# Synthesis, *In Vitro* Investigation and Molecular Docking Studies of Novel Quinoline Analogues: A Drug repositioning approach from Amodiaquine

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## ABSTRACT

Drug repositioning involves the investigation of existing drugs and their derivatives for new therapeutic purposes. Due to the widespread biological importance of quinoline, new series of novel quinolines (modified amodiaquine) analogues were synthesized. $^{1}$H NMR, $^{13}$C NMR and FT-IR were used for characterization. In antibacterial screening, analogue AA1 showed a 12 ± 1.1 mm and 14 ± 0.5mm zone of inhibition against *S. aureus* and *P. aeruginosa* respectively. Our analogues (AA1 and AA22) showed a 13 mm zone against *E. Coli* and AA2 has a 12 ± 0.5 mm zone against *Bacillus subtilis*. Excellent activity against fungal strains i.e. *C. albicans* (13 ± 0.5mm) zone was shown by analogue AA1 and AA2. Analogue AA2 showed 100% inhibition with $IC_{50}$ 11.1 ± 1.1 µg/ml against the HeLa cell line and binding energy against 1PFK (-6.123772 kcal/mol) and 1TUP (-6.512972 kcal/mol) in the docking study. New analogues have excellent activity against different microbial strains and Hela cell-line as compared to their parent molecule.

**Keywords:** Quinoline analogues, inhibitory concentration, MTT assay, amodiaquine.

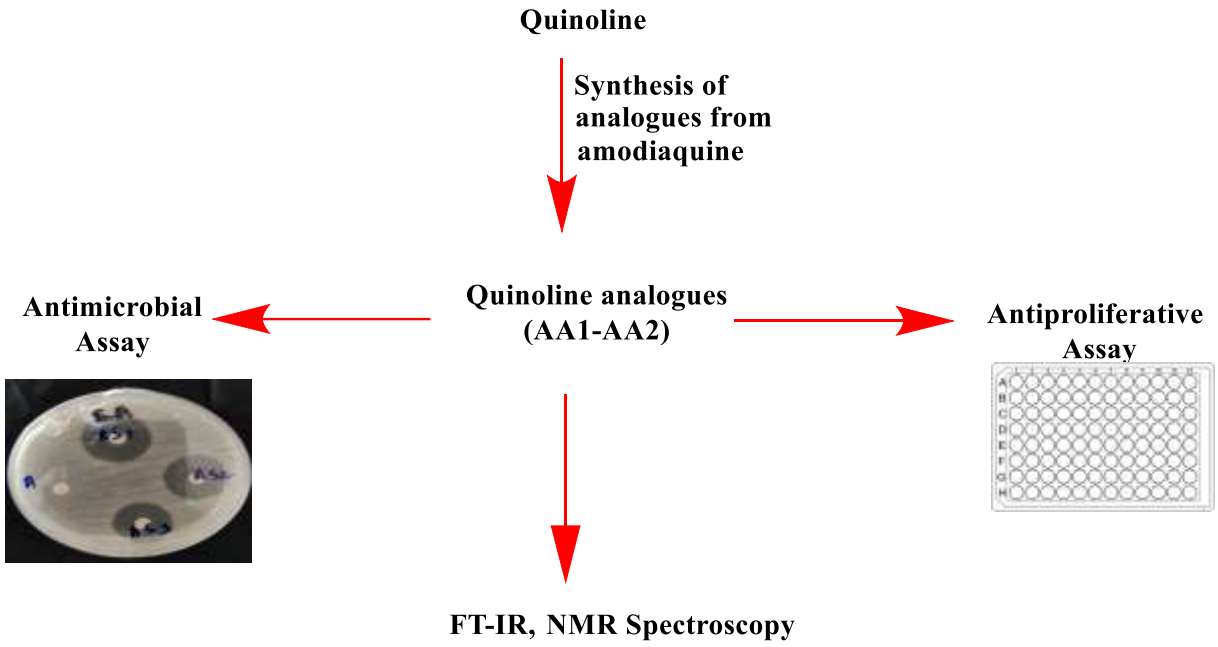



**Figure 1.** Graphical Abstract

## INTRODUCTION

Drug repositioning is a more efficient approach than traditional approaches in drug development. It has the potential to develop new drugs in pharmaceutical research and industry at a low cost. Searching for valid lead substances from FDA-approved drugs and structurally altering or derivatizing them has proven successful in drug development in recent decades by using a drug repositioning approach.[1,2] Heterocyclic compounds like quinoline analogues emerged as a vital class of biologically active N-heterocyclic compounds in the field of medicinal chemistry research for the development of newer biologically active molecules for treating several types of infections.[3] Medicinal chemists worked on their derivation due to their significant role in many pharmacological activities like anticonvulsant, anti-malarial, anti-bacterial, anti-tuberculosis, anti-fungal, anti-viral, anti-inflammatory, anti-plasmodial, antidiabetic and anticancer.[4]

