EFFICIENT SYNTHESIS OF 2-AMINO-4-(2-CHLORO-5-(4-SUBSTITUTED PHENYL) PYRIDIN-3-YL)-1-(4-SUBSTITUTED PHENYL)-7,7-DISUBSTITUTED-5-OXO-1,4,5,6,7,8-HEXAHYDROQUINOLINE-3-CARBONITRILE DERIVATIVES AND THEIR MICROBIAL SCREENING

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ABSTRACT

The work reports derivatives of 2-amino-4-(2-chloro-5-(4-substituted phenyl) pyridine-3-yl)-1-(4-substituted phenyl)-7,7-disubstituted-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile Q(1-14) have been synthesized by the reaction of 5,5-disubstituted-1,3-cyclohexanedione and 4-substituted aniline in the presence of ethanol and piperidine. The structure of synthesized compounds is characterized by spectroscopic methods such as IR, 1H NMR, Mass Spectroscopy, and elemental analysis. The efficient novel derivatives Q(1-14) with different effective substituent (-CH₃, -NO₂, -OCH₃, -Br) was screened against antibacterial and antifungal effectively and showed notable action against tested microbes.

Keywords: Carbonitrile Derivative, Cyclohexanedione, Hexahydroquinolin, Microbial Screening, Spectroscopic Methods, Substitutedphenyl.

INTRODUCTION

Heterocyclic compoundshave received the attention of chemists and biologists owing to their wide range of promising biological activities. The efficient synthesis of quinoline and its derivatives attracted the attention of various research communities because of its structural privileged that is found in a variety of natural products and therapeutics. In recent times quinolone-based heterocycles are a diverse part of people’s lives. The hydroquinoline skeleton is thus an effective structure for the development of the new synthesis of biologically active heterocyclic compounds. They are naturally found in plant-based chemical and biochemical systems. Quinoline-based derivatives have acquired notable attention due to their structural advantage, plenty of biological processes and various derivatives of quinolines are reported to possess wide biological activities such as neoplastic agent activity and especially derivatives of quinoline, due to this, it has a strong metal chelating property. Furthermore, quinolone-based heterocycles have specific potency towards anti-inflammatory, antifungal, and anti-cancer agents. In the last few years, drug repositioning has gained notable attention in drug discovery and development. The search for new effective lead heterocyclic derivatives and repositioning of the known drugs of hydroquinoline for medicinal applications are the main challenges. The work reported here was to synthesize quinoline derivatives by using hexahydroquinoline carbonitriles as parent motif. The preparation of those molecules plays really an important role in their organic synthesis. The purpose of the reported work is to synthesize derivatives of new hexahydroxyquinoline-based carbonitrile derivatives to investigate their biological activity against antifungal and antibacterial screening.
EXPERIMENTAL

Material and Methods
Measured melting points are uncorrected on the basis of available melting apparatus and present in degree Celsius. The observation of the progress of all reactions of all synthesized compounds was administered by TLC. TLC was run using TLC aluminum sheet silica gel 60F254(Merck) and visualization was done using an iodine/UV lamp for the detection of the spots. Elemental analysis (% C, H, N) was carried out by a Perkin Elmer 2400 CHN analyzer. IR spectra of all the compounds have been recorded on a Shimadzu FT-IR 8401 spectrophotometer in KBr disks. The $^1$H-NMR spectra have been recorded on a Bruker AC 400F (400MHz) instrument using TMS as an internal standard in DMSO-d$_6$ as a solvent, with chemical shifts in δ ppm. Mass spectra, JEOL-JMS 600 spectrometer. The solvent was removed under reduced pressure using a Buchi rotary evaporator. Chemicals were purchased from AR grade and substituted anilines, malononitrile are commercial products and were used without further purification.

Preparation of 5-(4-substituted phenyl)-2-chloro-3-formylpyridine
Charged Dimethylformamide (9.56 mL, 59mmol), and N-(2-arylethenyl) acetamide (4.8 mmol) during a three-necked glass round-bottomed flask equipped with a thermometer, condenser, guard tube, and mechanical stirrer. The reaction mixture cooled to 0°C. To it, phosphorous oxychloride (39 mmol) was added dropwise with stirring over a period of 35-40 minutes at 0-55°C. Stirred the reaction mixture for 1 hour at normal room temperature and then stirred at 90°C for 3.5 hours. After the completion of the reaction, the reaction yield cooled to 25°C temperature and poured into ice water, and neutralized with sodium acetate salt. The crude yield was filtered and washed with water, the basic liquid was extracted with chloroform and set to evaporate to dryness. The final crude solid was crystallized from Diethyl ether to give the desired derivatives.

