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Design, synthesis and antimicrobial activity of new biquinoline derivatives

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Abstract: A simple and efficient method has been developed for the synthesis of some novel biquinoline derivatives bearing a thiazole moiety through a one-pot three-component condensation of 2-chlro-3-formylquinolines, ethyl cyanoacetate and β-enaminone using catalytic amount of piperidine in refluxing ethanol. These molecules were evaluated *in vitro* for their antibacterial and antifungal activity. Most of the compounds exhibited moderate antibacterial and antifungal activity against all the tested strains.

Keywords: quinoline; thiazole; antibacterial; antifungal.

INTRODUCTION

Quinoline nucleus is one of the most important and widely exploited heterocyclic ring for the development of bioactive molecules. Recent literature is enriched with progressive findings about the synthesis and pharmacological actions of quinoline and its derivatives. A number of quinoline derivatives are known to possess antimicrobial, antimycobacterial, antidepressant, antimalarial, anticonvulsant, antiviral, anticancer, hypotensive and anti-inflammatory activities.[1]

Compounds containing thiazole rings have remarkable medicinal value due to their potential chemotherapeutic,[2] fungicidal,[3] antiviral[4] and pesticidal[5] properties. In addition, 2-aminothiazole derivatives are reported to exhibit significant biological activities such as anti-tuberculosis[6], anti-inflammatory,[7] enzyme inhibition [8] and antitumor activities.[9]

After the extensive literature search, it was observed that quinoline and thiazole are the important pharmacophore, but till date enough efforts have not been made to combine these two moieties as a single molecular scaffold. So, our aim was to synthesize and make a biological screening of a series of new compounds incorporating these moieties.

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RESULTS AND DISCUSSION

Chemistry

Following our interest on synthesizing biologically potent antimicrobials[10] we report herein a new series of biquinoline synthesized by one pot three component cyclocondensation reaction of 2-chloro-3-formylquinolines **2(a-d)**, ethyl cyanoacetate and 3-(4-arylthiazol-2-ylamino)cyclohex-2-enone (enaminone) **3(a-c)**. The synthetic route depicted in **Scheme 2** outlines the chemistry part of the present work. The key intermediates 2-chloro-3-formyl quinolines **2(a-d)** were prepared according to literature method[11] (Scheme 1). Solid phase reaction of 4-substituted acetophenone, thiourea and iodine for 4 h at 120°C afford to give 2-amino-4-arylthiazole[12] (Scheme 1). The required β-enaminones **3(a-c)** were prepared by the reaction β-diketone with 2-amino-4-arylthiazole by refluxing in methanol in presence of catalytic amount of acetic acid.

To choose the most appropriate medium to synthesize compounds **4(a-l)**, several reaction conditions were investigated. Looking for the optimal reaction solvent, the reaction was examined in ethylene glycol, DMF, HOAc, THF, and ethanol as solvent under reflux, respectively. The reaction in ethanol resulted in higher yields and shorter reaction time than others, so ethanol was chosen as the appropriate solvent. Moreover, to further improve the reaction yields, different bases like NaOH, K_2CO_3, DMAP, Et_3N, and piperidine were examined in ethanol. Piperidine afforded the target product **4a** in an 87 % yield. Therefore it was chosen as the most suitable base for all further reactions.

The reaction occurs via an *in situ* initial formation of the heterylidenenitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between 2-chloro-3-formyl quinolines and ethyl cyanoacetate by loss of water molecules. Finally, Michael addition of or to the initially formed unsaturated nitrile, i.e. nucleophilic attack of enaminone to the cyano olefins affords cyclized quinoline derivatives **4(a-l)**.

The structures of the compounds were confirmed on the basis of elemental analysis and spectral data. As an example, the IR spectra of compound **4d** (R_1=Cl, R_2=H) shows band at 3445 cm^{-1} for asym. N-H stretching, 3345 cm^{-1} for sym. N-H stretching and 1660 & 1640 cm^{-1} for C=O stretching of carbonyl group. ^{1}H-NMR spectra of **4d** showed a triplet signal at δ 1.01 for methyl group, a multiplet signal at δ 1.71-2.25 for three methylene group, a quartet signal at 3.92 for OCH_2 group, one singlet at δ 5.30 and δ 8.45 for methine group and amine group respectively and a multiplet due to the aromatic protons around at δ 7.37-8.24. The ^{13}C-NMR spectrum of **4d** was in good agreement with the structure assigned. The peak at δ 14.74 attributed to one methyl group, peak at 21.19, 27.46 and 36.58 attributed to three methylene carbons, δ 35.82 is attributed to methine carbon. The peak at 77.93 is assigned to carbon attached

with carboxylate and the peaks at δ 114.17-156.98 are attributed to aromatic carbon. The peak at δ 168.99 and 195.77 are assigned to carbonyl carbon. Mass spectra of the compounds **4d** and **4j** showed an M^++1 peaks in agreement with their exact mass or molecular weight.

