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Molecular docking and antifungal evaluationof some newly synthesized 4-amino-7-chloroquinoline derivativeson resistant *Candida albicans.*

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ABSTRACT:
An abrupt rise in the prevalence of infections brought on by fungi of *Candida* species has occurred over the past few decades as a result of concurrent rises in the immunocompromised population and the use of antifungal medications. As an antifungal agent against the potent commercial drug, three series of 21, 4-amino-7-chloroquinoline derivatives(2a-b, 3a-c, and 4a-b) bearing a quinoline backbone have been designed, synthesised, and tested in this paper.The docking studies revealed that the compounds24(2c), 25(2d), 27(2e), 29(2g), 35(2j), 38(2m), 42(2o), 32(3a), 39(3b), 40(3c) and 26(4a) have greater binding energy and several molecular interactions towards the target than the standarddrug fluconazole and co-crystallized ligand, and were responsible for the observed affinity. The results revealed that the quinoline ring attached with NH and C=O in39(3b)shows strong hydrogen bonding interaction with the amino acid residues of the B chain of receptor protein**1M78**, and it binds the same way as fluconazole and co-crystallized ligand which clearly advocates its better antifungal efficacy. Compound (29)2g and (38)2mwere found to be the most potent of all the compounds tested, with an MIC value of 1µg/mLagainst *Candida albicans*NICM-3102,ATCC-2091. Hence 4-amino-7-chloroquinoline derivatives are the type of potential small molecules that could be beneficial for future antifungal therapies.

Keywords: Antifungal, Molecular Docking, 4-amino-7-chloroquinoline, 1M78

1. Introduction:

Only 400 of the estimated 1.5–5.1 million types of fungi on Earth are identified as potentially dangerous to individuals[1,2].Over 1.6 million fatalities and one billion infections are caused by these pathogenic fungi each year around the globe. The major portion of documented global fungal deaths are caused by infections caused by the *Candida* species of human fungal pathogens[3]. An abrupt rise in the prevalence of infections brought on by *Candida* species has occurred over the past few decades as a result of concurrent rises in the immunocompromised population and the use of antifungal medications[3,4].When a microorganism develops tolerance to an antimicrobial drug that it was previously sensitive to, antimicrobial resistance results from the use and abuse of antimicrobial medications. Numerous pathogenic fungi, such as *Candida* spp., have both primary and acquired (or secondary) resistance to antifungal medications[5,6].

One of the most common commensal fungi in the human microbiota, *Candida albicans* colonises healthy people's epidermis, genitourinary tract, and gastrointestinal system without causing any symptoms[7,8,9].Opportunistic pathogens like Candida species pose a danger to public health because they are a leading cause of morbidity and mortality across the globe[10,11,12].Additionally, *Candida* species can result in systemic infections, vaginitis, mouth candidiasis, cutaneous candidiasis, and candidemia[13].There are only three main classes of antifungal medications used to treat invasive fungal infections: azoles, echinocandins, and polyenes. This contrasts with the diversity of antibiotic drug classes that are accessible for use against bacterial pathogens. The most popular type of antifungal

medications used to treat both systemic and superficial fungal infections is the azole family, which includes drugs like fluconazole[14].

Drug resistance in the treatment of *C. albicans* infections has emerged as a result of the widespread use of a small number of antifungal agents, especially azoles medications, and is now a problem of growing significance. New antifungal medications are therefore urgently required for the effective treatment of *candida* infections[15].However, because both fungi and humans are eukaryotes, it is difficult to create medications that specifically inhibit fungal growth without adverse effects on patients. The heavily researched approach in this field has been the discovery of synthetic analogues for possible therapeutic use[16].Therefore, there is a greater likelihood that this class of medications will encounter cross resistance, making the creation of new, promising molecules that do not include azoles urgently necessary. The effectiveness of antifungal agents is impacted by drug resistance, narrow spectrum, decreased bioavailability at the target tissues, and toxicity, which also restricts the range of available treatments. When considered as a whole, these results have significant ramifications for the creation of new, potent, broad-spectrum, and low-toxic antifungal agents.

