Evaluation of antimalarial and antibacterial activities of some newly synthesized 4-aminoquinoline analogs.

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ABSTRACT:

In this article, three series of 21, 4-aminoquinoline derivatives bearing a quinoline moiety (2a-b, 3a-c, and 4a-b) have been designed, synthesized, and evaluated as an antimalarial and antibacterial agent against the potent marketed drug. Compound (24)2c, (31)2i, 42(2o) and 32(3a) were found to be the most potent of all of the compounds tested, with an MIC value of 1μg/mL to 32μg/mL against several Gram-positive (*Staphylococcus aureus* 5345, *Salmonella typhi* 2501), *Bacillus cereus* 2217 and Gram-negative *Escherichia coli* 2931 strains of bacteria. In addition, compound 32(3a) showed potent inhibitory activity (MIC = 1μg/mL) against *Salmonella typhi* 2501, *Bacillus cereus* 2217 and Gram-negative *Escherichia coli* 2931 strains of bacteria indicating that its antibacterial spectrum is similar to those of the positive control ciprofloxacin. Structure-activity relationships (SAR) analyses and docking studies implicated the amino and amido side chain group formation responsible for in increasing the antimicrobial potency of the quinoline compounds. Five analogs were tested for inhibition of β-hematin formation as an antimalarial activities using standard chloroquine shows a good inhibition.

Keywords: Antimalarial, antibacterial, quinoline, β-hematin

1. Introduction:

Different and dangerous diseases are caused by protozoan and bacterial infections. A number of variables, such as newly emerging infectious diseases and an increase in the number of microbial pathogens that are multi-drug resistant, contribute to the fact that treating infectious diseases continues to be a significant and difficult problem. Despite the abundance of antibiotics and chemotherapeutics now used in medicine, the emergence of both old and new forms of antibiotic resistance produced in recent decades highlighted a significant medical need for new classes of antimalarial and antimicrobial drugs. It is widely acknowledged that novel compounds with antimalarial and antimicrobial activity are urgently needed.[1]

The mortality rates from malaria have declined over the past ten years, but they are still incredibly high for a disease that is entirely avoidable and treatable. The majority of drugs used in clinical settings, such as chloroquine, amodiaquine, pamaquine, and mefloquine, are subject to the development and spread of drug resistance, which is a substantial contributing factor to the problem. If new medications are not introduced to the market, malaria could become unmanageable and have catastrophic consequences.[2]

The identification of new antibacterial drugs can help to lessen these medical issues. In the modern era, one of the primary objectives of medicinal chemistry research is the creation of novel antimicrobial drugs with innovative structural design and molecular targets. The development of multi-drug resistant pathogenic bacteria, which quickly become resistant to commonly used antibiotics and other classes of drugs for the treatment of human diseases, is one of the greatest issues and challenge facing health care providers in the future[3-10].

Quinoline-based compounds are a significant class of heterocyclic compounds that are present in a wide range of both natural and artificial products having diverse biological activities. According to pharmacological investigations, the quinoline ring system exhibits a wide range of biological actions, including antimalarial, anticancer[11], antiviral[12], antibacterial[13] antifungal[14], anti-inflammatory[15], and antiplatelet aggregation[16].

The design of the synthesised molecules was based on two ideas. First, numerous reports have confirmed the effectiveness of quinolines as antibacterial medications, alongside ciprofloxacin and Gentamycin. The second and most important method for developing new medications is to alter the chemical makeup of an existing substance, combine two or more pharmacophoric moieties into a single molecular scaffold to produce a synergistic effect, or create new, reasonably priced derivatives with novel modes of action[17].

2. Materials and Methods:

2.1 Molecular Docking Studies

To learn more about its potential mode of action, a molecular docking investigation of the recently synthesized derivatives (2a-b, 3a-c, and 4a-b) was carried out on Intel Core i5 11th Generation CPU, with 8 GB DDR2 RAM and SSD512 system. All molecules were drawn using the ChemBioDraw 14.0. Employing the UCSF Chimera tool, all the drawn compounds were prepared for docking studies. Further, the ligands were desalted, and tautomers were generated. Specified chiralities were retained and stereoisomers were generated per ligand[18]. The generated ligand molecules' 2D structure was transformed into a 3D structure that reduced energy and was then used for docking. The X-ray crystal structures of the protein validated as antibacterial target was retrieved from protein data bank (PDB). Escherichia coli K-12 (**PDB ID: 6ZHV**), was imported into the protein preparation wizard tool of BIOVIA Discovery Studio. In the first step, imported protein is checked for any missing residues or loops. Then, hydrogens are added, bond-order corrected, and water molecules removed. In the next step, added hydrogens are optimized, and protein is put for restrained energy minimization using CGenFF forcefield[18].