Biological evaluation

Antimicrobial activity. Reviewing of the antibacterial activities (Table 1) of biquinolines indicate that among all the compounds **4a** (R_1 = H, R_2 = H), **4c** (R_1 = OCH_3, R_2 = H), **4d** (R_1 = Cl, R_2 = H), **4e** (R_1 = H, R_2 = Cl), **4h** (R_1 = Cl, R_2 = Cl), **4k** (R_1 = OCH_3, R_2 = OH) and **4l** (R_1 = Cl, R_2 = OH) exhibited good antibacterial activity against the bacterial strain *Escherichia coli*. Similarly compounds **4a** (R_1 = H, R_2 = H), **4c** (R_1 = OCH_3, R_2 = H), **4d** (R_1 = Cl, R_2 = H), **4g** (R_1 = OCH_3, R_2 = Cl), **4h** (R_1 = Cl, R_2 = Cl), **4k** (R_1 = OCH_3, R_2 = OH) and **4l** (R_1 = Cl, R_2 = OH) against Gram-positive bacterial species *Bacillus subtilis* and compounds **4a** (R_1 = H, R_2 = H), **4c** (R_1 = OCH_3, R_2 = H), **4d** (R_1 = Cl, R_2 = H), **4f** (R_1 = OCH_3, R_2 = Cl), **4h** (R_1 = Cl, R_2 = Cl), **4k** (R_1 = OCH_3, R_2 = OH) and **4l** (R_1 = CH_3, R_2 = Cl) against Gram-positive bacterial species *Staphylococcus aureus* showed good activity. Remaining compounds showed moderate activity against tested bacterial strain.

The antifungal evaluation of the synthesized compounds revealed that compounds **4c** (R_1 = OCH_3, R_2 = H), **4g** (R_1 = OCH_3, R_2 = Cl) and **4k** (R_1 = OCH_3, R_2 = OH) displayed excellent fungal activity towards *Fuserium oxysporum*. Against *Aspergillus niger* compounds **4c** (R_1 = OCH_3, R_2 = H), **4g** (R_1 = OCH_3, R_2 = Cl) **4h** (R_1 = Cl, R_2 = Cl), **4k** (R_1 = OCH_3, R_2 = OH) and **4l** (R_1 = Cl, R_2 = OH)and compounds **4c** (R_1 = OCH_3, R_2 = H), **4d** (R_1 = Cl, R_2 = H), **4g** (R_1 = OCH_3, R_2 = Cl), **4h** (R_1 = Cl, R_2 = Cl) and **4k** (R_1 = OCH_3, R_2 = OH) towards *Rhizopus oryzae* showed good activity. Remaining compounds showed mild to moderate antifungal activity.

A close examination of the structures of the active compounds in Table 1 revealed that, their antimicrobial activity is strongly bound to the nature of the substituent at the quinoline-C_6, together with the substituent linked to the arylthiazole part of the structure. In general, it could be clearly recognized that compound **4c** without a substituent in the arylthiazole ring (R_2 = H) and quinoline containing a methoxy substituent (R_1 = OCH_3) show greater activity compared to the other compounds studied. Moreover, compound **4k** quinoline containing a methoxy substituent (R_1 = OCH_3) and with a hydroxyl substituent in the arylthiazole ring (R_2 = OH) have good antimicrobial profile while compound **4g** quinoline also containing a methoxy substituent (R_1 = OCH_3) and with a chloro substituent in the arylthiazole ring (R_2 = Cl) exhibited moderate to good antimicrobial activity. On the other hand, introduction of methyl group at position-6 of the quinoline (compounds **4b**, **4j** and **4f**) resulted in a noticeable

decrease in the antimicrobial potential of these compounds. Compounds **4a** (R_1 = H, R_2 = H), **4h** (R_1 = Cl, R_2 = Cl) and **4l** (R_1 = Cl, R_2 = OH) showed a good antibacterial activity together with a moderate antifungal profile. It is worthy to mention that the biological activity of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the substituents and may also upon their spatial relationships. Here, thiazole moiety is introduced along with biquinoline ring for activity reinforcement. However, based on the observation, it is immature to arrive at any conclusion on structure activity aspect of these molecules and further evaluation is needed.

EXPERIMENTAL

Chemistry

Solvents used were of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates precoated with silica gel, 60F254, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds; eluent-hexane:ethyl acetate 6:4. UV radiation and/or iodine were used as the visualizing agents. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and all compounds were within ±0.4% of calculation. The IR spectra were recorded in KBr pellet on a Perkin-Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA), and only the characteristic peaks were reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO- d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using solvent peak as internal standard at 400 MHz and 100 MHz respectively. Chemical shifts were reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

General procedure for the synthesis of 2-amino-4-arylthiazole

2-amino-4-arylthiazole was synthesized, according to literature procedure[12] by the solid phase reaction of thiourea, 4-substituted acetophenone and iodine (Scheme 1)

*General procedure for the synthesis of 3-(4-arylthiazole-2-ylamino)cyclohex-enone **3(a-c)***

1,3-Dicarbonyl compound (30 mmol), 2-amino-4-arylthiazole (30 mmol), methanol (15 mL) and 2 drops of acetic acid were charged in 100 mL round bottom flask equipped with refluxing condenser. The reaction mixture was slowly heated and refluxed for 1 h. On completion of reaction, monitored by TLC using 30% EtOAc in toluene as eluent, the reaction mixture was cooled to room temperature and the solid separated was filtered and washed with methanol to obtain the pure compounds.