Quinoline scaffold is present in numerous groups of other biologically active substances that are used as antifungals, antibacterial, and antiprotozoal drugs[17,18,19,20].Many different quinoline-based synthetic processes have been documented, and a large selection of quinoline-based starting materials are inexpensively offered by well-known vendors. Its activity against some fungal strains is relatively high[3]while its toxicity is low, which is a clear benefit in the context of antifungal design.Therefore, using quinoline as a scaffold and diaminoalkane as a linker with addition of different building blocks, total 21 analogs were synthesized against the *Candida albican* species and tested for activity.

2. Materials andMethods:

2.1 Molecular Docking Studies

Using a Dell Intel Core i5 11th Generation CPU, 8 GB DDR2 RAM, and SSD512 system, a molecular docking study of the freshly synthesized compounds (2a-b, 3a-c, and 4a-b) was conducted to learn more about its overall interaction with the protein. ChemBioDraw 14.0 was used to sketch every chemical structure. All the drawn compounds were readied for docking experiments using the UCSF Chimera tool where the water molecules were removed and other non-standard components excluding co-crystallized ligands get deleted. Tautomers were also produced after the ligands were desalted. Per ligand, stereoisomers were produced while retaining the specified chiralities.The 2D structure of the produced ligand molecules was converted into a 3D structure that conserved energy and was used for docking. From the protein data repository, the X-ray crystal structures of the protein identified as an antifungal target were obtained, *Candida albicans* Dihydrofolate reductase complexed with dihydro-nicotinamide-adenine-dinucleotide phosphate (NADPH) and CLZ-5-chloryl-2,4,6-quinazolinetriamine(**PDB ID 1M78**), was imported into the protein preparation wizard tool of BIOVIA Discovery Studio. The received protein is initially examined for any loops or residues that might be absent. Hydrogens are then introduced, the bond order is adjusted, and the water molecules are eliminated. The added hydrogens are optimised in the following phase, and protein is placed using the CGenFF forcefield for restrained energy minimization.

By redocking the co-crystallized ligand CLZ-5-chloryl-2,4,6-quinazolinetriamine with PDB ID-1M78, the binding site was confirmed. All the test compounds' molecular docking computations were done using Auto Dock Vina19[21]. For study, the configuration with the least binding free energy was chosen. In Figures 1 to 4, docked pictures of the molecules with the greatest binding energy and association, namely 39(3b) are displayed.

2.2 Chemistry

Using lab-grade chemicals and solvents, all the recent 21 derivatives were produced, and thin layer chromatography was used to occasionally verify that the reactions had completed. Following that, the compounds underwent recrystallization to be made pure, and an appropriate analytical method like HPLC was used to verify the purity. By IR, NMR, and LCMS, all the substances were subsequently identified and verified.

3. Antifungal study

3.1 Strains and media

Candida albicans NICM-3102, ATCC-2091 used in this study is received from Adarsh Scientific Research Center and Testing Laboratory Pvt. Ltd, Panvel, Navi Mumbai. Microorganisms was sub cultured in RPMI-1640 Medium and incubated for 24hrs at 37ºC.

3.2Spread plate method

A well with a diameter of 8 mm is punched aseptically with8mm boarer and a standard Fluconazole and synthesized compounds solution at desired concentration introduced into the well. Then, agar plates are incubated at 37°C for 24 hrs. The antifungal agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.Such 333 petri plates were prepared for *Candida albican*. 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].