The binding site was verified by redocking the ligand, QLB-2-[2-(2-octoxyethoxy)ethoxy]ethanol. Molecular docking calculations of all the test compounds were carried out with Auto dock vina[19]. The conformation with the lowest binding free energy was used for analysis. Docked images of the compounds with highest binding energy and interaction i.e., 39(3b) and 32(3a) are shown in Fig. 1 to 4. Interaction in the form of binding energy of all the docked compounds including the standard drugs is presented in Table 1.

2.2 Chemistry

All the newer 21 derivatives were synthesized using lab grade reagents and solvents, reaction completion was periodically checked by using thin layer chromatography. Then the

derivatives were purified by recrystallization and the purity was checked by using suitable analytical technique like HPLC.

All the compounds then characterized and confirmed by IR, NMR and LCMS.

2.3 Qualitative evaluation of inhibition of β-hematin formation (antimalarial)

2.3.1 Stock solution preparation:

Hematin was made into a solution by mixing 5mg of hemin (bovine, ≥ 90.0%, obtained from Hi Media) in 1.0 mL of 0.1 M NaOH. This solution was incubated at 60°C, followed by the addition of previously incubated 0.1 mL 1.0 M HCl and 0.58 mL of 12.9 M acetate (pH 5) at 60°C. The mixture was incubated at 60°C for 1hr. After cooling in an ice bath for 5 min, the solid obtained was filtered using 8µm cellulose acetate/nitrate filter disk Millipore filter and washed thoroughly with water. The solid collected was dried under vacuum for several hours before analysis by infrared spectroscopy in a *Bruker* FT-IR spectrometer. For the study of the compounds, 3 or 5 molar equivalents of the studied compounds were added to the solution prior to the acidification step and continued as specified above[20,21].

2.4 Antibacterial study

2.4.1 Strains and media

Three Gram-positive bacteria *Staphylococcus aureus* 5345, *Salmonella typhi* 2501, *Bacillus cereus* 2217 and Gram-negative *Escherichia coli* 2931 strains were used in this study. All microorganisms were sub cultured in nutrient agar medium and incubated for 24hrs at 37ºC.

2.4.2 Preparation of subculture media for inoculum:

In a conical flask 13g of nutrient broth powder was added to 1L of distilled water. It was mixed to dissolve completely. The dissolved medium is then autoclaved at 15lbs pressure at 121ºC for 15 minutes. Once the autoclaving process is complete, the conical flask is taken out and cooled to temperature of about 40-45^{o}C. A loop full of microorganism was transferred from laboratory-maintained culture (agar slant) into the conical flask containing sterilized nutrient broth medium. The tubes were incubated for 24hrs at 37ºC.[22]

2.4.3 Preparation of nutrient agar and spread plates:

In a conical flask 28g of nutrient agar powder was added to 1L of distilled water. It was mixed to dissolve completely. The dissolved medium is then autoclaved at 15lbs pressure (121ºC) for 15 minutes. Once the autoclaving process is complete, the conical flask is taken out and cooled to temperature of about 40-45ºC. Spread plate method is used for uniform distribution of bacterial suspension on solid agar by glass spreader. 0.1mL of prepared subculture was poured on sterile nutrient agar plate. Then these dilutions were spread with help of glass spreader, which was sterilised by flaming after dipping in alcohol and it is allowed to become cool between two burners.

2.4.4 Spread plate method

A well with a diameter of 8 mm is punched aseptically with 8mm boarer and a standard ciprofloxacin and synthesized compound solution at desired concentration introduced into the well. Then, agar plates are incubated at 37°C for 24 hrs. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. Such 333 petri plates were prepared for each causative agent. On each plate 5 wells of 8mm were punched aseptically. For each newly synthesized drug analogs the concentration in triplicate were taken and their average was calculated[22].