*General procedure for the synthesis of Ethyl 2-amino-4-(2-chloro-6-(un)substituted(3-quinolyl))-5-oxo-1-(4-arylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinolne-3-carboxylate **4(a-l)***

A mixture of 2-chloro-3-formylquinolines (1 mmol), ethyl cyanoacetate (1 mmol), and appropriate β-enaminone (1 mmol) in ethanol (10 ml) containing catalytic amount of piperidine was slowly heated and refluxed for 3-4 h. On completion of reaction, monitored by TLC (ethyl acetate:toluene::3:7), the reaction mixture was cooled to room temperature and the solid separated was filtered and washed with mixture of chloroform and methanol (1:1) to

obtain the pure compounds. Analytical and spectroscopic characterization data of the synthesized compounds are given below:

Ethyl 2-amino-4-(2-chloro(3-quinolyl))-5-oxo-1-(4-phenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4a)**. Yield: 87 %; m.p. 245-247 °C; Anal. Calcd. for $C_{30}H_{25}ClN_4O_3S$: C, 64.68; H, 4.52; N, 10.06 %. Found: C, 64.82; H, 4.48; N, 09.90 %. IR (KBr, ν, cm^{-1}): 3425 & 3280 (asym. & sym. str. of -NH_2), 1678 (C=O str.), 1640 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.11 (3H, t, J = 7.16 Hz, CH_3), 1.71-2.20 (6H, m, 3×CH_2), 3.90 (2H, q, J = 7.16 Hz, OCH_2), 5.36 (1H, s, CH), 7.31-8.28 (11H, m, Ar-H), 8.42 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.45 (CH_3), 21.30, 26.82 (2C, CH_2), 35.40 (C4), 36.12 ($\underline{C}H_2$-CO), 57.32 (OCH_2), 78.25 ($\underline{C}$-COOEt), 113.31, 117.78, 119.89, 126.65, 126.96, 128.54, 129.12, 129.41, 131.23, 131.30, 133.30, 140.28, 144.93, 150.41, 150.60, 152.53, 152.73, 152.82, 156.91 (19C, Ar-C), 168.75 ($\underline{C}$OOEt), 195.88 (C=O).

Ethyl 2-amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-(4-phenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4b)**. Yield: 89 %; m.p. 169-171 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_3S$: C, 65.20; H, 4.77; N, 09.81 %. Found: C, 65.28; H, 4.72; N, 09.93 %. IR (KBr, ν, cm^{-1}): 3437 & 3333 (asym. & sym. str. of -NH_2), 1672 (C=O str.), 1620 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.09 (3H, t, J = 7.4 Hz, CH_3), 2.23 (3H, s, CH_3), 1.71-2.20 (6H, m, 3×CH_2), 3.93 (2H, q, J = 7.2 Hz, OCH_2), 5.31 (1H, s, CH), 7.29-8.25 (10H, m, Ar-H), 8.38 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.23 (CH_3), 20.14 (CH_3), 21.41, 26.02 (2C, CH_2), 35.10 (C4), 36.32 ($\underline{C}H_2$-CO), 57.10 (OCH_2), 78.74 ($\underline{C}$-COOEt), 113.14, 117.14, 119.41, 126.41, 126.47, 128.25, 129.45, 129.74, 131.13, 131.15, 133.25, 140.47, 144.78, 150.41, 150.45, 152.13, 152.71, 152.92, 156.45 (19C, Ar-C), 168.79 ($\underline{C}$OOEt), 195.45 (C=O).

Ethyl 2-amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-(4-phenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4c)**. Yield: 83 %; m.p. 221-223 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_3S$: C, 63.42; H, 4.64; N, 09.54 %. Found: C, 63.58; H, 4.72; N, 09.73 %. IR (KBr, ν, cm^{-1}): 3446 & 3240 (asym. & sym. str. of -NH_2), 1668 (C=O str.), 1635 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.01 (3H, t, J = 7.4 Hz, CH_3), 1.71-2.25 (6H, m, 3×CH_2), 3.89 (3H, s, OCH_3), 3.90 (2H, q, J = 7.1 Hz, OCH_2), 5.35 (1H, s, CH), 7.29-8.22 (10H, m, Ar-H), 8.32 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.64 (CH_3), 21.23, 27.12 (2C, CH_2), 35.14 (C4), 36.00 ($\underline{C}H_2$-CO), 57.23 (OCH_3), 59.10 (OCH_2), 77.74 ($\underline{C}$-COOEt), 105.27, 114.41, 119.32, 126.40, 126.84, 128.23, 129.18, 129.78, 131.41, 131.97, 133.41, 140.40, 144.68, 150.74, 150.92, 152.44, 152.64, 152.90, 156.01 (19C, Ar-C), 168.14 ($\underline{C}$OOEt), 195.38 (C=O).

