derivatives possess significant antimicrobial activity against most of the tested organisms used in these assays. The results of antimicrobial screening revealed that most of the tested pyrazoles displayed variable inhibitory effects on the growth of tested Gram-positive, Gram-negative bacterial and fungal strains. In general, most of the studied compounds revealed better activity against the Gram-positive rather than Gram-negative bacterial strains. It was also observed that transformation of chalcone derivatives to the respective pyrazole derivatives increase the antibacterial activity. As anticipated, a clear difference in antimicrobial activity is noted between compounds within and between each series, pointing to the reinforcing and opposing effects of substitution at pyrazole moiety. The results of the antimicrobial screening demonstrated the following assumptions about the structural activity relationship (SAR). Inhibition of the diameter zones values of antifungal activity showed similar trend as antibacterial activity. Key precursor, chalcone **2** showed poor antimicrobial activity. For that reason, chalcone derivatives **2** and **6a,b** were chemically transformed to pyrazoline derivatives hoping to get more potent antimicrobial agents. The cyclized target pyrazoline showed significant activity. Regarding the effect of substitution at pyrazole moiety, it is evident that varying such a unit may have a dramatic effect on antimicrobial activity, which may be augmented or reduced depending on whether a matching or mismatching relationship exists with the substitution at pyrazole moiety. The type of the substitutions on the pyrazoline ring is important. Using the general structure provided in Fig. 1, certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

Figure 1:

The results depicted in Table 1 and Fig. 2 revealed that the pyrazoline derivative **3** showed moderate to good activity against the growth of the different tested organisms except *K. pneumonia*, and its best result was observed against Gram-positive bacteria. Also, their analogue, *N*-phenyl substituted pyrazoline derivative **4,** showed moderate activity against all the screened bacteria and fungi except *B. subtilis* and their potencies were weaker than their

analogue, *N*-unsubstituted pyrazoline derivative **3.** The moving a functionalized substitutions around the pyrazole moiety to the other position resulted in retention of biological activity. The moving functionalized substitutions in compound **4** around the pyrazole moiety to the other position in case of compound **8b** resulted in the highest antimicrobial activity among all the compounds investigated in this study. Compound **8b** exhibited the highest antibacterial activity against most of the organisms, compound **8b** exhibited activities near to the references drugs (Ampicillin, Gentamycin and Amphotericin B). On the other hand, the moving functionalized substitutions in compound **3** around the pyrazole moiety to the other position in case of compound **7b** did not improve the antimicrobial activity. The results depicted in Fig. 2 revealed that the compound **7b** showed moderate activity against the growth of the different organisms. Further introduction of 2-chlorophenyl at position 5 of pyrazoline, as in **7a**, had a good effect on antibacterial activity, **7a** showed strong activity against the different organisms. Compound **7a** showed better results when compared with reference drug as revealed from their inhibition zone values. It showed near the reference drug. Interestingly, unlike **7a**, their analogue, *N*-phenyl substituted pyrazoline derivative **8a,** showed moderate activity against most of the screened bacteria and fungi. Compound **8a** resulted in the lowest antimicrobial activity among all the compounds investigated in this series. Unfortunately, compound **8a** was completely inactive against *S. Epidermidis, S. flexneri* and *A. clavatus*. By investigating the biological activity of this series as antimicrobial agents (Fig. 2), it was observed that compounds **7a** and **8b** were highly active with broad spectrum activity against all the screened microbes.

Figure 2:

Concerning the antimicrobial activity of pyrazoline derivatives **10a-c**, the results displayed that pyrazoline derivatives **10a-c** were highly active against all screened organisms. The results depicted in Fig. 3 revealed that the antimicrobial activity of pyrazoline derivatives **10a-c** was nearly equipotent to the reference drug against all screened organisms (Fig. 3). Regarding the effect of changing the substituent on C-(3) of pyrazole from phenyl to chlorophenyl to *p*-tolyl (**10a** → **10b** → **10c**); the presence of chlorophenyl moiety (**10b**) resulted in the highest antibacterial activity. The presence of *p*-tolyl moiety resulted in the lowest antibacterial activity. The presence of phenyl moiety resulted in the lowest antifungal activity.

Figure 3:

Pyrazoline series **11a,b, 12a,b** and **13a,b** bearing quinolin-2-yl group at *N*-(1), amino group at position 5 of pyrazole moiety, various substituent at position 3 and others at position 4 of pyrazole moiety. The effects of each substituent at position 3 and those at position 4 on these

activities was studied and make a comparative study between them to deduce a structure activity relationship. Using the general structure provided in Fig. 3, certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

Figure 4:

Introduction of methyl group at position 3 and carbonitrile at position 4 of pyrazoline, as in **11a**, had a good effect on antibacterial activity, compound **11a** showed good activities against the different organisms. Changing the substituent at position 4 of pyrazoline from carbonitrile to ethyl carboxylate and let the substituent position 3 not changed, as in **11b**, had slight effect on antibacterial activity. Introduction of methylthio group at position 3 and carbonitrile at position 4 of pyrazoline, as in **12a**, had a good effect on antibacterial activity, compound **12a** showed good activities against the different organisms. Changing the substituent at position 4 of pyrazoline from carbonitrile to ethyl carboxylate and let the substituent position 3 not changed, as in **12b**, had slight effect on antibacterial activity against gram positive Bactria. On the other hand, this changing resulted bad effect on antibacterial activity against gram-negative bacteria. Compound **12b** showed no activities against gram-negative bacteria. Alternative, **12b** showed improve the antifungal activity. Changing the substituent at position 3 in **11a**, from methyl to methylthio and let the substituent at position 4 (carbonitrile) of pyrazoline not changed as in **12a**, had slight effect on antibacterial activity. Compound **12a** was weaker than their analogue **11a**. Changing the substituent at position 3 in **11b**, from methyl to methylthio and let the substituent at position 4 (ethyl carboxylate) of pyrazoline not changed as in **12b**, had slight effect against gram positive bactria and fungi and bad effect against gram-negative bacteria. Compound **12b** showed no activities against gram-negative bacteria. Pyrazoline series **13a,b** bear carbonitrile at position 4, and 3-chlorophenyl (or 2,4-dichlorophenyl) substituent at position 3 of pyrazole moiety. The presence of 2,4-dichlorophenyl (**13b**) resulted in the highest antimicrobial activity among all the compounds investigated in this series. The presence of 2,4-dichlorophenyl moiety exhibited the highest antibacterial activity against most of the organisms, 2,4-dichlorophenyl moiety exhibited activities near to the references drug. Unlike **13b**, their analogue, 3-chlorophenyl moiety **13a** showed moderate activity against most of the screened bacteria and fungi. Compound **13a** resulted in the lowest antimicrobial activity among all the compounds investigated in this series. Among this series, compounds **13b** showed good inhibitory activity against all the screened Gram-positive bacteria (Fig. 5). Compounds **13b** showed better results when compared with ampicillin as revealed from their inhibition activities.

Figure 5:

2.2.2. MIC of the most active compounds

The minimal inhibitory concentrations (MICs) for compounds that showed significant growth inhibition zones were determined. The synthesized compounds **7a**, **8b**, **10a-c, 11a,b**, **13b** and reference drugs was then evaluated *in vitro* using the twofold serial dilution technique [18]. The lowest concentration showing no growth was taken as the minimum inhibitory concentration. The results of minimum inhibitory concentration were reported in Table **2**.

Table 2:

Results revealed that majority of synthesized compounds showed varying degrees of inhibition against the test panel of species. The obtained antimicrobial activity of tested compounds could be correlated to structural variations and modifications. Pyrazole derivative **13b** showed better results when compared with the reference drugs as revealed from their MIC values (0.12-0.98 μg/mL). The pyrazole derivative **13b** showed fourfold potency of Gentamycin in inhibiting the growth of *S. flexneri* (MIC 0.12 μg/mL). Also, compound **13b** showed fourfold potency of Amphotericin B in inhibiting the growth of *A. clavatus* (MIC 0.49 μg/mL) and *C. albicans* (MIC 0.12 μg/mL), respectively. Compound **13b** showed twofold potency of Gentamycin in inhibiting the growth of *P. vulgaris* (MIC 0.98 μg/mL), equipotent to the Ampicillin and Amphotericin B in inhibiting the growth of *S. epidermidis* (MIC 0.49 μg/mL), *A. fumigates* (MIC 0.98 μg/mL), respectively. Pyrazole derivative **13b** displayed 50% less activity compared to Gentamycin against K. pneumonia (MIC 0.49 μg/mL). Moreover, Pyrazole derivative **13b** showed narrow spectrum of activity of Ampicillin toward S. aureus and B. subtilis on contrast to its activity toward the other organisms. The comparison between the minimal inhibitory concentrations (MICs) of compound **13b** and standard drugs against the used Gram positive, Gram negative bacteria and fungi was represented in Fig 6. Also, pyrazole derivative **8b** showed better results when compared with reference drugs as revealed from their MIC values (0.06-9.9 μg/mL). Pyrazole derivative **8b** showed twofold potency of Ampicillin in inhibiting the growth of *S. epidermidis* (MIC 0.24 μg/mL); equipotent to the Gentamycin and Amphotericin B in inhibiting the growth of *S. flexneri* (MIC 0.12 μg/mL) and *C. albicans* (MIC 0.48 μg/mL), respectively. Pyrazole derivative **8b** displayed 50% less activity compared to Amphotericin B against *A. fumigates* (MIC 1.95 μg/mL), and 25% of the potency of Ampicillin against *S. aureus* with MIC values (0.24 μg/mL). Pyrazole derivative **10c** is equipotent to Amphotericin B in inhibiting the growth of *A. fumigatus* and *C. albicans* (MIC 0.98 and 0.49 μg/mL, respectively). Pyrazole derivative **10c** displayed 50% less activity compared to Ampicillin against *S. epidermidis* (MIC 0.98 μg/mL). Pyrazole derivative **7a** showed twofold potency of Gentamycin in inhibiting the growth of *P. vulgaris* (MIC 0.97 μg/mL). pyrazole derivative **9** displayed 50% less activity compared to ampicillin against *S. epidermidis* (MIC 0.97 μg/mL).