Preparation of {[2-chloro-5-(4-substituted phenyl) pyridin-3-yl]methylidene} propanedinitrile
5-phenyl-2-chloro-3-formyl pyridine (0.01mol) malononitrile and (0.01mol) ethanol (10.5 ml) were charged in R.B.F with mechanical stirrer and condenser. The reaction mixture was gradually heated. When the entire compound was dissolved in the mixture, 2-3 drops of triethylamine were added to the mixture and refluxed for about 1 hr. After the completion of the reaction (checked by TLC), the yield was filtered and washed with cold ethanol. The product was recrystallized with methanol.

Synthesis of enaminone
A mixture of 5, 5-disubstituted-1, 3-cyclohexanenedione (0.01 mol), and 4- substituted aniline (0.01 mol) was heated in an oil bath for 1 hr. and cooled to room temperature. The isolated solid mass was filtered and washed with ether and dried.

Synthesis of 2-amino-4-(2-chloro-5-(4-substitutedphenyl) pyridin-3-yl)-1-(4-substitutedphenyl)-7, 7-disubstituted-5-oxo-1, 4, 5, 6, 7, 8- hexahydroquinoline-3-carbonitrile derivatives (Q-1 to Q-14)
Charged enaminone (0.01mole), {[2-chloro-5-(4-substitutedphenyl) pyridin-3-yl] methylidene} propanedinitrile (0.01mole), piperidine (3-4 drops) and ethanol (10 ml) in a three-necked glass R.B.F. with a thermometer pocket, reflux condenser and mechanical stirrer. The reaction mass was refluxed with constant stirring. The reaction was monitored by TLC, after the completion of the reaction, it had been cooled to temperature and stirred for 10-15 min, and the resulting solid mass was filtered, washed with a small amount of ethanol, and dried. The crude product was purified by leaching in an equimolar mixture of chloroform and methanol to get the pure derivatives.

RESULTS AND DISCUSSION
Scheme-1 outlines the synthesis of intermediates I,II,III and IV used for the preparation of final compounds and outlines the synthesis of new quinoline derivatives (Q-1 to Q-14). The structures of the derivatives (Fig.-1) were confirmed on the basis of elemental analysis and spectral data studies.
Scheme-1: Synthesis of 2-Amino-4-(2-Chloro-5-(4-Substitutedphenyl) Pyridin-3-Yl)-1-(4-Substitutedphenyl)-7, 7-Disubstituted-5-Oxo-1, 4, 5, 6, 7, 8-Hexahydroquinoline-3-Carbonitrile Derivatives

Q1: M.P. 272-275°C, Yield 82%, IRcm⁻¹ 3463 (asym. N-H str.), 3333 (sym. N-H str.), 3025 (aromatic C-H str.), 2178 (-CN str.), 1660 (C=O str.), 1555 & 1345 (N=O str. of Ar-NO₂), 1568 & 1472 (C=C str. of aromatic ring), 722 (C-Cl str.), ¹H NMR δ ppm 2.20 (s, 3H, CH₃), 1.85-2.55 (m, 6H, 3xCH₂), 4.65 (s, 1H, CH), 5.60 (s, 2H, NH₂), 7.45-8.38 (m, 11H, Ar-H), Mol. For. C₂₇H₂₀ClN₅O₃, Mol.Wt. 497, Anal.data. (Cal/Found) C% 65.13/64.30, H% 4.05/4.26, N% 14.06/13.76. (Where R = H, Me, Cl; R₁, R’₁ = H, CH₃; R₂ = H, CH₃, OCH₃, Cl, Br₂, NO₂).

Q2: M.P. 278-282°C, Yield 77%, IRcm⁻¹ 3479 (asym. N-H str.), 3323 (sym. N-H str.), 3020 (aromatic C-H str.), 2183 (-CN str.), 1672 (C=O str.), 1565 & 1459 (C=C str. of aromatic ring), 497
705 (C-Cl str.), $^1$H NMR $\delta_{1}$ ppm 1.63 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$), 1.80-2.50 (m, 6H, 3xCH$_2$), 4.62 (s, 1H, CH), 5.49 (s, 2H, NH$_2$), 7.31-7.62 (m, 11H, Ar-H), Mol. For. C$_{28}$H$_{23}$ClN$_4$O, Mol. Wt. 466, Anal. data. (Cal/Found) C% 72.02/71.49, H% 4.96/4.76, N% 12.00/12.54. (Where R, R$_1$/R'$_1$, R$_2$ = -CH$_3$, -H, -H).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{R = H, Me, Cl, R$_1$/R'$_1$ = H, CH$_3$, R$_2$= H, CH$_3$, OCH$_3$, Cl, Br, NO$_2$}
\end{figure}