Ethyl 2-amino-4-(2-chloro-6-chloro(3-quinolyl))-5-oxo-1-(4-phenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4d)**. Yield: 76 %; m.p. 232-234 °C; Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_3S$: C, 60.92; H, 4.09; N, 09.47 %. Found: C, 60.86; H, 4.05; N, 09.60 %. IR (KBr, ν, cm^{-1}): 3445 & 3345 (asym. & sym. str. of -NH_2), 1660 (C=O str.), 1640 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.01 (3H, t, J = 7.12 Hz, CH_3), 1.71-2.25 (6H, m, 3×CH_2), 3.92 (2H, q, J = 7.12 Hz, OCH_2), 5.30 (1H, s, CH), 7.37-8.24 (10H, m, Ar-H), 8.45 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.74 (CH_3), 21.19, 27.46 (2C, CH_2), 35.82 (C4), 36.58 ($\underline{C}H_2$-CO), 59.24 (OCH_2), 77.93 ($\underline{C}$-COOEt), 114.17, 117.70, 119.34, 126.61, 126.85, 128.22, 129.37, 129.87, 131.05, 131.81, 133.95, 140.70, 144.35, 150.66, 150.93, 152.55, 152.76, 152.87, 156.98 (19C, Ar-C), 168.99 ($\underline{C}$OOEt), 195.87 (C=O). MS: M^++1 591.1.

Ethyl 2-amino-4-(2-chloro(3-quinolyl))-5-oxo-1-(4-chlorophenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4e)**. Yield: 87 %; m.p. 195-197 °C; Anal.

Calcd. for $C_{30}H_{24}Cl_2N_4O_3S$: C, 60.92; H, 4.09; N, 09.47 %. Found: C, 60.96; H, 4.00; N, 09.65 %. IR (KBr, ν, cm^{-1}): 3441 & 3260 (asym. & sym. str. of -NH_2), 1668 (C=O str.), 1645 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.10 (3H, t, J = 7.16 Hz, CH_3), 1.70-2.21 (6H, m, 3×CH_2), 3.94 (2H, q, J = 7.12 Hz, OCH_2), 5.40 (1H, s, CH), , 7.30-8.22 (10H, m, Ar-H), 8.40 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.98 (CH_3), 21.78, 26.54 (2C, CH_2), 35.45 (C4), 36.12 ($\underline{C}H_2$-CO), 57.98 (OCH_2), 78.32 ($\underline{C}$-COOEt), 113.87, 117.36, 119.74, 126.45, 126.65, 128.54, 129.56, 129.74, 131.36, 131.70, 133.12, 140.56, 144.56, 150.23, 150.45, 152.03, 152.65, 152.85, 156.19 (19C, Ar-C), 168.23 ($\underline{C}$OOEt), 195.98 (C=O).

Ethyl 2-amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-(4-chlorophenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4f)**. Yield: 81 %; m.p. 208-210 °C; Anal. Calcd. for $C_{31}H_{26}Cl_2N_4O_3S$: C, 61.49; H, 4.33; N, 09.25 %. Found: C, 61.41; H, 4.23; N, 09.35 %. IR (KBr, ν, cm^{-1}): 3442 & 3280 (asym. & sym. str. of -NH_2), 1678 (C=O str.), 1660 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.07 (3H, t, J = 7.2 Hz, CH_3), 1.72-2.45 (6H, m, 3×CH_2), 2.47 (3H, s, CH_3), 3.87 (2H, q, J = 7.2 Hz, OCH_2), 5.25 (1H, s, CH), 7.25-8.31 (9H, m, Ar-H), 8.48 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.12 (CH_3), 20.23 (CH_3), 21.39, 27.28 (2C, CH_2), 35.17 (C4), 36.36 ($\underline{C}H_2$-CO), 59.65 (OCH_2), 78.54 ($\underline{C}$-COOEt), 113.07, 114.78, 116.89, 122.54, 125.41, 126.65, 128.10, 128.32, 129.21, 140.14, 141.74, 141.90, 147.12, 149.23, 152.00, 152.36, 156.03, 158.36, 158.96 (19C, Ar-C), 169.19 ($\underline{C}$OOEt), 195.37 (C=O).

Ethyl 2-amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-(4-chlorophenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4g)**. Yield: 79 %; m.p. 225-227 °C; Anal. Calcd. for $C_{31}H_{26}Cl_2N_4O_4S$: C, 59.91; H, 4.22; N, 09.01. Found: C, 59.82; H, 4.42; N, 09.23 %. IR (KBr, ν, cm^{-1}): 3445 & 3285 (asym. & sym. str. of -NH_2), 1670 (C=O str.), 1650 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.01 (3H, t, J = 7.2 Hz, CH_3), 1.75-2.41 (6H, m, 3×CH_2), 3.85 (2H, q, J = 7.1 Hz, OCH_2), 3.95 (3H, s, OCH_3), 5.32 (1H, s, CH), 7.29-8.24 (9H, m, Ar-H), 8.46 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.78 (CH_3), 21.89, 27.96 (2C, CH_2), 35.65 (C4), 36.54 ($\underline{C}H_2$-CO), 56.41 (OCH_3), 59.12 (OCH_2), 78.23 ($\underline{C}$-COOEt), 105.32, 114.21, 116.14, 116.45, 122.55, 125.65, 128.85, 128.96, 129.74, 140.17, 141.20, 141.39, 147.23, 149.11, 152.75, 152.85, 156.63, 158.74, 158.32 (19C, Ar-C), 169.32 ($\underline{C}$OOEt), 195.97 (C=O).