Some of the prominent quinoline based analogues levofloxacin, ciprofloxacin, moxifloxacin, norfloxacin, ofloxacin, chloroquine, amodiaquine, hydroxychloroquine, mefloquine, quinine, quinidine, streptonigrin etc. According to WHO, annually 13.6% of deaths are caused due to several microbial infections, which is a serious health issue. Globally almost 15 million deaths per year in 2030 were estimated due to different cancers.[5] In developing countries and spread of cancer is a major threat to the world's public health care system soon.[6] Some of the novel 3-(quinolin-4-ylamino) benzene-sulfonamidesAQ3 have good inhibitory potential against carbonic anhydrase[7]. In another study, the synthesized benzene sulfonyl-substituted fluoroquinolones have been reported with good antibacterial properties.[8] Most of the quinoline-based anticancer drugs target the topoisomerase enzyme system.[9] They can be administered with other anti-cancer drugs to potentiate the drug's effects.[10] Pelitinib, a well-known quinoline derivative, got approved as an anti-cancer drug due to its irreversible inhibition of epidermal growth factor receptors through DNA intercalation.[11] Now researchers synthesized several other quinoline derivatives that are considered promising anti-tumour agent.[12]

In the present study, we reported new series of synthetic quinoline-based analogues from already antimalarial drugs i.e., amodiaquine as a drug repositioning approach. These quinoline analogues were evaluated *In vitro* for their antimicrobial potential against different strains and for cytotoxic potential against the HeLa cell line using an MTT assay. Most active

antiproliferative compounds were further processed for docking studies to find their binding energy against 1TUP and 1PFK targets,

## MATERIAL AND METHODS

### General

The chemical 4-((7-chloroquinolin-4-yl) amino)-2-( (diethylamino) methyl) phenol (AS0) used in this study was gifted by Delux Chemical. All the sulfonyl chlorides and acyl chlorides used in this study were purchased from Merck KGaA, Darmstadt, Germany. All the solvents including methanol, acetonitrile, n-Hexane and Ethyl acetate were purchased by Merck KGaA, Darmstadt, Germany. The melting point of all synthetic quinoline-based analogues was calculated by the melting point apparatus. Silica gel (60–120 mesh) Merck was used for purifying the synthesized quinoline analogues. During the reaction progress of the reaction was evaluated by Thin Layer Chromatography. A UV lamp was used and the RF value was calculated. NMR analyses were carried out on Bruker Avance 400 MHz spectrometer using $CDCl_3$, as a solvent. For the interpretation of spectra coupling constants, *J* in Hz was directly calculated from the spectra. Splitting patterns are designated as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), and *m* (multiplet). FTIR spectra were to detect the presence of new functional groups.[13]

### Statistical Analysis

The mean zone of inhibition, standard deviation and LD50 values were calculated using Microsoft Excel 2015.[14] The results (per cent inhibition and IC50 value) of quinoline analogues were analyzed by using software i.e., Soft-Max Pro (Molecular Device, USA).[15]