**Q3:** M.P. 284-287$^\circ$C, Yield 75%, IRcm$^{-1}$ 3474 (asym. N-H str.), 3339 (sym. N-H str.), 3015 (aromatic C-H str.), 2192 (-CN str.), 1668 (C=O str.), 1579 & 1455 (C=C str. of aromatic ring), 718 (C-Cl str.), $^1$H NMR $\delta_{1}$ ppm 1.60 (s, 3H, CH$_3$), 2.22 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$), 1.81-2.48 (m, 6H, 3xCH$_2$), 4.58 (s, 1H, CH), 5.51 (s, 2H, NH$_2$), 7.27-7.66 (m, 10H, Ar-H), Mol. For. C$_{28}$H$_{23}$ClN$_4$O, Mol. Wt. 480, Anal. data. (Cal/Found) C% 72.42/73.08, H% 5.24/5.43, N% 11.65/11.44. (Where R, R$_1$/R'$_1$, R$_2$ = -CH$_3$, -H, -H).

**Q4:** M.P. 277-280$^\circ$C, Yield 72%, IRcm$^{-1}$ 3481 (asym. N-H str.), 3345 (sym. N-H str.), 3025 (aromatic C-H str.), 2158 (-CN str.), 1671 (C=O str.), 1580 & 1452 (C=C str. of aromatic ring), 1235 & 1028 (C-O-C asym. & sym. str. of -OCH$_3$), 703 (C-Cl str.), $^1$H NMR $\delta_{1}$ ppm 1.58 (s, 3H, CH$_3$), 2.10 (s, 3H, CH$_3$), 1.81-2.51 (m, 6H, 3xCH$_2$), 4.58 (s, 1H, CH), 5.52 (s, 2H, NH$_2$), 7.27-7.59 (m, 10H, Ar-H), Mol. For. C$_{29}$H$_{25}$ClN$_4$O$_2$, Mol. Wt. 496, Anal. data. (Cal/Found) C% 70.08/69.29, H% 5.07/5.20, N% 11.27/10.95. (Where R, R$_1$/R'$_1$, R$_2$ = -CH$_3$, -H, -OMe).

**Q5:** M.P. 274-278$^\circ$C, Yield 69%, IRcm$^{-1}$ 3470 (asym. N-H str.), 3329 (sym. N-H str.), 3029 (aromatic C-H str.), 2157 (-CN str.), 1672 (C=O str.), 1560 & 1475 (C=C str. of aromatic ring), 708 (C-Cl str.), 568 (C-Br str.), $^1$H NMR $\delta_{1}$ ppm 1.69 (s, 3H, CH$_3$), 2.15 (s, 3H, CH$_3$), 1.80-2.50 (m, 6H, 3xCH$_2$), 4.66 (s, 1H, CH), 5.53 (s, 2H, NH$_2$), 7.30-7.82 (m, 10H, Ar-H), Mol. For. C$_{29}$H$_{25}$BrClN$_4$O, Mol. Wt. 545, Anal. data. (Cal/Found) C% 61.61/60.95, H% 4.06/4.26, N% 10.26/10.78. (Where R, R$_1$/R'$_1$, R$_2$ = -CH$_3$, -H, -Br).

**Q6:** M.P. 269-272$^\circ$C, Yield 81%, IRcm$^{-1}$ 3462 (asym. N-H str.), 3329 (sym. N-H str.), 3027 (aromatic C-H str.), 2160 (-CN str.), 1677 (C=O str.), 1572 & 1460 (C=C str. of aromatic ring), 715 (C-Cl str.), $^1$H NMR $\delta_{1}$ ppm 2.17 (s, 3H, CH$_3$), 1.65-2.34 (m, 6H, 3xCH$_2$), 4.64 (s, 1H, CH), 5.29 (s, 2H, NH$_2$), 7.10-7.65 (m, 11H, Ar-H), Mol. For. C$_{29}$H$_{25}$Cl$_2$N$_4$O, Mol. Wt. 487, Anal. Data. (Cal/Found) C% 66.54/63.65, H% 4.14/3.89, N% 11.50/10.98. (Where R, R$_1$/R'$_1$, R$_2$ = -Cl, -H, -H).