Figure 6:

3. Conclusion

The aim of the present investigation is to synthesize different series of quinoline derivatives which bearing a pyrazole moiety at position 2- to achieve the antimicrobial effect. The results of this work clearly revealed that quinoline derivatives bearing a moiety pyrazole exhibited good antimicrobial activity. Introduction *α,β*-unsaturated ketone at position 2 of quinoline moiety decreased the antimicrobial activity. Transformation of *α,β*-unsaturated ketone group into pyrazolines increases the antimicrobial activity. It is interesting to point out that quinoline moiety incorporating substituted pyrazole moiety showed good antimicrobial activity. The type of substituent on pyrazole moiety is important. The moving a functionalized substitutions around the pyrazole moiety to the other position resulted in retention of biological activity.

4. Experimental Section

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and may be uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a JASCO spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectrometers (at 500 MHz) and are reported relative to deuterated solvent signals in deuterated dimethylsulfoxide (DMSO-d_6). ^{13}C NMR spectra were recorded on Bruker Spectrometers (at 125 MHz) in deuterated dimethylsulfoxide (DMSO-d_6). The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

4.1. Synthesis of 4-(quinolin-2-yloxy)benzaldehyde (1)

A mixture of 2-choloroquinoline (1.63 g, 0.01mol) and 4-hydroxybenzaldehyde (1.22 g, 0.01mol) were dissolved in DMF (50 mL) containing anhydrous potassium carbonate (2.0 g). The mixture was heated in a water bath for 6 h. The mixture allowed to cool and poured into crushed ice-cold water. The obtained product was collected by filtration and crystallized from ethanol to give **1**. Yield: 82%; m.p.: 106-108 °C; IR: ν/cm^{-1}: 1695 (C=O); ^{1}H NMR: 7.33 (d, 1H, *J* = 9.0 Hz, quinoline -H), 7.47 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 7.50-7.57 (m, 1H, quinoline-H), 7.66-7.68 (m, 2H, quinoline-H), 7.97 (d, 1H, *J*= 8.0 Hz, quinoline-H), 8.00 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 8.45 (d, 1H, *J*= 9.0 Hz, quinoline-H), 10.02 (s, 1H, CHO); ^{13}C NMR: 113.6, 122.3 (2C), 125.8, 126.3, 127.6, 128.3, 130.7, 131.9, 133.3 (2C), 141.4, 146.0, 159.0, 161.0, 192.3 (CHO); Anal. Calcd for $C_{16}H_{11}NO_2$ (249.26): C, 77.10; H, 4.45; N, 5.62; Found: C, 77.32; H, 4.35; N, 5.43 %.

4.2. Synthesis of 1-(4-methoxyphenyl)-3-(4-(quinolin-2-yloxy)phenyl)prop-2-en-1-one (2)

The aldehyde **1** (2.49 g, 0.01mol) and 4-methoxyacetophenone (1.50 g, 0.01mol) were taken in ethanol (25 mL), cooled 10 % aqueous NaOH solution (2.5 mL) was added to the above

solution with constant stirring, until the turbidity appears. The reaction mixture was further stirred for 2 h and left overnight. The mixture was carefully acidified using dilute hydrochloric acid to obtain solid precipitate. The obtained product was filtered, washed with water and crystallized from ethanol to give chalcone **2**. Yield: 78 %; m.p.: 154-156 °C; IR: ν/cm^{-1}: 2927 (CH-aliph.), 1658 (C=O), 1602 (olefin C=C); ^{1}H NMR: 3.88 (s, 3H, OCH_3), 7.09 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 7.30 (d, 1H, *J* = 9.0 Hz, quinoline-H), 7.35 (d, 2H, *J* = 8.0 Hz, AB Ar-H), 7.49 (s, 1H, quinoline-H), 7.66-7.68 (m, 2H, quinoline-H), 7.78 (d, 1H, *J* = 15.5 Hz, olefinic CH), 7.98-8.00 (m, 4H, Ar-H + olefinic CH), 8.20 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 8.45 (d, 1H, *J* = 8.5 Hz, quinoline-H); ^{13}C NMR: 56.1 (OCH_3), 113.5, 114.5 (2C), 122.1, 122.4 (2C), 125.6, 126.1, 127.5, 128.3, 130.6, 130.8 (2C), 131.0, 131.4 (2C), 132.0, 141.2, 142.9, 146.0, 155.6, 161.5, 163.7, 187.8 (C=O); Anal. Calcd for $C_{25}H_{19}NO_3$ (381.42): C, 78.72; H, 5.02; N, 3.67; Found: C, 78.54; H, 4.92; N, 3.47 %.

4.3. Synthesis of 2-(4-(3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)phenoxy)quinoline (3)

To a solution of the chalcone **2** (3.81 g, 0.01mol) in ethanol (60 mL), hydrazine hydrate (0.6 mL, 0.012mol) was added. The reaction mixture was heated under reflux for 4 h. After cooling the separated solid was filtered and crystallized from ethanol to give the pyrazoline **3**. Yield: 80 %; m.p.: 112-114 °C; IR: ν/cm^{-1}: 3341 (NH), 2836 (CH-aliph.), 1599 (C=N); ^{1}H NMR: 2.89 (dd, 1H, J_{AX} = 9.76 Hz, J_{AB}= 16.43 Hz, pyrazoline C_4-H_A), 3.84 (dd, 1H, J_{BX} = 7.11 Hz, J_{BA} = 16.31 Hz, pyrazoline C_4-H_B), 3.88 (s, 3H, OCH_3), 4.87 (dd, 1H, J_{AX} = 7.22 Hz, J_{BX} = 9.71 Hz, pyrazoline C_5-H_X), 6.95 (d, 2H, *J* = 9.0 Hz, AB Ar-H), 7.22-7.26 (m, 3H, Ar-H), 7.44-7.51 (m, 4H, Ar-H), 7.60 (d, 2H, *J* = 9.0 Hz, AB Ar-H), 7.64-7.68 (m, 2H, quinoline-H), 7.95 (d, 1H, *J*= 10.0 Hz, NH exchangeable by D_2O), 8.41 (d, 1H, *J*= 9.0 Hz, quinoline-H); ^{13}C NMR: 41.2 (CH_2), 55.6 (OCH_3), 63.6, 113.4, 114.5, 121.9, 125.4, 126.0, 127.0, 127.4, 127.5, 127.6, 128.3, 128.4, 130.5, 139.9, 140.9, 146.1, 149.4, 159.9, 161.8 (C=N); Anal. Calcd for $C_{25}H_{21}N_3O_2$ (395.45): C, 75.93; H, 5.35; N, 10.63; Found: C, 75.87; H, 5.26; N, 10.78 %.

4.4. Synthesis of 2-(4-(3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenoxy)quinoline (4)

A mixture of chalcone **2** (3.81 g, 0.01mol) and phenylhydrazine (1.08 g, 0.01mol) in ethanol (60 mL) was heated under reflux for 6 h. After cooling the separated solid was filtered and crystallized from ethanol to give the pyrazolinone **4**. Yield 75 %; mp 139-141 °C; IR: ν/cm^{-1}: 2913 (CH-aliph.), 1593 (C=N); ^{1}H NMR: 3.08 (m, 1H, pyrazoline C_4-H), 3.87 (s, 3H, OCH_3), 3.94 (m, 1H, pyrazoline C_4-H), 5.32 (m, 1H, pyrazoline C_5-H), 6.70 - 6.90 (m, 3H, Ar-H), 7.00 - 7.15 (m, 4H, Ar-H), 7.24 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.30-7.35 (m, 3H, Ar-H), 7.52 (m, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 7.84 (d, 2H, *J* = 7.0 Hz, Ar-H), 8.01 (d, 1H, *J* = 7.0 Hz, Ar-H), 8.54 (d, 1H, *J* = 8.0 Hz, Ar-H); ^{13}C NMR: 43.5, 55.5 (OCH_3), 63.2, 113.4, 113.4 (2C), 114.8 (2C), 118.9, 122.3 (2C), 125.5, 126.0, 127.5 (3C), 127.5 (2C), 128.2, 129.3 (2C), 129.5, 130.5, 134.9, 141.0, 144.8, 146.0, 147.2, 154.2, 158.9 (C=N), 161.6 (C=N); Anal.