Ethyl 2-amino-4-(2-chloro-6-chloro(3-quinolyl))-5-oxo-1-(4-chlorophenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4h)**. Yield: 75 %; m.p. 198-200 °C; Anal. Calcd. for $C_{30}H_{23}Cl_3N_4O_4S$: C, 57.56; H, 3.70; N, 08.95. Found: C, 57.72; H, 3.85; N, 08.77 %. IR (KBr, ν, cm^{-1}): 3439 & 3281 (asym. & sym. str. of -NH_2), 1670 (C=O str.), 1635 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.03 (3H, t, J = 7.4 Hz, CH_3), 1.72-2.41 (6H, m, 3×CH_2), 3.87 (2H, q, J = 7.16 Hz, OCH_2), 5.20 (1H, s, CH), 7.25-8.28 (9H, m, Ar-H), 8.40 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.98 (CH_3), 21.23, 27.45 (2C, CH_2), 35.32 (C4), 36.89 ($\underline{C}H_2$-CO), 59.97 (OCH_2), 78.56 ($\underline{C}$-COOEt), 112.71, 114.82, 116.10, 116.98, 122.38, 125.30, 128.56, 128.91, 129.13, 140.17, 141.88, 141.99, 147.06, 149.12, 152.58, 152.30, 156.85, 158.06, 158.12 (19C, Ar-C), 169.87 ($\underline{C}$OOEt), 195.74 (C=O).

Ethyl 2-amino-4-(2-chloro(3-quinolyl))-5-oxo-1-(4-hydroxyphenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4i)**. Yield: 73 %; m.p. 198-200 °C; Anal. Calcd. for $C_{30}H_{25}ClN_4O_4S$: C, 62.88; H, 4.40; N, 09.78. Found: C, 63.07; H, 4.56; N, 09.67 %. IR (KBr, ν, cm^{-1}): 3439 & 3295 (asym. & sym. str. of -NH_2), 1675 (C=O str.), 1640 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.05 (3H, t, J = 7.12 Hz, CH_3), 1.68-2.44 (6H, m, 3×CH_2), 3.90 (2H, q, J = 7.12 Hz, OCH_2), 5.21 (1H, s, CH), 7.20-8.26 (10H, m, Ar-H), 8.29 (2H, s, NH_2), 9.65 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.11 (CH_3),

20.92, 27.14 (2C, CH_2), 35.47 (C4), 36.85 ($\underline{C}H_2$-CO), 59.52 (OCH_2), 78.36 ($\underline{C}$-COOEt), 113.36, 114.69, 116.78, 116.92, 122.12, 125.98, 128.32, 128.87, 129.40, 140.71, 141.43, 141.61, 147.91, 149.73, 152.82, 152.49, 156.93, 158.71, 158.25 (19C, Ar-C), 169.61 ($\underline{C}$OOEt), 195.40 (C=O).

Ethyl 2-amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-(4-hydroxyphenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4j)**. Yield: 64 %; m.p. 236-238 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_4S$: C, 63.42; H, 4.64; N, 09.54 %. Found: C, 63.22; H, 4.48; N, 09.74 %. IR (KBr, ν, cm^{-1}): 3442 & 3280 (asym. & sym. str. of -NH_2), 1678 (C=O str.), 1630 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.03 (3H, t, J = 7.2 Hz, CH_3), 1.70-2.41 (6H, m, 3×CH_2), 2.45 (3H, s, CH_3), 3.89 (2H, q, J = 7.16 Hz, OCH_2), 5.24 (1H, s, CH), 7.25 -8.23 (9H, m, Ar-H), 8.30 (2H, s, NH_2), 9.62 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.23 (CH_3), 20.98 (Ar-CH_3), 21.12, 27.78 (2C, CH_2), 35.45 (C4), 36.98 ($\underline{C}H_2$-CO), 59.56 (OCH_2), 78.89 ($\underline{C}$-COOEt), 112.54, 114.32, 116.36, 116.52, 122.00, 125.35, 128.34, 128.47, 129.10, 140.87, 141.71, 141.99, 147.40, 149.59, 152.81, 152.99, 156.63, 158.25, 158.86 (19C, Ar-C), 169.10 ($\underline{C}$OOEt), 195.32 (C=O). MS: M^++1 586.6.