### General Scheme

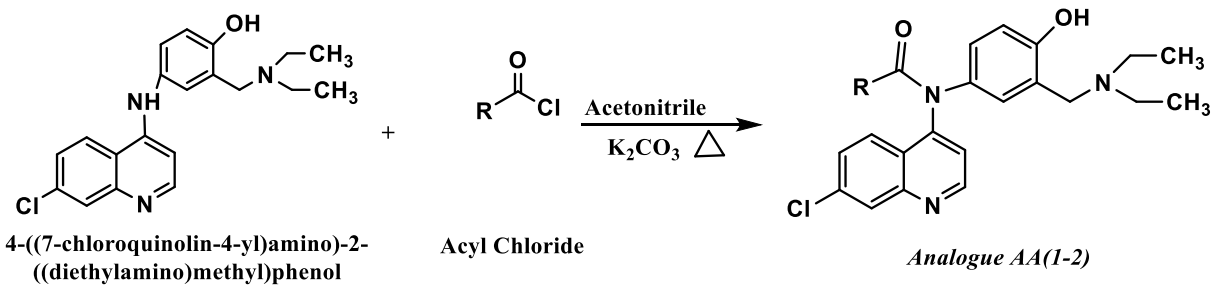



**Figure 2. General Scheme. Synthesis of Quinoline Analogue AA (1-2)**

Acetonitrile was used as a solvent in the pre-dried round bottom flask in which we added 5mmol 4-((7-chloroquinolin-4-yl)amino)-2-((diethylamino)methyl)phenol (AS0) and refluxed it for two hours. After two hours we added potassium bicarbonate 5mmol and reflux continued for more than one hour, then we added 5mmol of suitable Acyl chloride in the reaction flask and stirring was continued for almost 36 to 60 hours at approximately 70 °C. Furthermore, the column chromatography technique was used for the purification of the product.[13]

***AA1: Synthesis of N-(7-chloroquinolin-4-yl)-N-(3-((diethylamino)methyl)-4-hydroxyphenyl)benzamide***

Acetonitrile was used as a solvent in the pre-dried round bottom flask along with 5mmol 4-((7-chloroquinolin-4-yl)amino)-2-((diethylamino)methyl)phenol (AS0) and refluxed it for two hours. After two hours we added potassium bicarbonate (5mmol) and reflux again continued for more than one hour. After that, we added 5mmol suitable 4-(chlorocarbonyl)benzene-1-ylium in the reaction flask and stirring was continued for almost 36 hours at approximately 70 °C.

***AA2: Synthesis of N-(7-chloroquinolin-4-yl)-N-(3-((diethylamino)methyl)-4-hydroxyphenyl)-2-(trifluoromethyl)benzamide***

We added 5mmol 4-((7-chloroquinolin-4-yl)amino)-2-((diethylamino)methyl)phenol (AS0) in acetonitrile and refluxed it for two hours, then we added potassium bicarbonate 5mmol and the reflux continued for more than one hour. Finally, we added (5mmol) trifluromethyl benzoyl chloride in the reaction flask and stirring was continued for almost 60 hours at approximately 70 °C.

**Antibacterial Assay**

In this work, the antibacterial activity of the synthesized analogue AA1 and AA2 was investigated using the disc diffusion method on two Gram-positive (*Staphylococcus aureus ATCC#6538, Bacillus subtilis ATCC#6633*). The two Gram-negative bacterial strains i.e., *Escherichia coli ATCC#15224, and Pseudomonas aeruginosa ATCC#4619*. Nutrient Agar was dissolved in distilled water to prepare the medium. Media was poured into sterile plates (25 mL) and placed till solidification. Concentrations of 2 mg/mL in DMSO were used for sample

preparation. Then 5 µL of dissolved samples were loaded onto a filter paper disc and incubate for 24 hours. The diameter (mm) of the zone surrounding the disc was measured. As a reference, AS0 and as a positive control cefixime were used, while DMSO was used as a negative control.[14]

**Antifungal Assay**

The antibacterial activity of our synthesized quinoline-based analogues was measured in mm by disc diffusion method with Islam et al 2013 protocols, against two strains of fungal species: two *Candida albicans* (ATCC 9002) and *Candida parapsilosis*). Sterile Sabouraud Dextrose Agar in distilled water was poured into plates and plates were allowed to solidify. The synthesized quinoline analogues samples were prepared at the concentrations of 2mg per 1 ml DMSO. The 5 µL of the dissolved sample was poured onto discs and placed in a Petri plate. Incubated for 28 hrs at 37°C. The zone of inhibition around the disc was measured in (mm). Clotrimazole was used as a positive control, while DMSO was -ive control.[14]

**Brine Shrimp Lethality Assay**

The brine shrimp lethality screening of newly synthesized quinoline analogues was determined by using an already defined protocol with slight modification. The stock solution of samples was made by dissolving 1 mg of each synthesized quinoline analogue in 10µl of Dimethyl sulfoxide and making a final volume of up to 1ml with artificial seawater so the final concentration was 1 mg/ml. Lethality and lethal dose ($LD_{50}$) of the test samples were calculated, using Microsoft Excel software. The experiment was performed in triplicate and the mean value was used.[11,16]

**MTT assay**

Cytotoxic activity of all synthetic quinoline analogues was determined in a 96-well plate by using the MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5 diphenyltetrazolium bromide) colourimetric assay as defined by Mosmann,1983. HeLa cell lines were used and $IC_{50}$ of those samples was calculated whose inhibition value was above fifty per cent. The experiment was performed in triplicate and an inhibitory fifty per cent value was calculated.[15]

Per cent inhibition of sample =

$$100 - \frac{(\text{mean of O.D of synthetic quinoline analogue} - \text{mean of O.D of negative control}) * 100}{(\text{mean of O.D of positive control} - \text{mean of O.D of negative control})}$$

The results (per cent inhibition and IC50 value) of quinoline analogues were analyzed by using software i.e., Soft- Max Pro (Molecular Device, USA).