Calcd for $C_{31}H_{25}N_3O_2$ (471.55): C, 78.96; H, 5.34; N, 8.91; Found: C, 78.78; H, 5.21; N, 8.72 %.

4.5. Synthesis of 1-(4-(quinolin-2-yloxy)phenyl)ethanone (5)

A mixture of 2-choloroquinoline (1.63 g, 0.01mol) and 4-hydroxyacetophenone (1.36 g, 0.01mol) were dissolved in DMF (50 mL) containing anhydrous potassium carbonate (2 g). The mixture was heated under reflux for 12 h., then allowed to cool and poured into crushed ice-cold water. The obtained product was collected by filtration and crystallized from ethanol to give **5**. Yield: 85 %; m.p.: 117-119 °C; IR: ν/cm^{-1}: 2957 (CH-alph.), 1671 (C=O); ^{1}H NMR: 2.30 (s, 3H, CH_3), 7.31 (d, 1H, *J* = 9.0 Hz, quinoline-H), 7.38 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 7.49 (s, 1H, quinoline-H), 7.66-7.68 (m, 2H, quinoline-H), 7.96 (d, 1H, *J*= 8.0 Hz, quinoline-H), 8.05 (d, 2H, *J*= 8.5 Hz, AB Ar-H), 8.45 (d, 1H, *J* = 9.0 Hz, quinoline-H); ^{13}C NMR: 27.1 (CH_3), 113.5, 121.8, 125.7, 126.2, 127.6, 128.3, 130.6, 130.7, 133.9, 141.3, 146.0, 161.2, 162.8, 197.2 (C=O); Anal. Calcd for $C_{17}H_{13}NO_2$ (263.29): C, 77.55; H, 4.98; N, 5.32; Found: C, 77.71; H, 4.82; N, 5.46 %.

4.6. General method for the preparation of chalcone derivatives 6a,b

The acetophenone derivative **5** (2.63, 0.01mol) and the selected aldehydes (namely, 2-chlorobenzaldehyde and 4-methoxybenzaldehyde) (0.01 mol) were dissolved in ethanol (25 mL) and cooled. 10 % Aqueous NaOH solution (2.5 mL) was added to the above solution with constant stirring, until the turbidity appears. The reaction mixture was further stirred for 2 h and left overnight. The mixture was carefully acidified using dilute hydrochloric acid to obtain solid precipitate. The obtained product was filtered, washed with water and crystallized from ethanol to give the chalcone derivatives **6a,b**

4.6.1. 3-(2-Chlorophenyl)-1-(4-(quinolin-2-yloxy)phenyl)prop-2-en-1-one (6a): Yield: 77%; m.p.: 125-127 °C; IR: ν/cm^{-1}: 1657 (C=O), 1602 (olefin C=C); ^{1}H NMR: 7.30 (d, 1H, *J* = 9.5 Hz, quinoline-H), 7.43-7.54 (m, 5H, Ar-H), 7.57 (d, 1H, *J* = 7.0 Hz, olefinic CH), 7.66-7.68 (m, 2H, quinoline-H), 7.96 (d, 1H, *J* = 8.0 Hz, quinoline-H), 8.04-8.15 (m, 2H, Ar-H), 8.22 (d, 1H, *J* = 9.0, olefinic CH), 8.30 (d, 2H, *J* = 8.0 Hz, AB Ar-H), 8.46 (d, 1H, *J* = 8.5 Hz, quinoline-H); ^{13}C NMR: 113.6, 121.9, 125.2, 125.8, 126.3, 127.6, 128.1, 128.3, 129.1, 130.5, 130.7, 131.2, 132.4, 132.8, 134.2, 134.9, 138.9, 141.3, 146.0, 158.2, 161.1, 188.2 (C=O); Anal. Calcd for $C_{24}H_{16}ClNO_2$ (385.84): C, 74.71; H, 4.18, N, 3.63; Found: C, 74.58; H, 4.23, N, 3.47 %.

4.6.2. 3-(4-Methoxyphenyl)-1-(4-(quinolin-2-yloxy)phenyl)prop-2-en-1-one (6b): Yield: 67 %; m.p.: 128-130 °C; IR: ν/cm^{-1}: 2923 (CH-aliph.), 1652 (C=O), 1593 (olefin C=C); ^{1}H NMR: 3.83 (s, 3H, OCH_3), 7.02 (d, 2H, *J* = 8.5 Hz, AB Ar-H), 7.32 (d, 1H, *J* = 9.0 Hz, quinoline-H), 7.43 (d, 2H, *J* = 8.0 Hz, AB Ar-H), 7.49-7.51 (m, 1H, quinoline-H), 7.66-7.68 (m, 2H, quinoline-H), 7.77 (d, 1H, *J* = 14.3 Hz, olefinic CH), 7.85-7.92 (m, 3H, Ar-H + olefinic CH), 8.05 (d, 1H, *J* = 8.5 Hz, quinoline- H), 8.20 (d, 2H, *J* = 8.0 Hz, AB Ar-H), 8.48 (d, 1H, *J*= 8.5 Hz, quinoline-H); ^{13}C NMR: 55.9 (OCH_3), 113.6, 114.9, 119.9, 121.9, 125.8,

126.2, 127.6, 127.8, 128.3, 130.7, 130.9, 131.3, 134.8, 141.3, 144.4, 146.0, 157.8, 161.2, 161.9, 188.3 (C=O); Anal. Calcd for $C_{25}H_{19}NO_3$ (381.42): C, 78.72; H, 5.02; N, 3.67; Found: C, 78.64; H, 4.98; N, 3.73 %.

4.7. General method for the preparation of 4,5-dihydropyrazole derivatives 7a,b

To a solution of the chalcone derivatives **6a** or **6b** (0.01mol) in ethanol (60 mL), hydrazine hydrate (0.6 g, 0.012mol) was added. The reaction mixture was heated under reflux for 4 h. After cooling the separated solid was filtered and crystallized from ethanol.

4.7.1. 2-(4-(5-(2-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)quinoline (7a): Yield: 80 %; m.p.: 102-104 °C; IR: ν/cm^{-1}: 3317 (NH), 1594 (C=N); ^{1}H NMR: 2.89 (s, 1H, pyrazoline C_4-H_A), 3.80 (s, 1H, pyrazoline C_4-H_B), 4.87 (s, 1H, pyrazoline C_5-H_X), 7.00-7.51 (m, 9H, Ar-H), 7.60-7.68 (m, 4H, Ar-H), 7.87 (d, 1H, *J* = 10.0 Hz, NH exchangeable by D_2O), 8.45 (d, 1H, *J* = 9.0 Hz, quinoline-H); ^{13}C NMR: 41.1 (CH_2), 63.5, 113.7, 114.2, 121.5, 125.3, 126.1, 127.0, 127.3, 127.8, 127.6, 128.3, 128.9, 130.5, 139.9, 141.1, 146.1, 149.5, 160.2, 161.7; Anal. Calcd for $C_{24}H_{18}ClN_3O$ (399.87): C, 72.09; H, 4.54; N, 10.51; Found: C, 72.23; H, 4.42; N, 10.72 %.

4.7.2. 2-(4-(5-(4-Methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)quinoline (7b): Yield: 82%; m.p.: 129-131 °C; IR: ν/cm^{-1}: 3330 (NH), 1558 (C=N); ^{1}H NMR: 2.92 (s, 1H, pyrazoline C_4-H_A), 3.82 (s, 1H, pyrazoline C_4-H_B), 3.88 (s, 3H, OCH_3), 4.81 (s, 1H, pyrazoline C_5-H_X), 7.00-7.26 (m, 5H, Ar-H), 7.40-7.50 (m, 4H, Ar-H), 7.62 (d, 2H, *J* = 9.0 Hz, AB Ar-H), 7.60-7.70 (m, 2H, quinoline-H), 7.93 (d, 1H, *J* = 10.0 Hz, NH), 8.44 (d, 1H, *J* = 9.0 Hz, quinoline-H); ^{13}C NMR: 41.3, 55.5 (OCH_3), 63.4, 113.1, 114.4 (2C), 121.8 (2C), 125.2, 125.7, 126.8 (2C), 127.3, 127.6, 127.5, 128.2, 128.3 (2C), 130.6, 139.8, 140.8, 146.0, 149.3, 159.8 (C=N), 161.8 (C=N); Anal. Calcd for $C_{25}H_{21}N_3O_2$ (395.45): C, 75.93; H, 5.35; N, 10.63; Found: C, 76.08; H, 5.21; N, 10.74 %.