Ethyl 2-amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-(4-hydroxyphenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4k)**. Yield: 81 %; m.p. 244-246 °C; Anal. Calcd. for $C_{31}H_{27}ClN_4O_5S$: C, 61.74; H, 4.51; N, 09.29. Found: C, 61.88; H, 4.70; N, 09.07 %. IR (KBr, ν, cm^{-1}): 3445 & 3260 (asym. & sym. str. of -NH_2), 1680 (C=O str.), 1660 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.05 (3H, t, J = 7.4 Hz, CH_3), 1.70-2.41 (6H, m, 3×CH_2), 3.91 (2H, q, J = 7.2 Hz, OCH_2), 3.91 (3H, s, OCH_3), 5.28 (1H, s, CH), 6.87-8.17 (9H, m, Ar-H), 8.27 (2H, s, NH_2), 9.72 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.72 (CH_3), 21.26, 27.47 (2C, CH_2), 35.63 (C4), 36.60 ($\underline{C}H_2$-CO), 56.08 (Ar-OCH_3), 59.13 (OCH_2), 78.35 ($\underline{C}$-COOEt), 105.79, 114.48, 116.04, 116.25, 122.99, 125.28, 128.09, 128.59, 129.14, 140.27, 141.20, 141.90, 147.47, 149.01, 152.69, 152.91, 156.73, 158.10, 158.38 (19C, Ar-C), 169.06 ($\underline{C}$OOEt), 195.81 (C=O).

Ethyl 2-amino-4-(2-chloro-6-chloro(3-quinolyl))-5-oxo-1-(4-hydroxyphenylthiazol-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(4l)**. Yield: 79 %; m.p. 256-258 °C; Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_4S$: C, 59.31; H, 3.98; N, 09.22. Found: C, 59.50; H, 4.05; N, 09.47 %. IR (KBr, ν, cm^{-1}): 3440 & 3275 (asym. & sym. str. of -NH_2), 1675 (C=O str.), 1640 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.02 (3H, t, J = 7.12 Hz, CH_3), 1.70-2.41 (6H, m, 3×CH_2), 3.91 (2H, q, J = 7.12 Hz, OCH_2), 5.25 (1H, s, CH), 7.25-8.20 (9H, m, Ar-H), 8.26 (2H, s, NH_2), 9.70 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.12 (CH_3), 21.23, 27.36 (2C, CH_2), 35.32 (C4), 36.21 ($\underline{C}H_2$-CO), 59.45 (OCH_2), 78.56 ($\underline{C}$-COOEt), 105.65, 114.23, 116.10, 116.98, 122.34, 125.30, 128.23, 128.87, 129.13, 140.17, 141.78, 141.99, 147.56, 149.32, 152.23, 152.30, 156.30, 158.96, 158.32 (19C, Ar-C), 169.87 ($\underline{C}$OOEt), 195.96 (C=O).

Antimicrobial activity

The in vitro antimicrobial activity was carried out against 24 hr old cultures of three bacteria and three fungi by disc diffusion method [13-14]. Compounds **4(a-l)** have been tested for their antibacterial activity against Escherichia coli as Gram-negative bacteria and Bacillus subtilis and Bacillus cereus as Gram-positive bacteria and antifungal activity against Aspergillus niger, Fuserium oxysporum, and Rhizopus. Nutrient agar and potato dextrose were used to culture the bacteria and fungus respectively. The compounds were tested at 1000 ppm in DMF solution. Ciprofloxacin, Ampicillin and Griseofulvin were used as standards for comparison of antibacterial and antifungal activities respectively. Inhibition was recorded by

measuring the diameter of the inhibition zone at the end of 24 hr for bacteria at 35°C and 48hr for fungus at 28°C. The results are summarized in Table 1.

CONCLUSIONS

In conclusion, we have developed a simple and efficient method for the synthesis of biquinoline derivatives. The straightforward approach, simplicity and one-step method make it an interesting approach for the synthesis of said compounds. Most of the compounds showed better antibacterial activity, further optimization and development is needed in designing more potent antibacterial and antifungal agents for therapeutic use.

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ИЗВОД

ДИЗАЈН, СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НОВИХ БИХИНОЛИНСКИХ ДЕРИВАТА

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Развијен је једноставан и ефикасан поступак за синтезу нових бихинолинских деривата који садрже тиазолински структурни фрагмент. Поступак се састоји из тро-компонентне кондензације 2-хлор-3-формилхинолина, етил-цианоацетата и β-енаминона у једном реакционом кораку, катализован пиперидином у кључалом етанолу. Испитана је ин витро антибактеријска и антифунгална инхибиторна активност добијених једињења. Већина испитаних једињења показује умерену активност према испитиваним сојевима.