2.4.5 Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of the test compounds were evaluated by following Clinical and Laboratory Standards Institute (CLSI) recommended macro-broth dilution method M07-A9 and M07-A10. Test compounds (1mL), previously dissolved in 1% DMSO, was added to the test tube containing 1mL of broth media and serial dilutions were done to obtain the final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1µg/mL. Following serial dilutions, 1 mL of bacterial cultures, following standard inoculum of 105 CFU/mL, was added to each tube. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 24hrs, was determined visually after incubation for 24hrs at 37ºC. Tests using 1% DMSO and ciprofloxacin were also included as negative and positive controls respectively.[23]

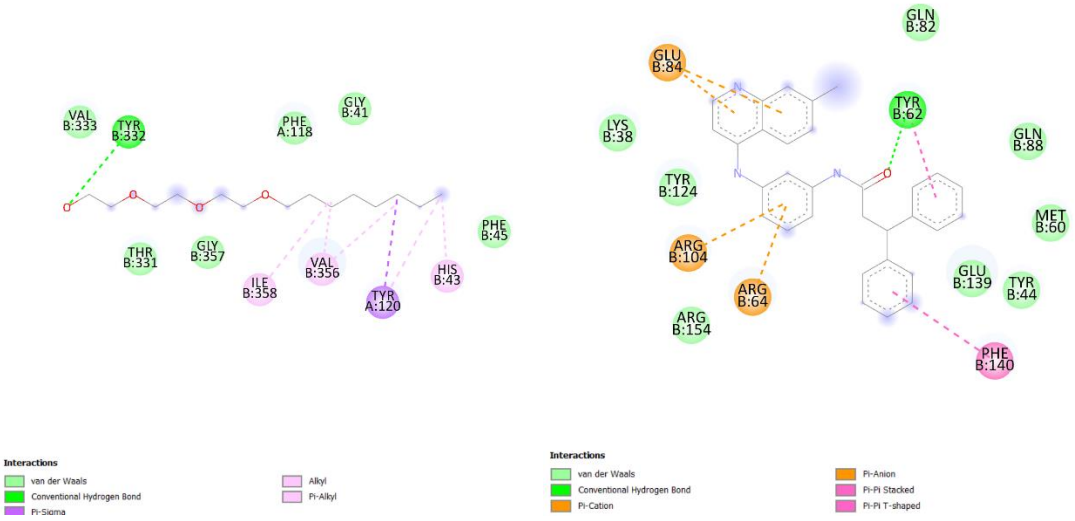
3. Results and Discussion

3.1 Docking Studies

The docking studies revealed that except compound 16(2a) all the synthesized molecules have greater binding energy and several molecular interactions towards the target than the standard, and were responsible for the observed affinity. The best docking energy model and most possible interaction mode of the active compound 39(3b) and 32(2a) with 6ZHV is shown in Fig. 1,2,3 and 4. It was observed that the compound **39(3b)** mainly interacts with the target enzyme by showing hydrogen bonding interaction with TYR62 residue, mimicking 2-[2-(2-octoxyethoxy)ethoxy]ethanol a co-crystallized ligand that shows interaction with TYR332 amino acid residue (see Fig. 1). Besides hydrophobic and Van der Waals interactions were also involved. Binding affinity value of the docked target compounds were found to be in the range -4.2 to -9.3 kcal mol^{-1}. The results revealed that the quinoline ring attached with NH and C=O in **39(3b)** shows strong hydrogen bonding interaction with the amino acid residues of the B chain of protein **6ZHV**, which clearly advocates its better antibacterial efficacy. From these results it can be inferred that compound probably shows its antibacterial activity in a similar way as that of the ciprofloxacin. Based on the docking analysis it can be concluded that the synthesized analogs (except 16(2a) not able to form a stable complex with the protein.) shows a **greater** binding score than the co crystallized ligands might inhibit many bacterial proteins.