4.8. General method for the preparation of 4,5-dihydropyrazole derivatives 8a,b

To a solution of the chalcone derivatives **6a** or **6b** (0.01mol) in ethanol (60 mL), phenylhydrazine (1.08mol) was added. The reaction mixture was heated under reflux for 8 h. After cooling the separated solid was filtered and recrystallized from ethanol to give 4,5 dihydropyrazole derivatives **8a,b**

4.8.1. 2-(4-(5-(2-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)quinoline (8a): Yield: 75 %; m.p.: 175-177 °C; IR: ν/cm^{-1}: 1591 (C=N); ^{1}H NMR: 3.13 (dd, 1H, J_{AX} = 9.76 Hz, J_{AB} = 16.43 Hz, pyrazoline C_4-H_A), 4.07 (dd, 1H, J_{BX} = 7.11 Hz, J_{BA} = 16.31 Hz, pyrazoline C_4-H_B), 5.78 (dd, 1H, J_{AX} = 7.22 Hz, J_{BX} = 9.71 Hz, pyrazoline C_5-H_X), 6.75 (t, 1H, *J* = 8.5 Hz, Ar-H), 6.95 (d, 2H, *J* = 9.0 Hz, Ar-H),7.15-7.36 (m, 8H, Ar-H), 7.51 (t, 1H, *J* = 8.5 Hz, Ar-H), 7.56 (d, 1H, Ar-H), 7.64-7.68 (m, 2H, Ar-H), 7.84 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.95 (d, 1H, *J* = 10.0 Hz, Ar-H), 8.42 (d, 1H, *J* = 9.0 Hz, Ar-H); ^{13}C NMR: 19.0, 56.5, 113.1, 113.4, 122.3, 125.5, 127.5, 127.7, 128.3, 128.4, 129.6, 129.8, 130.6, 141.1, 144.8,

146.1, 147.2, 154.2, 159.0, 161.7 (C=N); Anal. Calcd for $C_{30}H_{22}ClN_3O$ (475.97): C, 75.70; H, 4.66; N, 8.83; Found: C, 75.62; H, 4.61; N, 8.93 %.

4.8.2. 2-(4-(5-(4-Methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenoxy)quinoline (8b): Yield: 77 %; m.p.: 145-147 °C; IR: ν/cm^{-1}: 2835 (CH-aliph.), 1597 (C=N); ^{1}H NMR: 3.10 (dd, 1H, J_{AX} = 9.76 Hz, J_{AB} =16.43 Hz, pyrazoline C_4-H_A), 3.87 (s, 3H, OCH_3), 3.90 (dd, 1H, J_{BX} = 7.11 Hz, J_{BA}= 16.31 Hz, pyrazoline C_4-H_B), 5.44 (dd, 1H, J_{AX} = 7.22 Hz, J_{BX} = 9.71 Hz, pyrazoline C_5-H_X), 6.72 (s, 1H, Ar-H), 6.90 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.03 (d, 2H, *J*= 7.0 Hz, Ar-H), 7.16-7-20 (m, 2H, Ar-H), 7.23 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.29-7.32 (m, 3H, Ar-H), 7.50 (s, 1H, Ar-H), 7.66-7.69 (m, 2H, Ar-H), 7.82 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.95 (d, 1H, *J* =7.0 Hz, Ar-H), 8.42 (d, 1H, *J* = 8.0 Hz, Ar-H); ^{13}C NMR: 55.5 (OCH_3), 63.3, 113.4, 113.5, 114.8, 119.0, 122.3, 125.5, 126.1, 127.5, 127.6, 128.3, 129.3, 129.6, 130.6, 134.9, 141.1, 144.8, 146.1, 147.2, 154.2, 159.0, 161.7 (C=N); Anal. Calcd for $C_{31}H_{25}N_3O_2$ (471.55): C, 78.96; H, 5.34; N, 8.91; Found: C, 79.12; H, 5.41; N, 8.87 %.

4.9. General procedure for the condensation of the 2-hydrazinylquinoline 9 with formylpyrazoles to afford 10a-c

To a solution of the 2-hydrazinylquinoline **9** [15] (1.45g, 0.01mol) in ethanol (30 mL), formylpyrazoles (0.01 mol) were added and the reaction mixture was heated under reflux for 3 h. The solid obtained upon cooling was collected by filtration, dried and crystallized from dioxane.

4.9.1. 2-(2-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)quinoline (10a): Yield: 87 %; m.p.: 130-132 °C; IR: ν/cm^{-1}: 3162 (NH), 1608 (C=N); ^{1}H NMR: 7.28 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.37 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.48 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.53-7.63 (m, 7H, Ar-H), 7.76-7.80 (m, 3H, Ar-H), 8.01 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.14 (d, 1H, *J* = 9.0 Hz, Ar-H), 8.28 (s, 1H, Ar-H), 9.01 (s, 1H, CH=N), 11.12 (br, 1H, NH exchangeable by D_2O); ^{13}C NMR: 110.1, 118.5, 119.0, 122.8, 124.6, 126.2, 127.2, 127.3, 128.3, 128.9, 129.0, 130.0, 130.1, 133.1, 133.1, 138.2, 139.7, 147.7, 151.2, 156.3 (C=N); Anal. Calcd for $C_{25}H_{19}N_5$ (389.45): C, 77.10; H, 4.92; N, 17.98; Found: C, 77.23; H, 4.82; N, 17.91 %.

4.9.2. 2-(2-((3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)quinoline (10b): Yield: 88 %; m.p.: 295-297 °C; IR: ν/cm^{-1}: 3174 (NH), 1671 (C=N); ^{1}H NMR: 7.20-7.50 (m, 3H, Ar-H), 7.50-7.60 (m, 7H, Ar-H), 7.76-7.80 (m, 3H, Ar-H), 8.03-8.15 (m, 2H, Ar-H), 8.31 (s, 1H, Ar-H), 9.04 (s, 1H, CH=N), 11.17 (br, 1H, NH exchangeable by D_2O); ^{13}C NMR: 110.0, 118.4, 119.2 (2C), 122.6, 124.4, 126.0, 127.1, 127.3, 128.4, 128.8 (2C), 129.2 (2C), 130.0 (2C), 130.5, 132.0, 133.1, 133.7, 138.2, 139.7, 147.7, 151.2 (C=N), 156.3 (C=N); Anal. Calcd for $C_{25}H_{18}ClN_5$ (423.90): C, 70.84; H, 4.28; N, 16.52; Found: C, 70.76; H, 4.24; N, 16.45 %.

4.9.3. 2-(2-((1-Phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)quinoline (10c): Yield: 80 %; m.p.: 151-153 °C; IR: ν/cm^{-1}: 3183 (NH), 1603 (C=N); ^{1}H NMR: 2.01 (s, 3H, CH_3), 7.30-7.37 (m, 2H, Ar-H), 7.43-7.63 (m, 8H, Ar-H), 7.73-7.82 (m, 3H, Ar-H), 8.03-8.17

(m, 2H, Ar-H), 8.28 (s, 1H, Ar-H), 9.05 (s, 1H, CH=N), 11.17 (br, 1H, NH exchangeable by D_2O); ^{13}C NMR: 21.2, 110.1, 118.5, 119.3 (2C), 122.7, 124.2, 126.1, 127.4, 127.2, 128.1, 128.9 (2C), 129.2 (2C), 130.1 (2C), 130.8, 132.1, 133.5, 133.2, 138.4, 139.6, 147.4, 151.0 (C=N), 156.7 (C=N); Anal. Calcd for $C_{26}H_{21}N_5$ (403.48): C, 77.40; H, 5.25; N, 17.36; Found: C, 77.54; H, 5.31; N, 17.46 %.

4.10. General procedure of synthesis of ethyl 5-amino-3-(methylthio)-1*H*-pyrazole derivatives 11a,b

To a solution of 2-hydrazinylquinoline **9** (1.45g, 0.01mol) in ethanol (50 mL), 2-(1-ethoxyethylidene)malononitrile or 2-(1-ethoxyethylidene)ethyl cyanoacetate (0.01mol) were added and the reaction mixture was heated under reflux for 4 h. The solid obtained upon cooling was collected by filtration, dried and crystallized from butanol.

4.10.1. 5-Amino-3-methyl-1-(quinolin-2-yl)-1*H*-pyrazole-4-carbonitrile (11a): Yield: 78 %; m.p.: 246-248 °C; IR: ν/cm^{-1}: 3368, 3272 (NH_2), 2918 (CH-aliph.), 2218 (C≡N); ^1H NMR: 2.24 (s, 3H, CH_3), 7.59 (s, 1H, quinoline-H), 7.79 (s, 1H, quinoline-H), 8.00-8.11 (m, 3H, quinoline-H), 8.40-8.50 (m, 3H, quinoline-H + NH_2 exchangeable by D_2O); ^{13}C NMR: 13.2, 74.3, 113.2 (2C), 126.0, 126.7, 128.1, 128.4, 131.2 (2C), 140.3, 145.4, 151.7, 154.2; MS (m/z: %): 249 (M+, 100), 250 (M+1, 20.7), 242 (M-NH_2, 41.9), 184 (24.7), 143 (26.8), 129 (59.1) 102 (36.8), 77 (34.58); Anal. Calcd for $C_{14}H_{11}N_5$ (249.27): C, 67.46; H, 4.45; N, 28.10; Found: C, 67.57; H, 4.38; N, 28.27 %.