**Molecular Docking Studies**

For calculation of the binding energy crystal structure of the two targeted proteins i.e. 1TUP and 1PFK were retrieved from PDB protein bank. Structures were drawn in Chem Draw Professional 15.0. Analogues from each series with maximum activity and least $IC_{50}$ value were docked against 1TUP and 1PFK using MOE software to know the binding interaction as Quinoline analogues have good anticancer properties. Physicochemical properties and pharmacokinetic properties of all synthesized analogues were online predicted via http://www.swissadme.ch.[17]

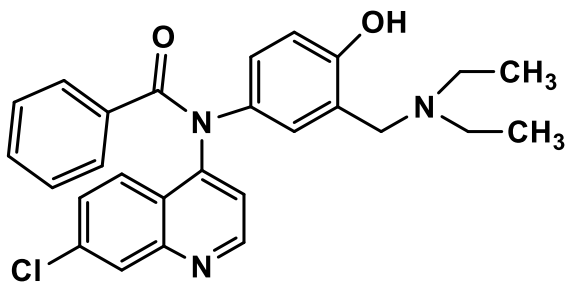
## RESULT AND DISCUSSION

### Synthesis

A series of quinoline analogues were made by using acyl chlorides in optimum reaction conditions to synthesize novel 4-amino quinoline analogues with potential anti-microbial (bacterial, fungal) and anti-cancer properties.[13]

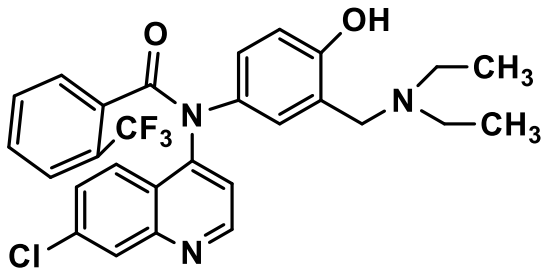
*AA1: N-(7-chloroquinolin-4-yl)-N-(3-((diethylamino)methyl)-4-hydroxyphenyl)benzamide:*

Purified product yield was 73% and RF value was 0.09. Its melting point was 172°C. IR (cm-1): 1680 (C=O), 787 (C-Cl) $^{1}$H NMR (400 MHz, Chloroform-d) δ 3.70 (s, 4H), 7.23 (d, J = 9.5 Hz, 3H), 7.91 (s, 2H), 8.42 (s, 1H), 8.10 (s, 1H), 7.47 (t, J = 7.3 Hz, 2H), 7.40 (t, J = 7.5 Hz, 4H), 7.15 (d, J = 2.6 Hz, 2H), 7.10 (d, J = 2.6 Hz, 1H), 6.82 (d, J = 8.6 Hz, 2H), 6.44 (s, 1H), 2.62 (d, J = 7.1 Hz, 4H), 1.10 (s, 6H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 172.62 1C, 157.24 1C, 153.50 1C, 143.57 1C, 141.29 1C, 138.35 1C, 134.82 1C, 131.45 1C, 129.67 2C, 128.50 1C, 128.03 3C, 126.75 1C, 125.41 1C, 124.96 1C, 122.54 1C, 121.51 1C, 117.21 1C, 116.03 1C, 99.67 1C, 55.67 1C, 46.25 2C., 29.68 1C, 10.73 2C.