**Q7:** M.P. 268-271$^\circ$C, Yield 68%, IRcm$^{-1}$ 3472 (asym. N-H str.), 3323 (sym. N-H str.), 3015 (aromatic C-H str.), 2185 (-CN str.), 1666 (C=O str.), 1560 & 1475 (C=C str. of aromatic ring), 1253 & 1018 (C-O-C asym. & sym. str. of -OCH$_3$), 711 (C-Cl str.), $^1$H NMR $\delta_{1}$ ppm 2.17 (s, 3H, CH$_3$), 3.81 (s, 3H, OCH$_3$), 1.65-2.34 (m, 6H, 3xCH$_2$), 4.64 (s, 1H, CH), 5.29 (s, 2H, NH$_2$), 7.10-7.65 (m, 10H, Ar-H), Mol. For. C$_{28}$H$_{22}$Cl$_2$N$_4$O$_2$, Mol. Wt. 517, Anal. Data. (Cal/Found) C% 65.00/66.27, H% 4.29/4.12, N% 10.83/10.41. (Where R, R$_1$/R'$_1$, R$_2$ = -Cl, -H, -OMe).

**Q8:** M.P. 270-273$^\circ$C, Yield 67%, IRcm$^{-1}$ 3468 (asym. N-H str.), 3314 (sym. N-H str.), 3022 (aromatic C-H str.), 2187 (-CN str.), 1657 (C=O str.), 1583 & 1464 (C=C str. of aromatic ring), 718 (C-Cl str.), 560 (C-Br str.), $^1$H NMR $\delta_{1}$ ppm 2.14 (s, 3H, CH$_3$), 1.62-2.34 (m, 6H, 3xCH$_2$), 4.53 (s, 1H, CH), 5.68 (s, 2H, NH$_2$), 7.27-7.66 (m, 10H, Ar-H), Mol. For. C$_{28}$H$_{22}$Cl$_2$N$_4$O$_2$, Mol. Wt. 517, Anal. Data. (Cal/Found) C% 65.00/66.27, H% 4.29/4.12, N% 10.83/10.41. (Where R, R$_1$/R'$_1$, R$_2$ = -Cl, -H, -OMe).

CARBONITRILE DERIVATIVES

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The prepared derivatives Q1-Q14 were screened against microorganism species at 1000 ppm concentration. The results of antibacterial screening reported in the Table-1 show that methyl group-containing compounds Q1-Q14 were active against bacterial species E. coli, NO3-group containing compounds Q1 and Q9 and methoxy group-containing compounds Q7 and Q12 are found active against B. subtilis. Br group-containing compounds Q8, Q10, and Q14 and NO2 group-containing compounds Q1 and Q13 are active against S. aureus. From the antifungal assay, it has been also noticed...
that compounds Q1, Q4, Q10, and Q11 are found to be active against \textit{F.oxy\textit{s}porium}. Compounds Q2, Q3, Q13, and Q14 having methyl substituents show good activity against \textit{A.niger} and. Halogen-containing compounds Q5, Q6, Q7, and Q10 show good activity against \textit{R.oryzae}. Compounds show significant efficacy against the conventional fungicidal Griseofulvin.

Table-1: Antimicrobial Activity of Synthesis of 2-amino-4-(2-chloro-5-(4-substitutedphenyl) pyridin-3- yl)-1-(4-substitutedphenyl)-7, 7-disubstituted-5-oxo-1, 4, 5, 6, 7, 8- hexahydroquinoline-3-carbonitrile derivatives

| Compound | Inhibition Zone (in mm) against |
|----------|-----------------------------|
|          | \\textit{E.coli} | \\textit{B.subtilis} | \\textit{S.aureus} | \\textit{F.oxyporum} | \\textit{A.niger} | \\textit{R.oryzae} |
| Q1       | 20  | 23  | 24  | 25  | 19  | 20  |
| Q2       | 24  | 18  | 19  | 21  | 22  | 19  |
| Q3       | 22  | 19  | 17  | 18  | 22  | 21  |
| Q4       | 23  | 17  | 19  | 13  | 20  | 18  |
| Q5       | 18  | 20  | 19  | 22  | 18  | 26  |
| Q6       | 25  | 18  | 19  | 18  | 17  | 24  |
| Q7       | 20  | 25  | 19  | 20  | 16  | 25  |
| Q8       | 18  | 20  | 22  | 20  | 19  | 24  |
| Q9       | 20  | 23  | 15  | 18  | 16  | 22  |
| Q10      | 20  | 20  | 23  | 23  | 18  | 25  |
| Q11      | 24  | 20  | 19  | 22  | 16  | 20  |
| Q12      | 23  | 24  | 17  | 20  | 18  | 21  |
| Q13      | 19  | 20  | 23  | 18  | 22  | 20  |
| Q14      | 16  | 25  | 22  | 19  | 22  | 20  |
| Ciprofloxacinc | 35  | 34  | 33  | --- | --- | --- |
| Ampicillin | 28  | 30  | 30  | --- | --- | --- |
| Griseofulvin | --- | --- | --- | 28 | 26 | 30 |

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