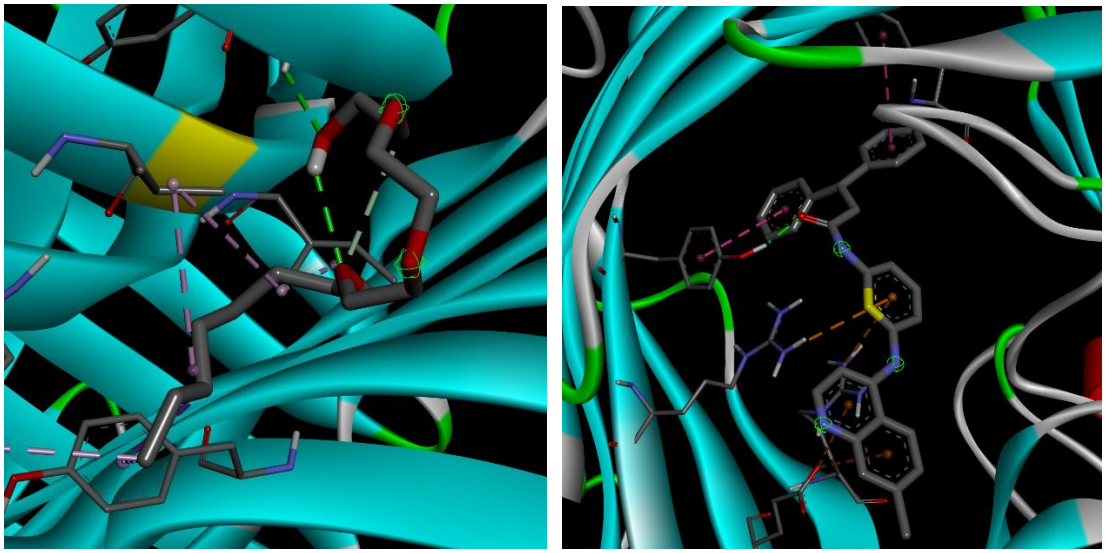
Table 1: Binding energy/Docking score of synthesized compounds with target.

Sr. No.	Compound	Docking Score	Sr. No.	Compound	Docking Score
1	Ciprofloxacin	-5.3	12	(36)2k	-6.3
2	(16)2a	-4.2	13	(37)2l	-7.1
3	(17)2b	-7	14	(38)2m	-7.1
4	(24)2c	-7.3	15	(41)2n	-7
5	(25)2d	-5.8	16	(42)2o	-6.3
6	(27)2e	-7.1	17	(5)2p	-6.1
7	(28)2f	-6	18	(32)3a	-8.1
8	(29)2g	-6.1	19	(39)3b	-9.3
9	(30)2h	-6.6	20	(40)3c	-7.8
10	(31)2i	-7.2	21	(26)4a	-7.2
11	(35)2j	-6.3	21	(34)4b	-6.6



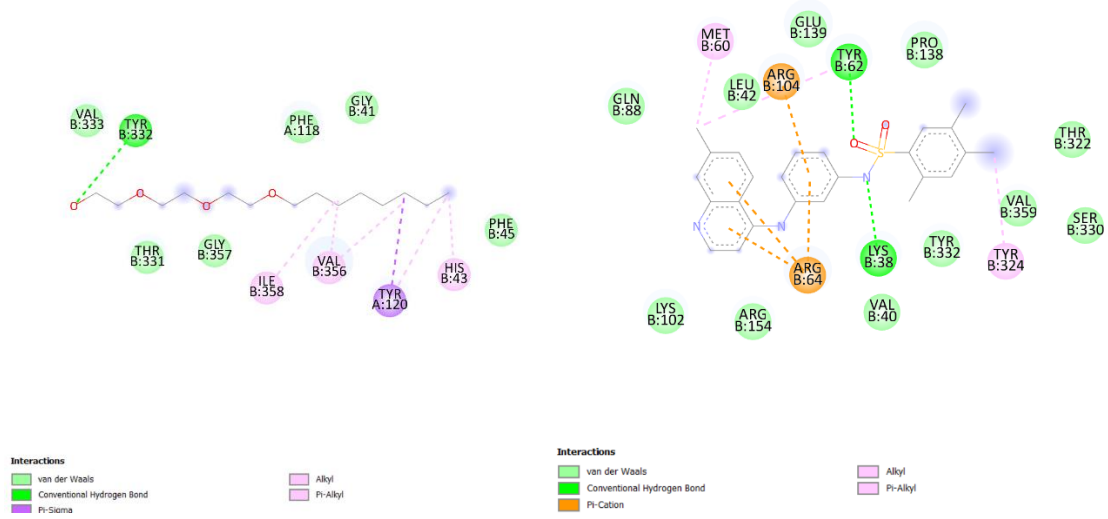
(A) (B)

Figure 1. Ligand interaction diagram (2D). (A) The co-crystallized ligand (PDB ID: 6ZHV) (B) Compound 39(3b) with protein receptor (Best docked).