4.10.2. Ethyl 5-amino-3-methyl-1-(quinolin-2-yl)-1*H*-pyrazole-4-carboxylate (11b): Yield: 74 %; m.p.: 154-156 °C; IR: ν/cm^{-1}: 3429, 3301 (NH_2), 2967, 2922 (CH-aliph.), 1668 (C=O); ^1H NMR: 1.23 (t, 3H, *J* = 6.9 Hz, CH_3-ester), 2.34 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 7.0 Hz, CH_2-ester), 7.59 (s, 1H, quinoline-H), 7.81 (s, 1H, quinoline-H), 8.00-8.11 (m, 4H, quinoline-H), 8.52 (br, 2H, NH_2 exchangeable by D_2O); ^{13}C NMR: 14.9 (CH_3), 15.1 (CH_3 ester), 59.5 (CH_2), 93.4, 113.2, 126.0, 126.5, 127.9, 128.4, 131.2, 140.2, 145.4, 151.0, 153.2 (C=O); MS (m/z: %): 296 (M^+, 86), 250 (M-$COOC_2H_5$, 20.7), Anal. Calcd for $C_{16}H_{16}N_4O_2$ (296.32): C, 64.85; H, 5.44; N, 18.91; Found: C, 64.74; H, 5.42; N, 18.78 %.

4.11. General procedure of synthesis of ethyl 5-amino-3-(methylthio)-1*H*-pyrazole derivatives 12a,b

To a solution of 2-hydrazinylquinoline **9** (1.45g, 0.01mol) in ethanol (50 mL), substituted ketene dithioacetals (0.01 mol) were added and the reaction mixture was heated under reflux for 4 h. The solid obtained upon cooling was collected by filtration, dried and crystallized from butanol.

4.11.1. 5-Amino-3-(methylthio)-1-(quinolin-2-yl)-1*H*-pyrazole-4-carbonitrile (12a): Yield: 80 %; m.p.: 233-235 °C; IR: ν/cm^{-1}: 3367, 3270 (NH_2), 2915 (CH-aliph.), 2217 (C≡N); ^1H NMR: 2.61 (s, 3H, SCH_3), 7.58 (t, 1H, *J* = 7.5 Hz, quinoline-H), 7.78 (t, 1H, *J* = 7.5 Hz, quinoline-H), 7.97 (d, 1H, *J* = 8.0 Hz, quinoline-H), 8.01 (d, 1H, *J*= 8.5 Hz,

quinoline-H), 8.47-8.52 (m, 4H, quinoline-H + NH_2 exchangeable by D_2O); ^{13}C NMR: 13.4 (CH_3), 73.5 (C=C-pyrazole), 113.1, 114.1, 126.0, 126.7, 128.1, 128.4, 131.2, 140.4, 145.2, 151.3, 152.5, 154.8; MS (m/z: %): 281 (M+, 79.4), 250 (M+1, 14.7), 234 (20.4), 144 (29.7), 143 (26.8), 128 (100) 109 (62.8), 77 (36.8); Anal. Calcd for $C_{14}H_{11}N_5S$ (281.34): C, 59.77; H, 3.94; N, 24.89; Found: C, 59.62; H, 3.89; N, 25.01 %.

4.11.2. Ethyl 5-amino-3-(methylthio)-1-(quinolin-2-yl)-1*H*-pyrazole-4-carbo-xylate (12b): Yield: 82 %; m.p.: 124-126 °C; IR: ν/cm^{-1}: 3439, 3311 (NH_2), 2977 (CH-aliph.), 1691 (C=O); ^1H NMR: 1.31 (t, 3H, *J* = 6.5 Hz, CH_3-ester), 2.54 (s, 3H, SCH_3), 4.24 (q, 2H, *J* = 6.5 Hz, CH_2-ester), 7.57 (t, 1H, *J* = 8.0 Hz, quinoline-H), 7.79 (t, 1H, *J*= 7.5 Hz, quinoline-H), 7.97 (d, 1H, *J* = 8.0 Hz, quinoline-H), 8.05 (d, 1H, *J*= 8.5 Hz, quinoline-H), 8.03-8.07 (m, 3H, quinoline-H + NH_2 exchangeable by D_2O), 8.52 (d, 1H, *J* = 9.0 Hz, quinoline-H); ^{13}C NMR: 12.8 (CH_3), 14.9 (CH_3), 59.7 (CH_2), 92.8 (C=C-pyrazole), 113.1, 126.0, 126.5, 127.9, 128.4, 131.2, 140.2, 145.3, 151.2, 152.8, 153.5 (C=N), 163.4 (C=O); Anal. Calcd for $C_{16}H_{16}N_4O_2S$ (328.39): C, 58.52; H, 4.91; N, 17.06; Found: C, 58.63; H, 4.93; N, 17.12 %.

4.12. General procedure of synthesis of ethyl 5-amino-3-(methylthio)-1*H*-pyrazole derivatives 13a,b

To a solution of 2-hydrazinylquinoline **9** (1.45g, 0.01mol) in ethanol (50 mL), aryledine derivatives (0.01mol) were added and the reaction mixture was heated under reflux for 4 h. The solid obtained upon cooling was collected by filtration, dried and recrystallized from butanol.

4.12.1. 5-Amino-3-(3-chlorophenyl)-1-(quinolin-2-yl)-1*H*-pyrazole-4-carbonitrile (13a): Yield: 75 %; m.p.: 211-213 °C; IR: ν/cm^{-1}: 3378, 3282 (NH_2), 2213 (C≡N); ^1H NMR: 7.56-7.60 (m, 3H, Ar-H), 7.80 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.92 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 8.00 (d, 1H, *J*= 8.0 Hz, Ar-H), 8.14 (d, 1H, *J* = 8.5 Hz, Ar-H), 8.18 (d, 1H, *J* = 9.0 Hz, Ar-H), 8.51-8.59 (m, 3H, Ar-H + NH_2); ^{13}C NMR: 71.8, 113.4, 115.4, 125.3, 126.3, 127.0, 128.2, 128.4, 130.0, 131.3, 131.3, 133.1, 134.1, 140.5, 145.2, 149.6, 152.7, 155.5 (2C=N); MS (m/z: %): 345 (M^+, 76): Anal. Calcd for $C_{19}H_{12}ClN_5$ (345.79): C, 66.00; H, 3.50; N, 20.25; Found: C, 66.12; H, 3.43; N, 20.37 %

.

4.12.2.5- Amino-3-(2,4-dichlorophenyl)-1-(quinolin-2-yl)-1*H*-pyrazole-4-carbonitrile (13b): Yield: 77 %; m.p.: 116-118 °C; IR: ν/cm^{-1}: 3359, 3230 (NH_2), 2200 (C≡N); ^1H NMR: 7.50-7.90 (m, 5H, Ar-H), 8.00-8.20 (m, 3H, Ar-H), 8.50-8.60 (m, 3H, Ar-H + NH_2 exchangeable by D_2O); ^{13}C NMR: 12.8 (CH_3), 14.9 (CH_3), 59.7 (CH_2), 92.8 (C=C-pyrazole), 113.1, 126.0, 126.5, 127.9, 128.4, 131.2, 140.2, 145.3, 151.2, 152.8 (C=N), 153.5 (C=N), 163.4 (C=O); Anal. Calcd for $C_{19}H_{11}Cl_2N_5$ (380.23): C, 60.02; H, 2.92; N, 18.42; Found: C, 60.14; H, 2.87; N, 18.54 %.

4.13. Antimicrobial activity

Chemical compounds were individually tested against a panel of gram positive, gram negative bacterial pathogens and fungi. Antimicrobial tests were carried out by the agar well diffusion method using 100 μL of suspension containing 1 x108 CFU/mL of pathological tested bacteria and 1 x106 CFU/mL of fungi spread on nutrient agar and Sabouraud dextrose agar media, respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made and loaded with 100 μL of tested compound solution prepared by dissolving 5 mg of the chemical compound in one mL of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C for bacteria, and 72 h at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicillin, Gentamycin and Amphotericin B (1 mg/mL) were used as standard for antibacterial and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

4.14. Minimal inhibitory concentration (MIC) measurement

The Minimum Inhibitory Concentration was determined by the macro-tube dilution technique following the guidelines of the National Committee for Clinical Laboratory Standards for bacteria and for fungi [18]. The bacteria and fungi were maintained on nutrient broth medium and malt extract broth medium, respectively. In this method 15 test tubes were utilized for each microorganism, where the first one was charged with 2.0 mL of DMSO and 10 mg of the tested compound. To the remaining tubes was added 1.0 mL of sterile broth medium. Subsequently, 1.0 mL was transferred from the first tube to the second one, followed by continuous dilutions in this manner to the 14^{th} tube, discarding 1.0 mL from the latter. The 15^{th} tube served as a control. Several colonies of tested culture were suspended to an appropriate turbidity in 5.0 mL of broth medium. Further dilution by transferring 0.2 mL of the suspension into 40 mL was done. 1.0 mL of the diluted culture suspension was then added to each of the tubes. Finally, Incubation at 37 °C overnight for bacteria and 30 °C and 72 h for fungi was performed. The tubes were examined for visible signs of microbial and fungal growth, where the highest dilution without growth is the MIC.