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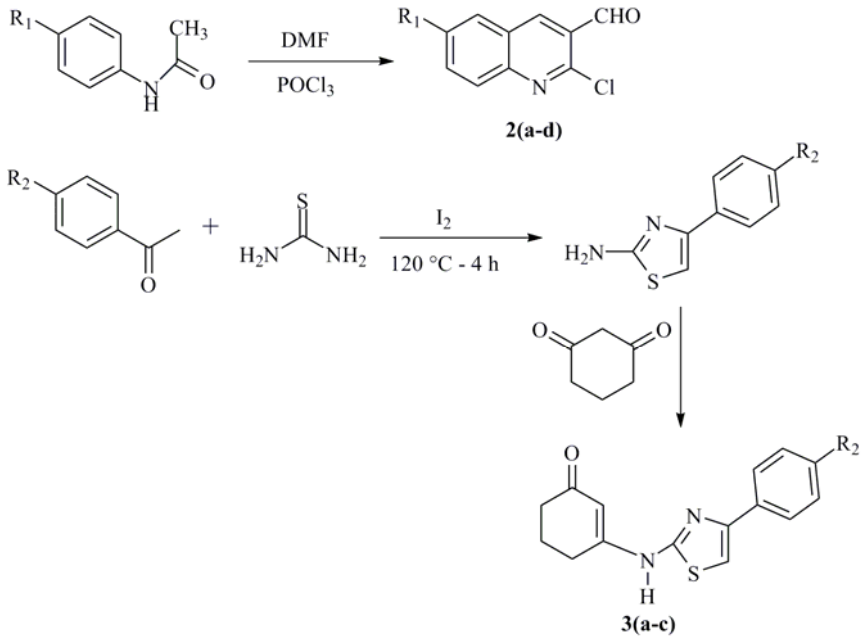
Table I. Antimicrobial activity of the compounds **4(a-l)**

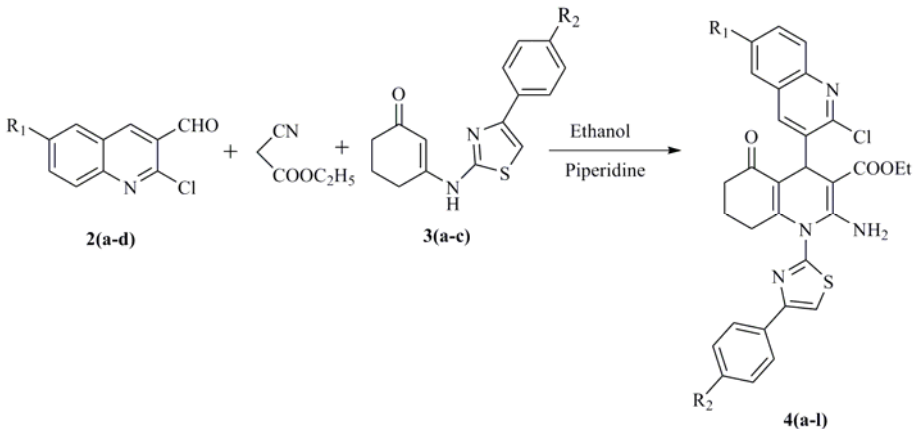
	Antibacterial activity Zone of inhibition in mm			**Antifungal activity Zone of inhibition in mm**		
Compd.	***E. coli***	***B. substilis***	***S. aureus***	***F. oxysporum***	***A. niger***	***R. oryzae***
4a	23	25	22	16	15	18
4b	17	19	18	15	19	16
4c	25	24	23	20	22	25
4d	24	23	25	14	16	21
4e	20	17	18	17	16	18
4f	18	19	20	16	18	17
4g	19	20	18	21	24	22
4h	25	22	24	19	20	21
4i	19	18	15	17	17	14
4j	18	17	16	15	14	18
4k	24	22	23	21	25	24
4l	21	23	25	19	20	17
Ampicillin	28	30	30	NT	NT	NT
Ciprofloxacin	35	34	33	NT	NT	NT
Griseofulvin	NT	NT	NT	26	28	30

NT = not tested.

Control (DMF) (-) – No activity



Scheme 1. Synthetic pathway for the intermediates 2(a-d) and 3(a-c)



Scheme 2. Synthetic pathway for the compounds 4(a-l)

Compd.	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l
R_1	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl
R_2	H	H	H	H	Cl	Cl	Cl	Cl	OH	OH	OH	OH

Scheme 2

Captions for Scheme:

Scheme 1: Synthetic pathway for the intermediates **2(a-d)** and **3(a-c)**

Scheme 2: Synthetic pathway for the compounds **4(a-l)**

Supplementary material

Physical data for compounds 2(a-d)

Compound	R_1	M.P. (^oC)	Mol.Wt. (gm)	Mol. Formula	Yield (%)
2a	H	145	191.61	$C_{10}H_6ClNO$	71
2b	Me	125-26	205.64	$C_{11}H_8ClNO$	73
2c	OMe	148-49	221.64	$C_{11}H_8ClNO_2$	68
2d	Cl	165-66	226.06	$C_{10}H_5Cl_2NO$	42

EXPERIMENTAL

Chemistry

Analytical and spectroscopic characterization data of 3-(4-arylthiazole-2-ylamino)cyclohex-enone **3(a-c)** are given below:

3-(4-Phenylthiazol-2-ylamino)cyclohex-2-enone **(3a)**. Yield: 74 %; m.p. 188-189 °C; Anal. Calcd. for $C_{15}H_{14}N_2OS$: C, 66.64; H, 5.22; N, 10.36 %. Found: C, 66.87; H, 4.98; N, 10.51 %. IR (KBr, ν, cm^{-1}): 3395 (NH str.), 1665 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.89-2.27 (6H, m, 3×CH_2), 5.23 (1H, s, CH), 6.72-7.85 (6H, m, Ar-H), 10.48 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.19, 26.98 (CH_2), 37.02 ($\underline{C}H_2$-CO), 106.41, 106.89, 112.47, 128.06, 132.79, 147.78, 153.56, 159.05, 161.67 (Ar-C), 197.28 (C=O).