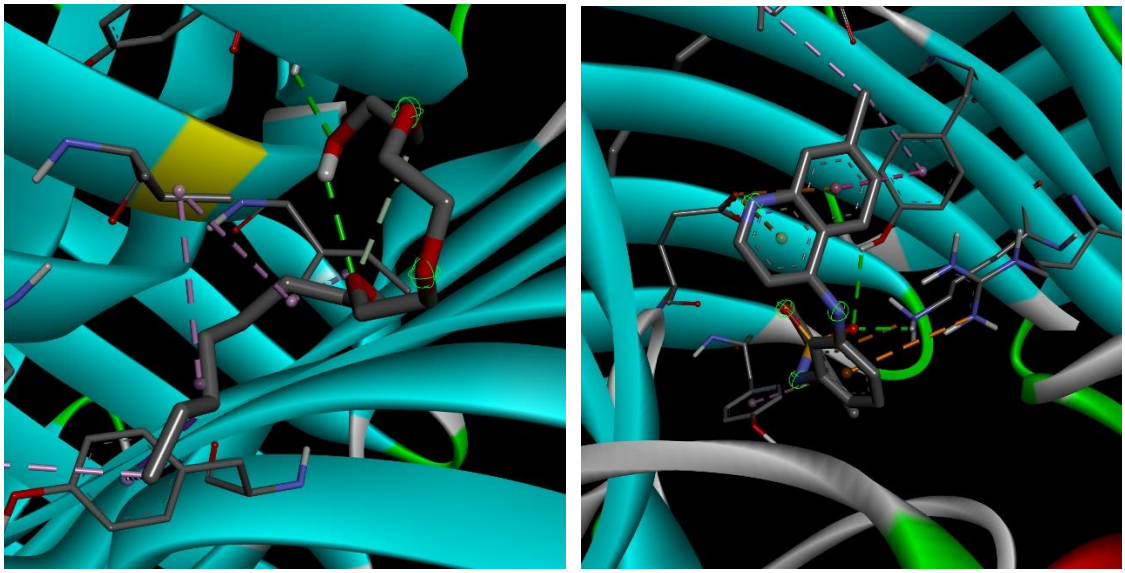
(C) **(D)**

Figure 2. Ligand interaction in 3D for the co-crystallized ligand (PDB ID: 6ZHV) (c) and compound 39(3b) with protein receptor.



(A) (B)

Figure 3. Ligand interaction diagram (2D). (A) The co-crystallized ligand (1J3K) (B) Compound 32(3a) with protein receptor (Best docked with good activity).



(C) **(D)**

Figure 4. Ligand interaction in 3D for the co-crystallized ligand (PDB ID 1J3K) (C) and compound 32(3a) (D) with protein receptor (Best docked with good activity).

3.2 Qualitative evaluation of inhibition of β-hematin formation

The ability of these substances to prevent the formation of β-hematin was also investigated in relation to their antimalarial characteristics. It was already mentioned, one of the known modes of action for drugs containing quinolines is inhibition of the naturally occurring formation of the "malaria pigment" hemozoin. It has been demonstrated that chloroquine, quinine, and amodiaquine block the synthesis of β-hematin (synthetic analogues of hemozoin). Using infrared spectroscopy, the compounds' capacity to prevent the production of β-hematin was assessed qualitatively. Hematin and β-hematin have different infrared spectra, which are used in the qualitative IR test. β-hematin can be produced from a solution of hematin even in the lack of proteins or enzymes.

The presence of two distinct sharp peaks at 1660 and 1207 cm^{-1} in the infrared spectrum of β-hematin peaks that are not present in all of the IR spectra gathered indicates a suppression of β -hematin synthesis.