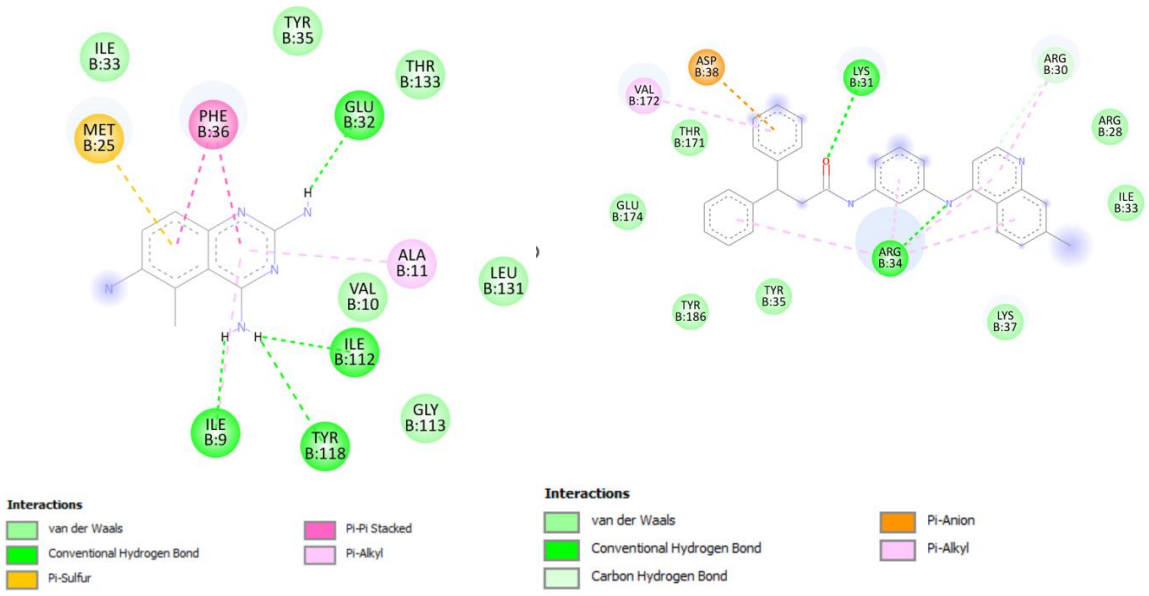
3.3Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of the test compounds were evaluated by following Clinical and Laboratory Standards Institute (CLSI) recommended macro-broth dilution method M27-A3. 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 Fluconazole were also included as negative and positive controls respectively[22,23,24].