*AA2: N-(7-chloroquinolin-4-yl)-N-(3-((diethylamino)methyl)-4-hydroxyphenyl)-2-(trifluoromethyl)benzamide:*

Its purified product yield was 62%, while RF value was 0.38. Its melting point was 127°C. IR (cm-1): 1680 (S=O), 1182 (C-F), 787 (C-Cl) $^{1}$H NMR (400 MHz, Chloroform-d) δ 8.83 – 8.70 (m, 1H), 8.42 (d, J = 5.6 Hz, 1H), 8.13 – 7.80 (m, 3H), 7.76 – 7.29 (m, 6H), 6.92 (d, J = 5.5 Hz, 1H), 3.68 (d, J = 59.3 Hz, 2H), 2.64 – 2.49 (m, 4H), 1.12 – 0.97 (m, 6H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 164.88 1C, 157.02 1C, 150.39 1C, 148.66 1C, 137.68 1C, 135.91 1C, 131.97 1C, 130.80 2C, 127.45 2C, 127.11 1C, 127.06 1C, 126.26 1C, 126.18 1C, 125.29 1C, 125.17 1C, 125.03 1C, 124.68 1C, 123.25 1C, 123.13 1C, 122.55 1C, 122.33 1C, 117.86 1C, 102.08 1C, 51.56 1C, 46.87 2C, 11.04 2C.




**The novelty of compounds:**

For confirming the novelty of compounds, we have conducted retro studies by using Reaxy along with online tools that are http://www.swisssimilarity.ch/ and http://www.chemspider.com/. It was confirmed that our compounds were novel and these compounds are not previously reported. Their data is not available on these data sources [17].

**Antibacterial assay**

In this study, our synthesized quinoline analogues have good activity against bacterial strains. Against *S. Aureus* analogue AA1 has a 12±1.1 mm zone of inhibition while analogue AA2 has a 1±0.5 mm zone of inhibition respectively. Analogue AA1 possess a 14±0.5 mm zone while analogue AA2 has a 12±0.5 zone of inhibition against *P aeruginosa* respectively. AA1 exhibit a 13±1.1 mm zone against *E coli*. Against *Bacillus subtilis* strain AA1 and AA2 showed a 11mm inhibitory zone. The parent compound (amodiaquine) did not show any activity against any bacterial strain at a given concentration as described in **Table 1**.

**Antifungal assay**

The antifungal potential was explained in **Table 1**. Our synthetic quinoline analogues showed

excellent activity against fungal strains. Analogue AA1 and AA2 showed a 13 mm inhibitory zone against *C albicans*, while AA1 and AA2 11mm zone of inhibition against *Candida parapsilosis.* as explained in Table 1.

**Table 1.** *In vitro* antimicrobial activity of newly synthesized quinoline analogues. Cefixime was used as a standard drug (*S. aureus*=20mm±0.5, *P. aeruginosa*=18mm±0.5, *E. coli*=17mm±0.5, *B. subtilis*=18mm±0.5) and Clotrimazole was used as a standard drug in the antifungal assay (*C. albicans*=22mm±0.1, *C. parapsilosis*=19mm±0.5)

| **Sample** | ***S.aureus*** | ***P. aeruginosa*** | ***E. Coli*** | ***B. subtilis*** | ***C. albicans*** | ***C. parapsilosis*** |
|---|---|---|---|---|---|---|
| AA1 | 12±1.1 | 14±0.5 | 13±1.1 | 10±0.5 | 13±0.5 | 11±0.5 |
| AA2 | 11±0.5 | 12±0.5 | 13±0.5 | 12±0.5 | 13±0.5 | 11±1 |
| AS0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DMSO | 0 | 0 | 0 | 0 | 0 | 0 |

**Brine shrimp lethality assay (BSLA*)***

As mentioned in **Table 2**, our synthesize quinoline analogues showed excellent activity against brine shrimps. Analogue AA1 has 100% mortality with $LD_{50}$ 3.1 µg/ml., while Analogue AA2 exhibited 9.4 µg/ml $LD_{50}$.

.**Table 2.** Brine shrimp lethality activity at 50µg/ml and $LD_{50}$ value, of quinoline analogues, Doxorubicin (standard drug) has $LD_{50}$=3.1 µg/ml.

| **Sample** | **Concentration** | **% Mortality** | **LD $_{50}$ µg/ml** |
|---|---|---|---|
| AA1 | 50µg/ml | 100 | 3.1 |
| AA2 | 50µg/ml | 100 | 9.4 |
| AS0 | 50µg/ml | 56.7 | 38.63 |
| DMSO | | 0 | 0 |

**MTT assay (Anti-Cancer Screening) against HeLa cell line**

Anticancer activity against HeLa cells was mentioned below in **Table 3**. Quinoline-based synthetic analogues showed good activity against cancer cell lines. Analogue AA1 has 87.4% inhibition with $1C_{50}$ 12.7 µg/ml, while analogue AA2 showed 100% inhibition with $IC_{50}$ 11.1 µg/ml. Amodiaquine (AS0) 67.7% inhibition against the HeLa cell line.