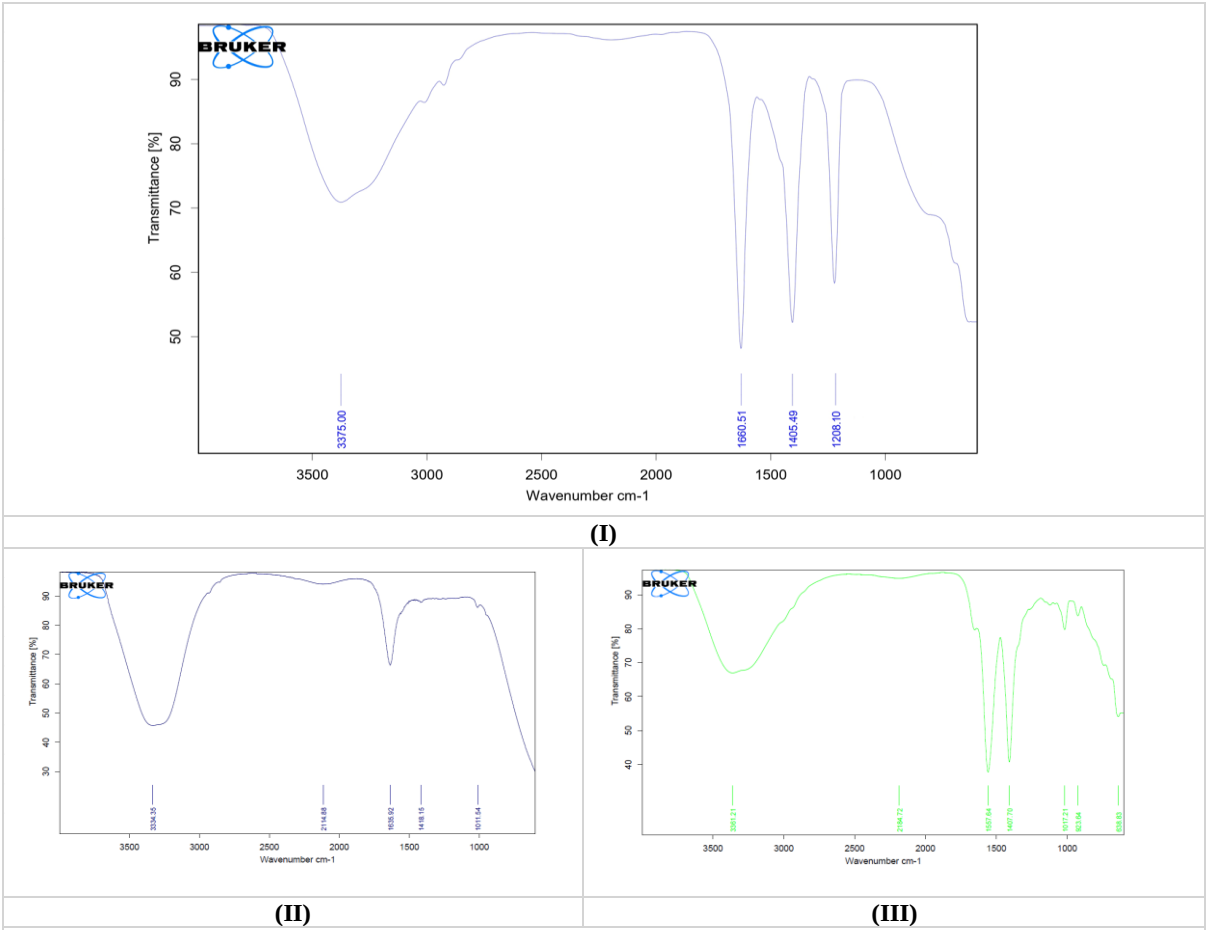


Figure: (I)-FTIR of β-Hematin control , (II)-FTIR of sample-39 and (III)-FTIR of sample-40

Figure 5: representation by IR for Inhibition of β-hematin by compound 39(3b) and 40(3c).