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Figure captions:

Scheme 1: Syntheses of the chalcones **2** and **6a,b**, and pyrazoline **3**,**4**, **7a,b** and **8a,b** derivatives. Reagents and reactions conditions: (a) 4-hydroxybenzaldehyde, DMF, K_2CO_3, 100 °C 6 h; (b) 4-methoxyacetophenone, ethanol, 10 % aq. NaOH, stirring 2 h; (c) hydrazine hydrate, ethanol, reflux 4 h; (d) phenylhydrazine ethanol, reflux 6 h; (e) 4-hydroxyacetophenone, DMF, K_2CO_3, reflux 12 h; (f) aldehydes (namely, 2-chlorobenzaldehyde and 4-methoxybenzaldehyde), ethanol, 10 % aq. NaOH, stirring 2 h.

Scheme 2: Synthesis of pyrazole derivatives **10a-c**; Reagents and reactions conditions: (a) hydrazine hydrate, ethanol, reflux, 12 h; (b) formylpyrazoles, ethanol, reflux 3 h.

Scheme 3: Synthesis of pyrazole derivatives **11-13;** reactions conditions: ethanol, reflux, 4 h.

Figure 1: General formula of the synthesized compounds **3**,**4**, **7a,b** and **8a**,**b**
Figure 2: The comparison between the antibacterial and antifungal activities of synthesized compounds **2-4**, **7a,b**, **8a,b** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi
Figure 3: The comparison between the antibacterial and antifungal activities of synthesized compounds **10a-c** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi
Figure 4: General formula of the synthesized compounds **11a,b**, **12a,b**, **13a,b**
Figure 5: The comparison between the antibacterial and antifungal activities of synthesized compounds **11a,b**, **12a,b**, **13a,b** and standard drugs
Figure 6: The comparison between the minimal inhibitory concentrations of compound **13b** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi
Table 1: Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay
Table 2: Minimum inhibitory concentration (µg/mL) of the more potent synthesized compounds against the pathological organisms

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Table 1: Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay

Compd. No	Gram +ve bacteria			Gram -ve bacteria			Fungi		
	S. aureus	*S. epidermidis*	*B. subtilis*	*P. vulgaris*	*K. pneumonia*	*S. flexneri*	*A. fumigatus*	*A. clavatus*	*C. albicans*
2	8.7 ±0.5	10.6±0.4	0.0	0.0	7.2±0.16	0.0	10.7±0.62	11.5±0.42	0.0
3	16.2 ±0.5	18.6±0.7	19.4±0.35	16.2±0.52	0.0	15.4±0.64	15.6±0.21	12.8±0.53	11.6±0.35
4	12.1 ±0.3	13.11±0.1	0.0	13.5±0.55	15.92±0.76	14.4±0.33	16.2±0.24	14.5±0.12	11.6±0.52
7a	23.9 ±0.4	25.3±0.3	24.2±0.61	23.6±0.17	22.8±0.28	23.6±0.32	21.4±0.36	17.5±0.28	18.5±0.24
7b	13.1±0.3	14.6±0.5	13.7±0.53	14.3±0.32	16.2±0.55	14.3±0.42	13.5±0.54	14.2±0.52	12.1±0.34
8a	14.8±0.3	0.0	16.4±0.25	12.4±0.63	13.3±0.50	0.0	12.3±0.52	0.0	9.3±0.24
8b	29.0±0.2	26.2±0.3	28.7±0.24	24.5±0.38	26.1±0.24	23.3±0.32	23.5±0.36	22.9±0.35	23.5±0.19
10a	22.2±0.5	23.4±0.5	23.2±0.31	20.2±0.18	21.2±0.25	20.1±0.33	17.5±0.35	18.4±0.35	19.6±0.45
10b	24.5±0.6	25.7±0.6	27.6±0.34	21.4±0.76	22.9±0.63	21.8±0.34	21.5±0.36	19.8±0.25	24.9±0.43
10c	20.3±0.6	20.7 ± 0.2	22.4 ± .036	16.2 ± .058	17.6 ± 0.58	18.3 ± 1.2	20.5 ± 0.22	18.3 ± 0.26	22.1 ± 0.15
11a	19.2 ± 0.2	20.6 ± 0.23	20.9 ± 0.63	11.6 ± 0.43	15.7 ± 0.56	15.9 ± 0.77	16.8 ± 025	18.3 ± 0.44	20.4 ± 0.63
11b	20.3± 0.5	17.3 ± 0.6	20.2 ± 0.44	13.6 ± 0.37	15.9 ± 0.37	16.8 ± 0.37	16.3 ± 0.44	18.6 ± 0.58	19.8 ± 0.25
12a	18.9 ± 0.1	16.2 ± 0.2	19.8 ± 0.42	12.3 ± 0.53	15.3 ± 0.53	16.2 ± 0.53	15.7 ± 0.33	15.9 ± 0.25	16.8 ± 0.34
12b	15.8± 0.4	17.8 ± 0.2	19.8 ± 0.22	0	0	0	18.7 ± 0.11	19.3 ± 0.23	20.3 ± 0.27
13a	10.3 ± 0.5	11.6 ± 0.6	13.3 ± 0.72	0	0	0	12.3 ± 0.25	13.2 ± 0.25	15.2 ± 0.58
13b	21.6 ± 0.2	22.3 ± 0.3	25.8 ± 0.25	20.6 ± 0.25	21.6 ± 0.19	23.2 ± 0.42	20.9 ± 0.25	22.3 ± 0.25	24.8 ± 0.58
Ampicillin	28.9±0.14	25.4±0.2	34.6±0.35	-----	-----	-----	-----	-----	-----
Gentamycin	-----	-----	-----	23.4±0.3	26.3±0.15	24.8±0.24	-----	-----	-----
Amphotericin B	-----	-----	-----	-----	-----	-----	23.7±0.10	21.9±0.12	26.4±0.20

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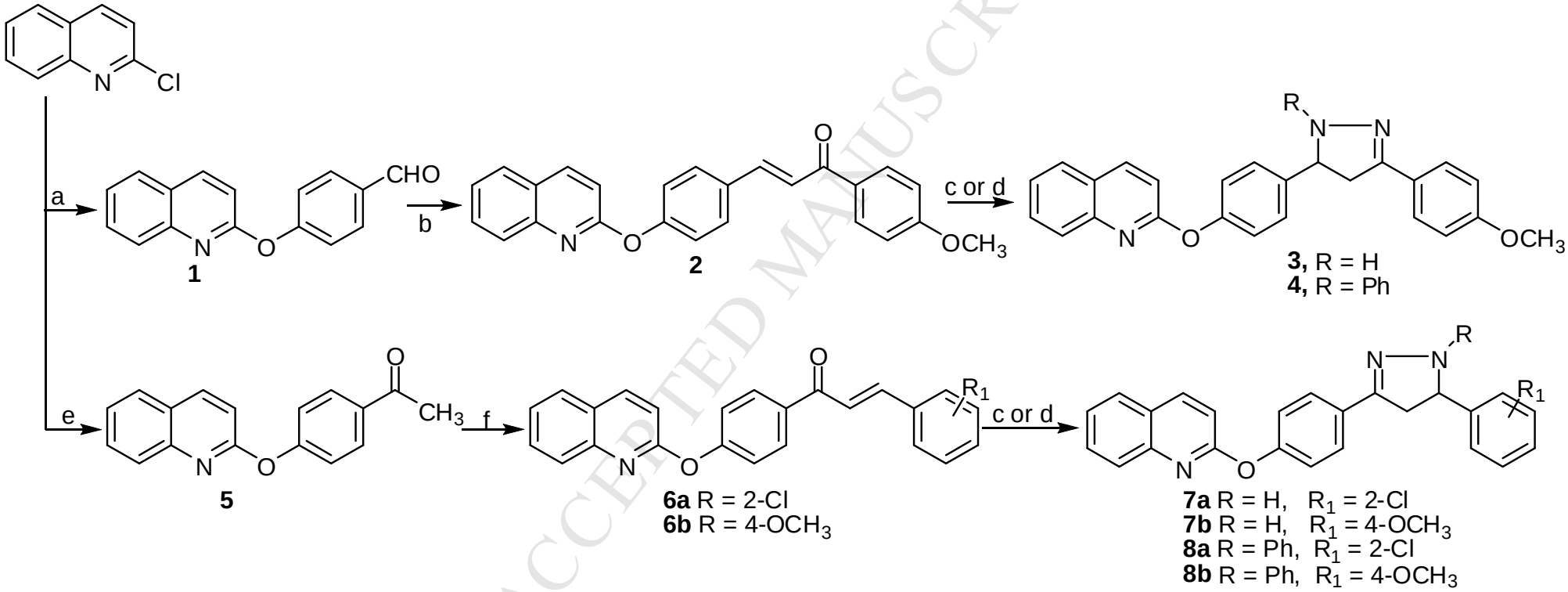
Table 2: Minimum inhibitory concentration (µg/mL) of the more potent synthesized compounds against the pathological organisms