**Table 3.** Antiproliferative activity against HeLa cell line using MTT assay of quinoline analogues. Doxorubicin (standard drug) has $IC_{50}$=0.9±0.14 µg/ml.

| Sample Code | Concentration µg/ml | % Inhibition | $IC_{50}$ µg/ml ± SD |
|---|---|---|---|
| AA1 | 30 | 87.4 | 12.7 ± 1.0 |
| AA2 | 30 | 100 | 11.1 ± 1.1 |
| AS0 | 30 | 67.7 | 24.1 ± 0.1 |

**Molecular Docking studies**

Docking study revealed that analogue AA21 has excellent affinity against 1TUP (Tumor suppressor P53 complexed with DNA) (Figure 2) and 1PFK (Crystal structure of the complex of phosphofructokinase from Escherichia Coli with its reaction product) (Figure 3) protein as presented in table 4. Both proteins have a prominent role in cancer. All their analogues have excellent inhibitions against the HeLa cell line and docking data also supports these results, as their binding energy is -6.123772 kcal/mol (IPFK) and -6.512972kcal/mol (1TUP). It also has a good lipophilic value and bioavailability score (0.55) as explained in Table 5.

**Table 4.** Molecular Docking Results; Binding affinity of newly synthesized quinoline analogues having best result in MTT assay, against 1PFK and 1TUP (proteins using MOE software)

| **Docking Results against 1PFK** | | **Docking Results against 1TUP** | |
|---|---|---|---|
| Analogue | Affinity (kcal/mol) | Analogue | Affinity (kcal/mol) |
| AA2 | -6.123772 | AA2 | -6.512972 |

**Table 5.** Physicochemical and Pharmacokinetic Properties

| **Physicochemical Properties** | **AA1** | **AA2** | **Pharmacokinetics Properties** | **AA1** | **AA2** |
|---|---|---|---|---|---|
| **Formula** | C27H26 ClN3O2 | C28H25 ClF3N3O2 | **GI absorption** | Low | Low |
| **Molecular weight** | 459.97 g/mol | 527.97 g/mol | **BBB permeant** | No | No |
| **Num. heavy atoms** | 33 | 37 | **P-gp substrate** | No | No |
| **Num. arom. heavy atoms** | 22 | 22 | **CYP1A2 inhibitor** | No | No |
| **Fraction Csp3** | 0.19 | 0.21 | **CYP2C19 inhibitor** | Yes | Yes |
| **Num. rotatable bonds** | 8 | 9 | **CYP2C9 inhibitor** | Yes | Yes |
| **Num. H-bond acceptors** | 4 | 7 | **CYP2D6 inhibitor** | Yes | Yes |
| **Num. H-bond donors** | 1 | 1 | **CYP3A4 inhibitor** | Yes | Yes |
| **Molar Refractivity** | 135.22 | 140.22 | **Log Kp (skin permeation)** | -5.60 cm/s | -5.55 cm/s |
| **TPSA** | 56.67 Å² | 56.67 Å² | **Bioavailability Score** | 0.55 | 0.55 |