3.3 Antibacterial Activity

Three series of 21, 4-aminoquinoline derivatives bearing a quinoline moiety (2a-b, 3a-c, and 4a-b) have been evaluated as an antibacterial agent against the potent marketed drug like ciprofloxacin. Compound (24)2c, (31)2i, 42(2o) and 32(3a) were found to be the most potent of all of the compounds tested, with an MIC value of 1µg/mL to 32µg/mL against several Gram-positive (*Staphylococcus aureus* 5345, *Salmonella typhi* 2501), *Bacillus cereus* 2217 and Gram-negative *Escherichia coli* 2931 strains of bacteria. In addition, compound (31)2i, and 32(3a) showed potent inhibitory activity (MIC=1µg/mL) against *Salmonella typhimurium* 2501, *Bacillus cereus* 2217 and Gram-negative *Escherichia coli* 2931 strains of bacteria indicating that its antibacterial spectrum is similar to those of the positive control ciprofloxacin.

Table-2: Antibacterial properties in terms of Zone of inhibition(mm) of target compounds 2a-b, 3a-c and 4a-b.

Compound	***S. aureus* (5345)**	***S. typhimurium* (2501)**	***B. Cereus* (2217)**	***E. coli* (2931)**
Ciprofloxacin	23.67 ± 0.07	23.97 ± 0.09	11.00 ± 0.06	25.33 ± 0.33
(16)2a	NA	NA	18.33 ± 0.58	17.67 ± 0.33
(17)2b	16.73 ± 0.37	19.13 ± 0.13	28.33 ± 0.33	14.33 ± 0.33

(24)2c	24.17 ± 0.17	26.33 ± 0.33	23.33 ± 0.33	18.67 ± 0.33
(25)2d	22.33 ± 0.33	27.63 ± 0.32	NA	19.33 ± 0.33
(27)2e	NA	10.47 ± 0.29	23.33 ± 0.33	16.33 ± 0.33
(28)2f	19.37 ± 0.32	17.93 ± 0.07	16.33 ± 0.33	22.33 ± 0.33
(29)2g	16.23 ± 0.39	16.63 ± 0.32	9.67 ± 0.33	24.67 ± 0.33
(30)2h	13.00 ± 0.00	14.93 ± 0.07	27.33 ± 0.33	18.67 ± 0.33
(31)2i	19.00 ± 0.00	26.50 ± 0.29	14.33 ± 0.33	23.67 ± 0.33
(35)2j	18.53 ± 0.29	19.00 ± 0.00	19.33 ± 0.33	NA
(36)2k	17.33 ± 0.33	15.10 ± 0.10	20.67 ± 0.33	12.00 ± 0.58
(37)2l	12.00 ± 0.00	15.27 ± 0.37	18.00 ± 0.33	10.33 ± 0.33
(38)2m	9.00 ± 0.00	10.13 ± 0.13	14.67 ± 0.00	8.33 ± 0.33
(41)2n	14.03 ± 0.03	16.47 ± 0.29	24.33 ± 0.33	NA
(42)2o	26.53 ± 0.29	24.27 ± 0.37	16.00 ± 0.33	22.00 ± 0.58
(5)2p	10.17 ± 0.17	19.83 ± 0.17	27.33 ± 0.58	26.33 ± 0.33
(32)3a	29.77 ± 0.39	27.53 ± 0.29	20.67 ± 0.33	29.67 ± 0.33
(39)3b	23.17 ± 0.44	22.80 ± 0.20	15.67 ± 0.67	15.33 ± 0.33
(40)3c	13.07 ± 0.07	12.50 ± 0.29	13.00 ± 0.33	13.33 ± 0.33
(26)4a	NA	10.80 ± 0.20	9.00 ± 0.58	NA
(34)4b	7.67 ± 0.33	15.47 ± 0.29	NA	NA