Compd. No	Gram +ve bacteria			Gram -ve bacteria			Fungi		
	S. aureus	*S. epidermidis*	*B. subtilis*	*P. vulgaris*	*K. pneumonia*	*S. flexneri*	*A. fumigatus*	*A. clavatus*	*C. albicans*
7a	0.97	0.97	1.95	0.97	1.95	1.95	7.81	15.63	1.95
8b	0.24	0.24	0.06	9.9	3.9	0.48	1.95	3.9	0.48
10a	3.9	3.9	1.97	7.81	3.9	7.81	7.81	15.63	7.81
10b	0.97	3.9	0.97	3.9	3.9	3.9	15.63	7.81	3.9
10c	1.95	0.98	0.49	31.25	7.81	7.81	0.98	7.81	0.49
11a	3.9	0.98	0.98	500	31.25	31.25	15.63	7.8	1.95
11b	1.95	15.63	1.95	125	31.25	15.63	31.25	3.9	1.95
12b	-----	-----	-----	-----	-----	-----	7.81	7.81	1.95
13b	0.49	0.49	0.12	0.98	0.49	0.12	0.98	0.49	0.12
Ampicillin	0.06	0.48	0.007	-----	-----	-----	-----	-----	-----
Gentamycin	-----	-----	-----	1.95	0.24	0.48	-----	-----	-----
Amphotericin B	-----	-----	-----	-----	-----	-----	0.97	1.95	0.48

Scheme 1: Syntheses of the chalcones **2** and **6a,b**, and pyrazoline **3**,**4**, **7a,b** and **8a,b** derivatives. Reagents and reactions conditions: (a) 4-hydroxybenzaldehyde, DMF, K_2CO_3, 100 °C 6 h; (b) 4-methoxyacetophenone, ethanol, 10 % aq. NaOH, stirring 2 h; (c) hydrazine hydrate, ethanol, reflux 4 h; (d) phenylhydrazine ethanol, reflux 6 h; (e) 4-hydroxyacetophenone, DMF, K_2CO_3, reflux 12 h; (f) aldehydes (namely, 2-chlorobenzaldehyde and 4-methoxybenzaldehyde), ethanol, 10 % aq. NaOH, stirring 2 h.



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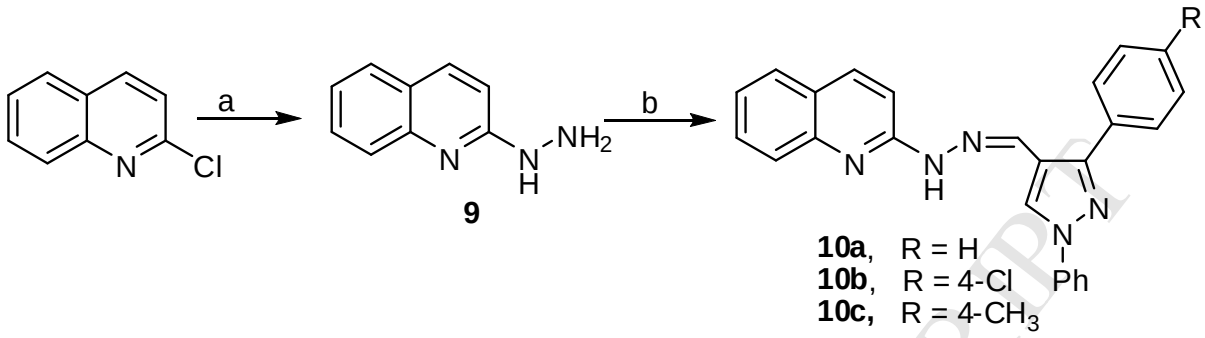
Scheme 2: Synthesis of pyrazole derivatives **10a-c**; Reagents and reactions conditions: (a) hydrazine hydrate, ethanol, reflux, 12 h; (b) formylpyrazoles, ethanol, reflux 3 h.



Scheme 3: Synthesis of pyrazole derivatives **11-13;** reactions conditions: ethanol, reflux, 4 h.

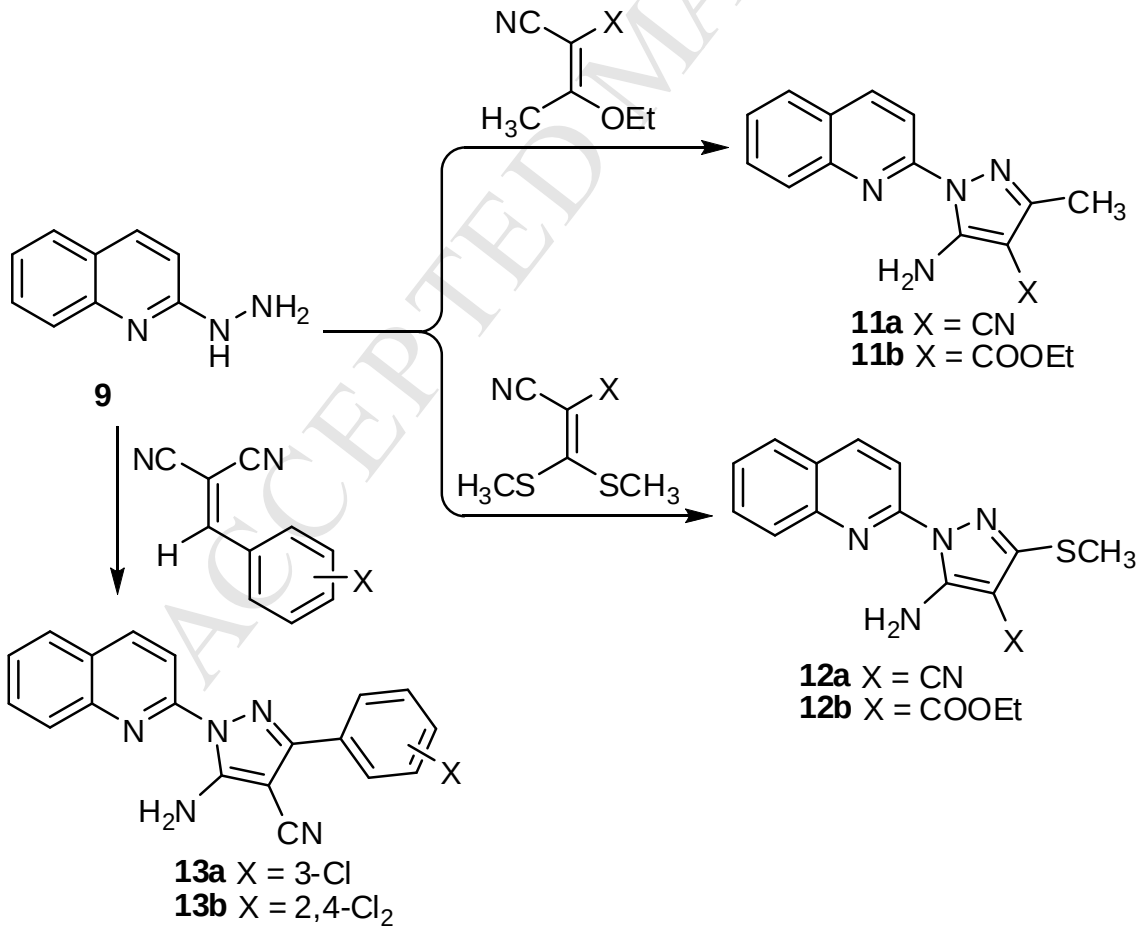


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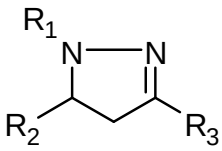
Figure 1: General formula of the synthesized compounds **3**,**4**, **7a,b** and **8a**,**b**



3, R_1 = H, R_2 = 2-phenoxyquinoline, R_3 = 4-methoxyphenyl
4, R_1 = Ph, R_2 = 2-phenoxyquinoline, R_3 = 4-methoxyphenyl
7a, R_1 = H, R_2 = 2-chlorophenyl R_3 = 2-phenoxyquinoline,
7b, R_1 = H, R_2 = 4-methoxyphenyl R_3 = 2-phenoxyquinoline,
8a, R_1 = Ph, R_2 = 2-chlorophenyl R_3 = 2-phenoxyquinoline,
8b, R_1 = Ph, R_2 = 4-methoxyphenyl R_3 = 2-phenoxyquinoline,

Figure 2: The comparison between the antibacterial and antifungal activities of synthesized compounds **2-4**, **7a,b**, **8a,b** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi.