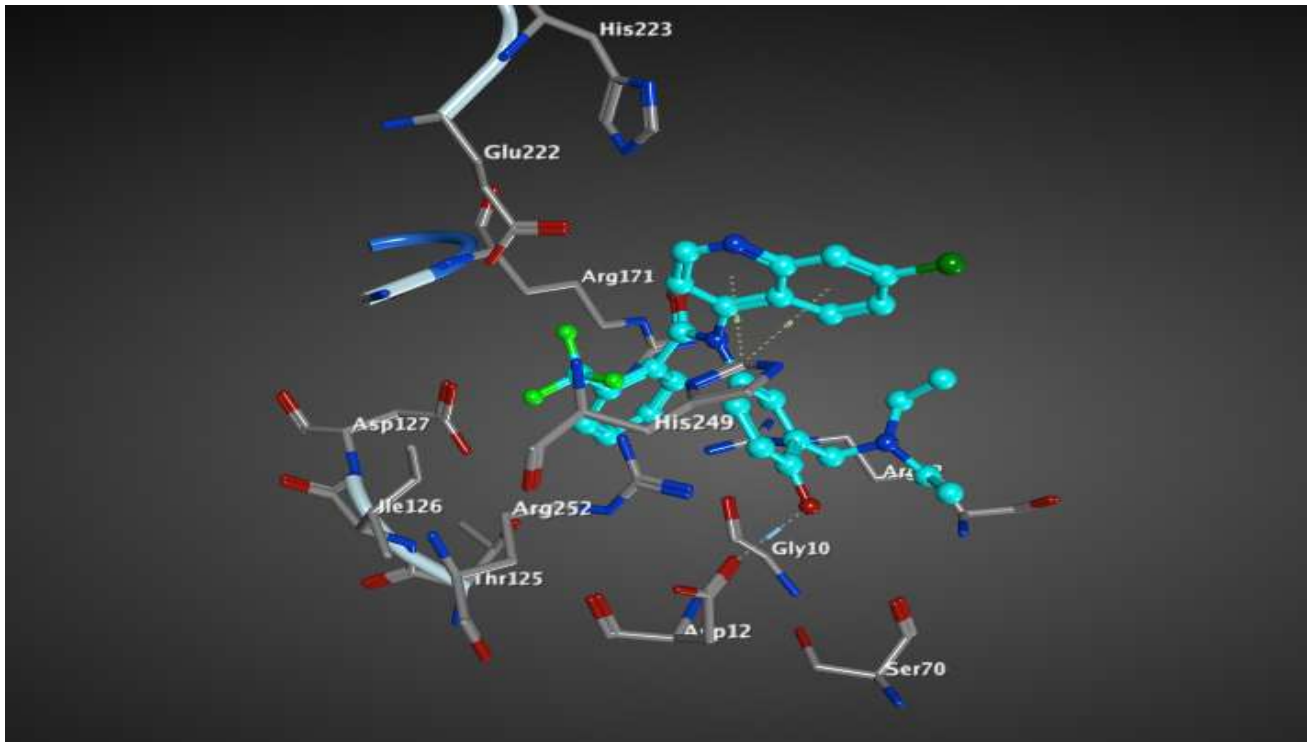



**Figure 3.** Analogue AA2 binding affinity with 1PFK protein (Crystal structure of the complex of phosphofructokinase from Escherichia Coli with its reaction product) is --6.1237721 kcal/mol using MOE software**.**