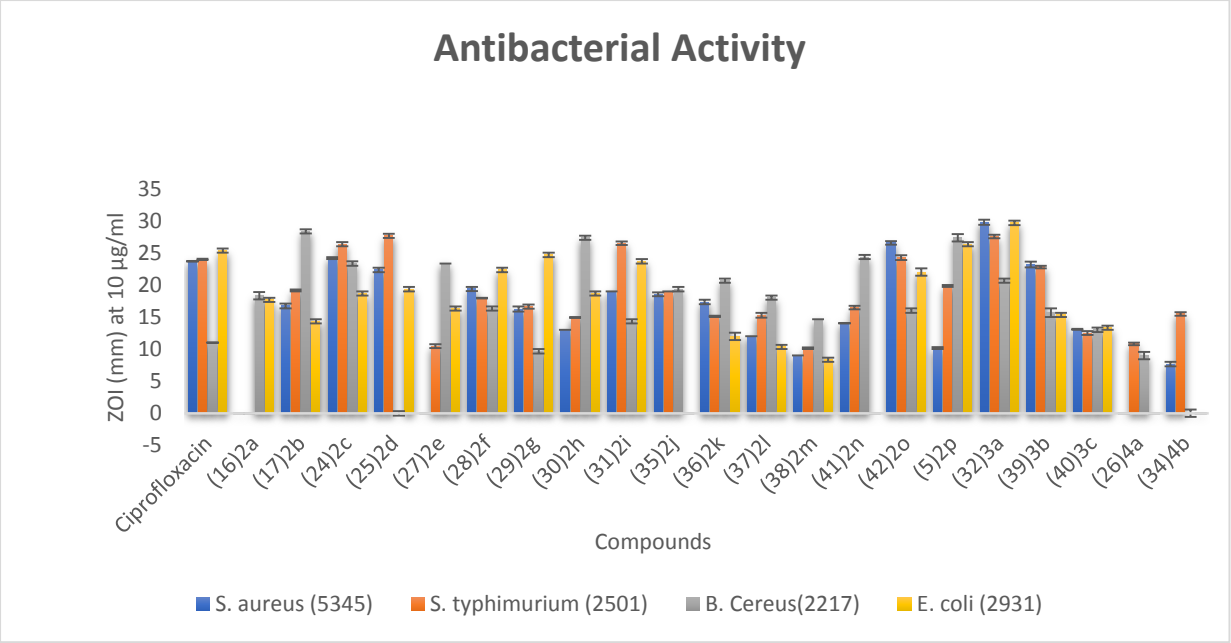


Figure 6: Graphical representation of antibacterial activity in terms of zone of inhibition in mm with SEM.

Table 3. Antibacterial data as MIC(µg/mL) for target compounds 2a-b, 3a-c and 4a-b.

Compound	Gram positive bacteria			Gram negative bacteria
	S. aureus 5345	*S. typhi* 2501	*B. cereus* 2217	*E. coli* 2931
Ciprofloxacin	16	1	2	1

(16)2a	256	256	64	32
(17)2b	8	4	4	2
(24)2c	16	1	1	4
(25)2d	32	1	2	4
(27)2e	128	64	256	8
(28)2f	32	8	2	2
(29)2g	32	32	32	8
(30)2h	64	64	128	2
(31)2i	32	1	1	1
(35)2j	32	4	16	256
(36)2k	32	8	4	64
(37)2l	64	4	4	64
(38)2m	64	64	4	128
(41)2n	128	4	16	256
(42)2o	8	1	2	2
(5)2p	256	2	16	1
(32)3a	4	1	1	1
(39)3b	16	2	4	8
(40)3c	64	64	16	16
(26)4a	256	128	64	256
(34)4b	64	32	128	256

4. Conclusion

In conclusion, we present a series of synthesised 4-aminoquinoline analogues that are easily accessible and exhibit good activity against multidrug-resistant protozoan, gram-positive *Staphylococcus aureus* 5345, *Salmonella typhi* 2501, *Bacillus cereus* 2217, and gram-negative *Escherichia coli* 2931 strains of bacteria. In addition, compared to ciprofloxacin, compounds (31)2i and (32)3a demonstrated strong inhibitory action (MIC=1µg/mL) against the bacterial strains *Salmonella typhi* 2501, *Bacillus cereus* 2217, and gram-negative *Escherichia coli* 2931. Hope for further creation of non-resistant antimalarial compounds is provided by the inhibition of β-hematin formation like chloroquine in all five compounds. When compared to ciprofloxacin, these compounds are easier to synthesise and more cost-effective, making them a desirable family of anti-infective agents that can fight drug-resistant bacterial and protozoal strains.

Conflict of Interest

None

Acknowledgement

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