3-(4-(4-chlorophenyl)thiazol-2-ylamino)cyclohex-2-enone **(3b)**. Yield: 70 %; m.p. 199-201 °C; Anal. Calcd. for $C_{15}H_{13}ClN_2OS$: C, 59.11; H, 4.30; N, 09.19 %. Found: C, 58.95; H, 4.44; N, 09.27 %. IR (KBr, ν, cm^{-1}): 3430 (NH str.), 1655 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.87-2.26 (6H, m, 3×CH_2), 5.27 (1H, s, CH), 6.87-7.94 (5H, m, Ar-H), 10.55 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.37, 27.60 (CH_2), 36.79 ($\underline{C}H_2$-CO), 105.35, 106.78, 127.93, 129.25, 131.82, 148.63, 154.41, 159.30, 162.11 (Ar-C), 197.49 (C=O).

3-(4-(4-hydroxyphenyl)thiazol-2-ylamino)cyclohex-2-enone **(3c)**. Yield: 75 %; m.p. 210-212 °C; Anal. Calcd. for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 09.78 %. Found: C, 63.07; H, 4.75; N, 09.66 %. IR (KBr, ν, cm^{-1}): 3415 (NH str.), 1680 (C=O str.). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.83-2.29 (6H, m, 3×CH_2), 5.25 (1H, s, CH), 6.81-7.71 (5H, m, Ar-H), 9.62 (1H, s, OH), 10.34 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.27, 27.33 (CH_2), 37.00 ($\underline{C}H_2$-CO), 105.21, 106.62, 118.21, 127.10, 129.53, 148.87, 157.65, 159.48, 162.02 (Ar-C), 197.66 (C=O).

Antimicrobial activity

Sample preparation for the study of antibacterial activity. A 1000 ppm solution of newly synthesized compounds has prepared in DMF.

Culture media for the study of antibacterial activity. The media: Nutrient broth (Hi-Media, Mumbai, India) having following composition is used for the preparation of inoculums for antibacterial study.

Ingredient	Gms/liter
Peptic digest of animal tissue	5.00
Yeast extract	1.50
Beef extract	1.50
Sodium chloride	5.00

Weighed quantities of all the components were dissolved in freshly prepared hot distilled water.

Sterilization. The sterilization of culture media, culture tubes, and other materials was done by autoclaving them at 15 lbs/sq.inch pressure for 15 min. The petri-dishes were sterilized by keeping them overnight in an electrically heated air oven at 140 ^{o}C.

Preparation of Nutrient Plates:

20 ml of this sterilized media was poured in each sterilized petri dish and allowed to solidify.

Preparation of Inoculum. The inoculum of organisms were prepared by transferring a loopful of corresponding organism from the stock culture into the sterile broth and incubated at 37 ^{o}C for 24 hrs under shaking conditions. The organisms were sub cultured on the nutrient agar slants. The inoculum was prepared by dispensing colonies in sterile distilled water to prepare a suspension.

Antibacterial susceptibility testing. A test tube containing sterile melted soft agar (approximately 15 ml) was cooled to 45 ^{o}C and inoculated with 0.2 ml suspension of the test culture, mixed thoroughly and poured in the petri dish containing sterile N-agar medium and allowed to solidify for five minutes. The cup-borer was sterilized by dipping into absolute alcohol and flaming it and then allowed to cool down. With the sterile cup-borer, cups were bored in the agar and marked. The cups were filled with 0.1 ml of respectively test sample solution and the test sample was allowed to diffuse for 10 to 15 min. in refrigerator. The plate was incubated at 37 ^{o}C for 24 hr and on the next day the zone of inhibition of surrounding each cup was observed and measured in mm.

Sample preparation for the study of antifungal activity. A 1000 ppm solution of newly synthesized compounds has prepared in DMF.

Culture media for the study of antifungal activity. The media: Potato-dextrose agar (Hi-Media, Mumbai, India) having following composition is used for the preparation of inoculum for antibacterial study.

Ingredient	Gms/liter
Potatoes infusion from	200.00
Dextrose	20.0
Agar	15.0

Weighed quantities of all the components were dissolved in freshly prepared hot distilled water.

All experimental conditions for antifungal activity are same as that of the antibacterial activity only difference is culture media and the plate was incubated at 28 ^{o}C for 48 hr and the zone of inhibition of surrounding each cup was observed and measured in mm.