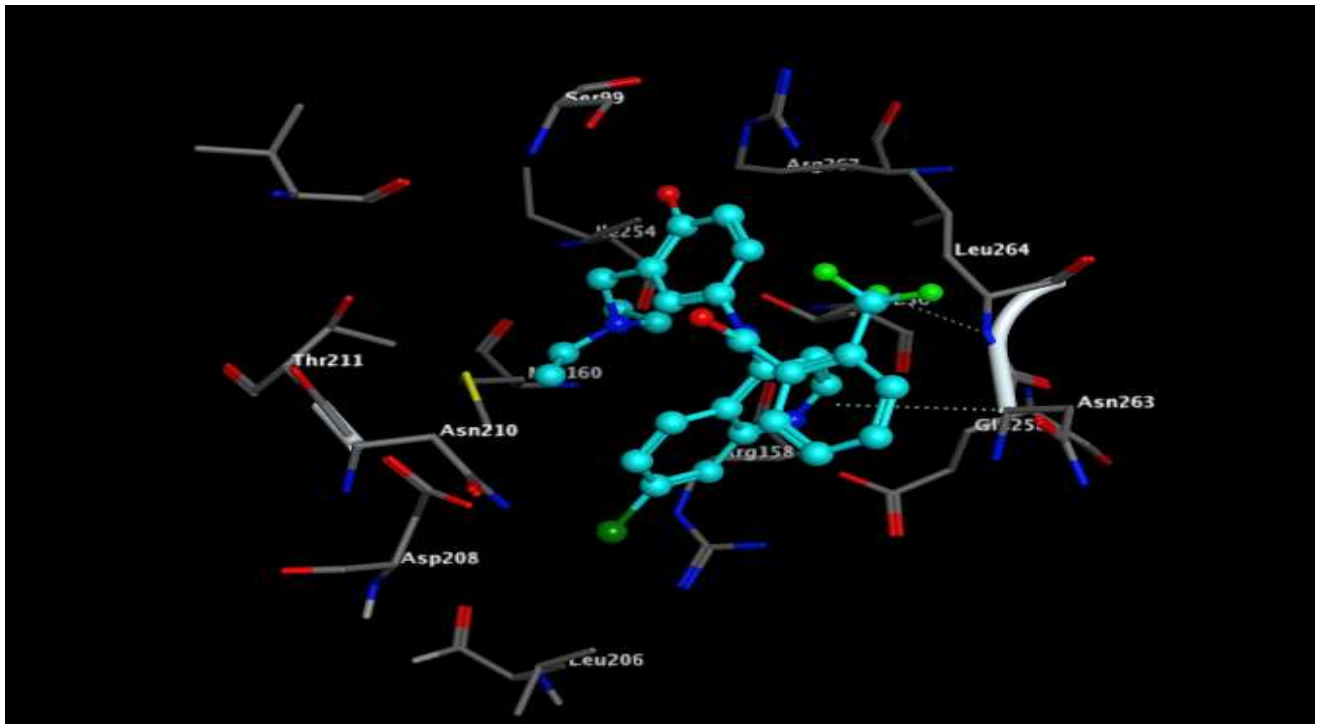



**Figure 4.** Analogue AA2 binding affinity with 1TUP (Tumor suppressor P53 complexed with DNA) is -6.5129719kcal/mol using MOE software**.**

Compounds with quinoline nuclei have well-known pharmacologically active properties, and are used to treat various lethal diseases, for example, malaria, and cancer. In our study, we synthesized biologically active quinoline analogues at 4 amino positions from commercially available drug *i.e*., amodiaquine as a drug repositioning approach. With the help of free online chemical databases., http://www.swisssimilarity.ch/ and http://www.chemspider.com/, it was confirmed that these compounds are not previously reported.[28,29] These two quinoline analogues were investigated (*in-vitro*) for their antibacterial significance against four Gram +ive and Gram -ive strains and two fungal strains. In the antimicrobial assay zone of inhibition in mm was measured to calculate the inhibitory effect of analogues. The results are already described in **Table 1** given above.

Our synthesized quinoline analogues have good antibacterial activity. We calculated a 14±0.5 mm inhibitory zone against bacteria *P. aeruginosa* and a 13 mm zone of inhibition against bacteria *E. coli* by analogue AA1 as compared to Cornett et. al. 1988 and Abdi et al. 2021.[18,19] Other studies also confirm that our quinoline-based analogues are more potent against bacterial strains as a zone of inhibition in our study was more. Substitution at the amino

position will improve the antimicrobial potential of quinolines.[20,13] In antifungal screening against two fungal strains our synthetic quinoline analogue AA1 has a 13 mm inhibitory zone against *C. albicans* and a 11 mm inhibitory against *C. parapsilosis* as described in **Table 1**. Our synthesized quinoline-based analogues showed good anti-fungal activity rather than Kumar et. al. 2009 study.[21]

In the brine shrimp lethality assay our quinoline analogues show good activity as shown in Table 2. Our quinoline analogue AA1 and AA2 showed 100% mortility in brine shrimp lethality assay as compared to other studies conducted by Khan et. al. 2000 and Zoonish et. al. 2020.[22,23] Our synthetic quinoline analogues showed excellent activity against the Hela Cell line (**cervical cancer cell line**). Quinoline analogue AA1 showed excellent activity with less $IC_{50}$ (11.1 ug/ml) value as compared to other studies.[24,25,26] As per predicted data, all analogues have a good bioavailable score and good lipophilicity that will improve their membrane permeation and will ultimately lead to improved levels in the bloodstream. For the mechanism of action, we used docking data, and it was proposed that analogues exhibit anticancer properties due to Tumor suppressor P53 complex and phosphofructokinase enzyme inhibition. [27]

## CONCLUSION

In conclusion, our novel quinoline analogues have a good zone of inhibition against bacterial and fungal strains. In anticancer screening against HeLa cell line analogue AA2 showed 100 % inhibition. The increase in activity as compared to the parent molecule is due to substitution at the 4-amino. These compounds can be further processed for antimicrobial and anticancer studies as they have good *in vitro* screening results as well as molecular docking results.

**Abbreviations**

| | |
|---|---|
| % I: | Percent inhibition |
| Amodiaquine | AS0 |
| *C albicans* | *Candida albicans* |
| *C krusei* | *Candida krusei* |
| DMSO: | Dimethyl sulphoxide |
| *E. coli:* | *Escherichia coli* |
| $IC_{50}$: | Concentration for 50 percent Inhibiton |
| LD: | Lethal dose |
| mm | millimeter |
| *P. aeruginosa:* | *Pseudomonas aeruginosa* |
| *S. aureus:* | *Staphylococcus aureus* |
| TLC: | Thin-layer chromatography |

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***Conflict of interest:*** The authors declare that there is no conflict of interest.

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