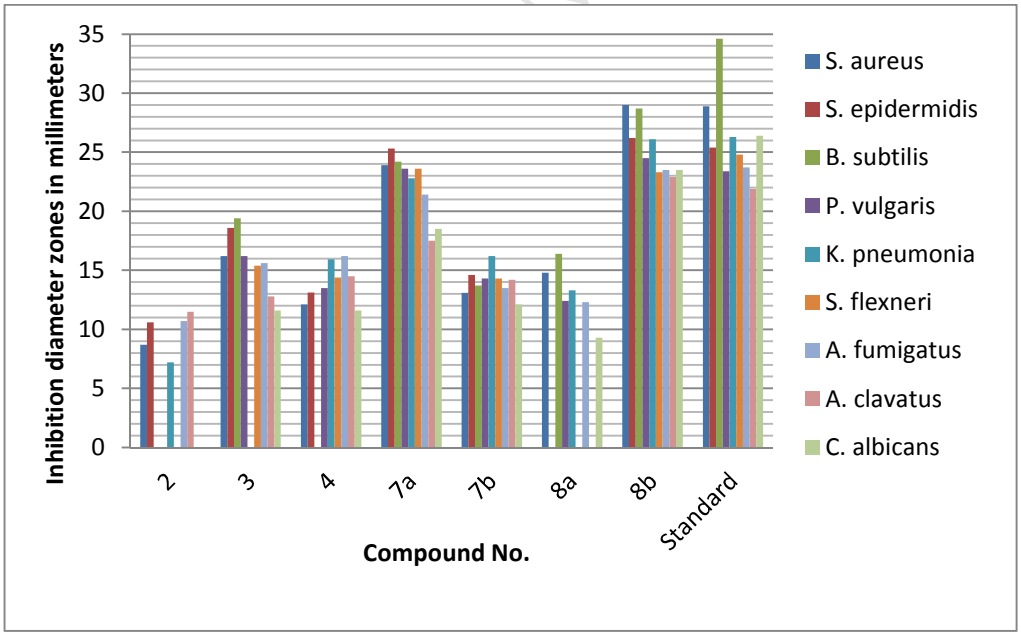


Figure 3: The comparison between the antibacterial and antifungal activities of synthesized compounds **10a-c** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi.

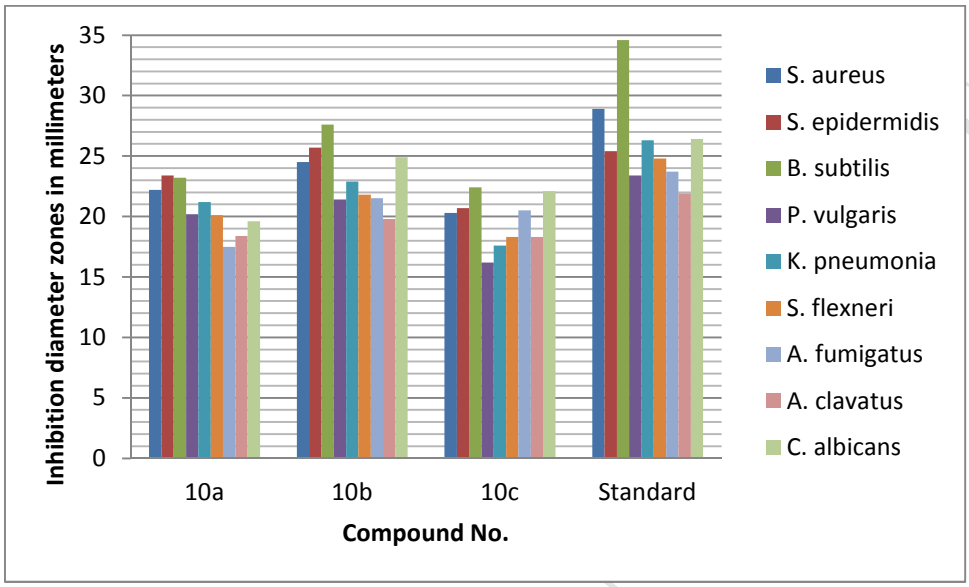
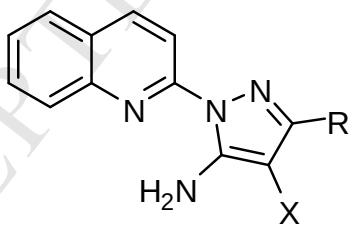


Figure 4: General formula of the synthesized compounds **11a,b**, **12a,b**, **13a,b**



11a X = CN, R = CH_3, **12a** X = CN, R = SCH_3, **13a** X = CN, R = 3-Cl-C_6H_4
11b X = COOEt, R = CH_3, **12b** X = COOEt, R = SCH_3, **13b** X = CN, R = 2,4-Cl_2-C_6H_3

Figure 5: The comparison between the antibacterial and antifungal activities of synthesized compounds **11a,b**, **12a,b**, **13a,b** and standard drugs

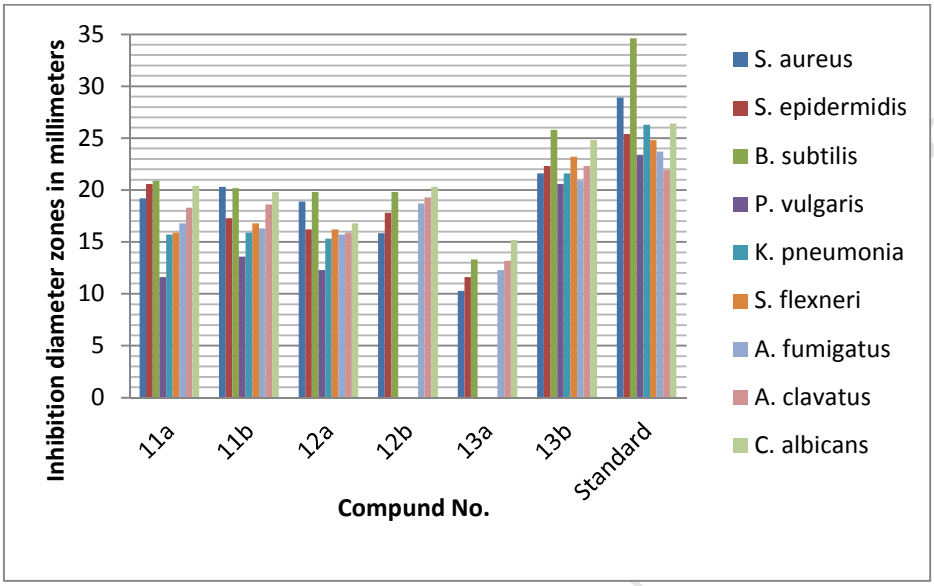
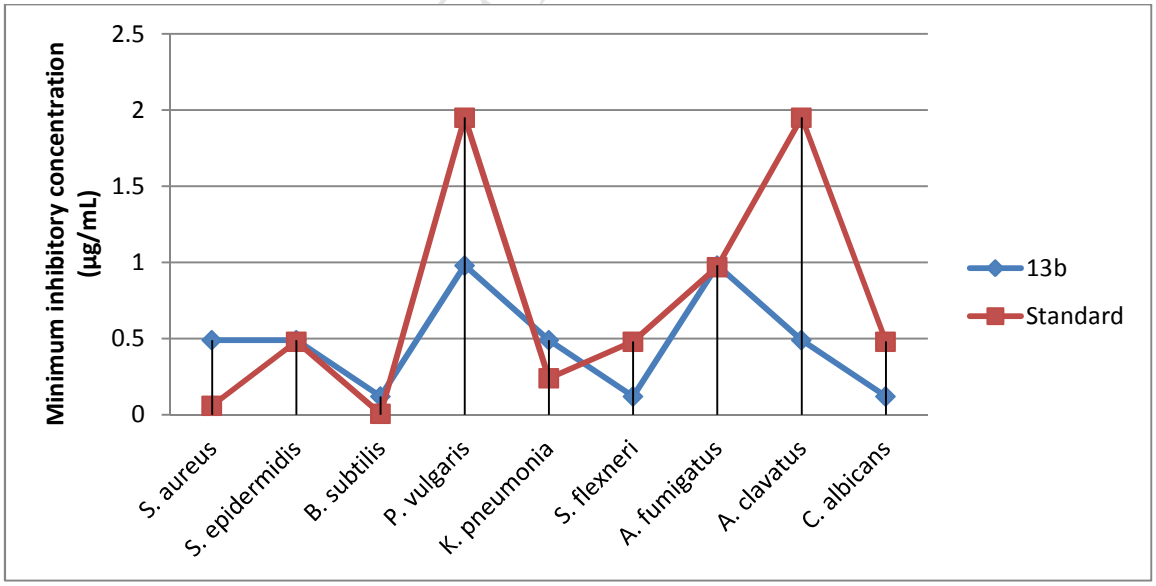


Figure 6: The comparison between the minimal inhibitory concentrations of compound **13b** and standard drugs against the used Gram-positive, Gram-negative bacteria and fungi



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- Synthesis of chalcones.
- Using the chalcones for synthesizing variously substituted pyrazoles.
- Synthesis of 2-hydrazinylquinoline.
- Using the 2-hydrazinylquinoline for synthesizing variously substituted pyrazoles.
- The antimicrobial activity assay was determined.

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