4. Results and Discussion

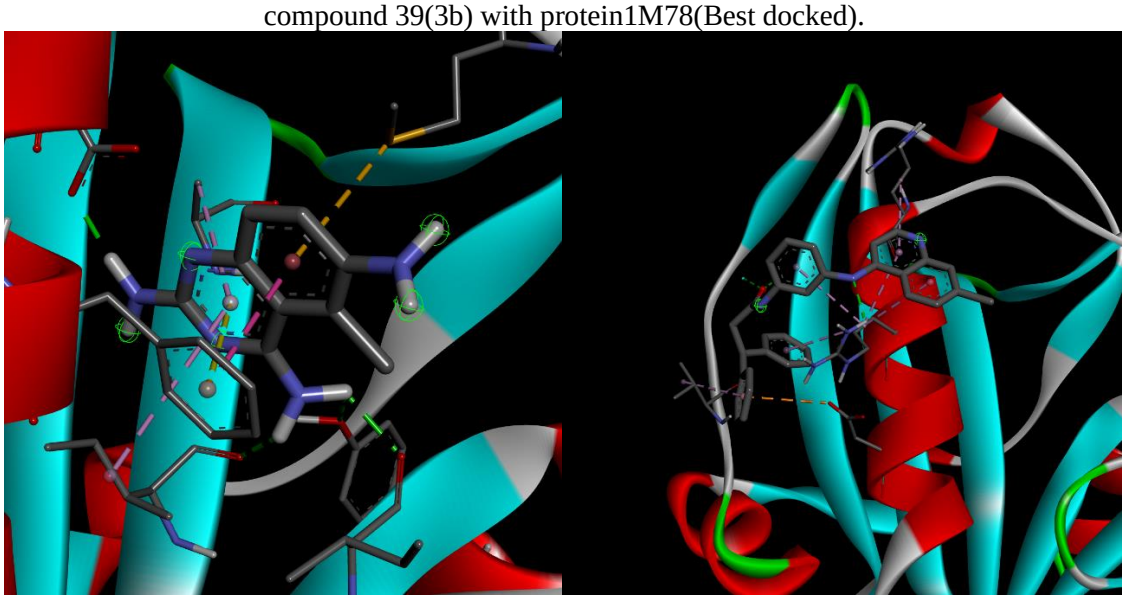
4.1 Docking Studies

The docking studies revealed that the compounds24(2c), 25(2d), 27(2e), 29(2g), 35(2j), 38(2m), 42(2o), 32(3a), 39(3b), 40(3c) and 26(4a) synthesized molecules have greater binding energy and several molecular interactions towards the target than the standard and co-crystallized ligand, and were responsible for the observed affinity.The best docking energy2D and 3D model and most possible interaction mode of the co-crystallized ligand with protein 1M78, standard fluconazole with protein receptor 1M78 and mostdocked active compound 39(3b) with1M78 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 conventional H- bonding interaction of -C=O of side chain with LYS31residue and -N- of side chain ARG34, mimicking H- bonding interaction of fluconazole. The van der Waals interaction in sample 39(3b) for TYR186, TYR35 AND THR171 is also exists with the standard fluconazole. The sample 39(3b) also has Pi anion interaction with ASP38 and Pi-alkyl interaction with ARG34. The co-crystallized ligandCLZ: 5-chloryl-2,4,6-quinazolinetriamineshows H-bonding interaction with ILE112, TYR118, ILE9, GLU32 and Pi-sulphur interaction with MET25, Pi-Pi stacked with PHE36 and Pi-alkyl interaction with ALA11 and ILE9 aminoacid residues (see Fig. 1). Binding affinity value of the docked target compounds were found to be in the range -5.3 to -7.4 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 protein1M78, and it binds the same way as fluconazole and co-crystallized ligand which clearly advocates its better antifungal efficacy. From these results it can be inferred that compound probably shows its antifungal activity in a similar way as that of the fluconazole. Based on the docking analysis it can be concluded that many synthesized analogs show a **greater** binding score than the co-crystallized ligand and Fluconazole might inhibit many bacterial proteins.



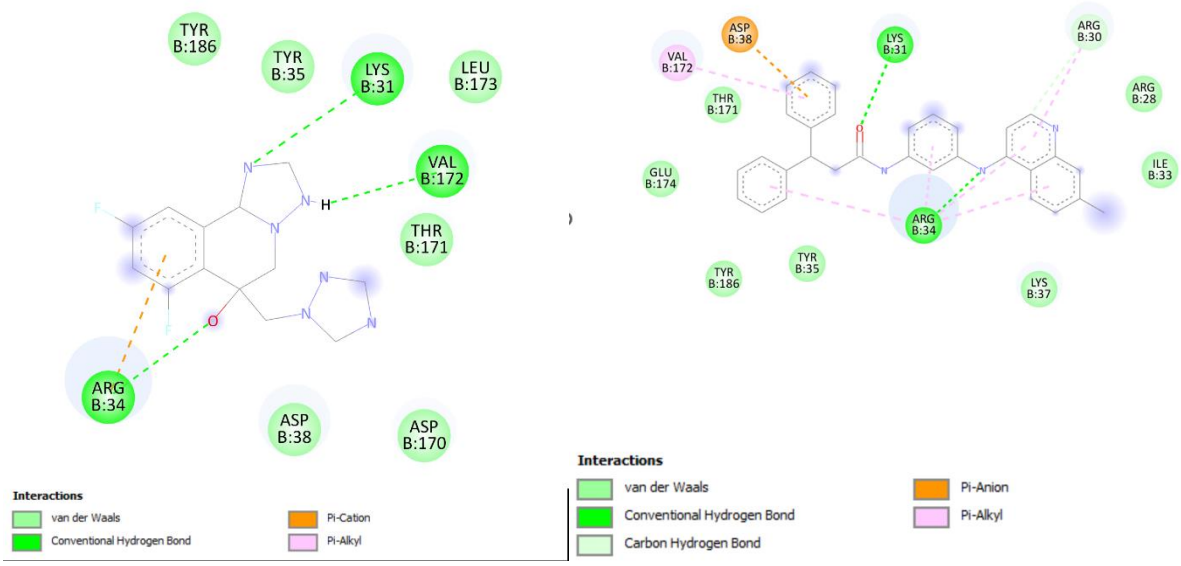
(A) (B)

Figure 1. Ligand interaction diagram (2D). (A) The co-crystallized ligand with protein1M78 (B) compound 39(3b) with protein1M78(Best docked).



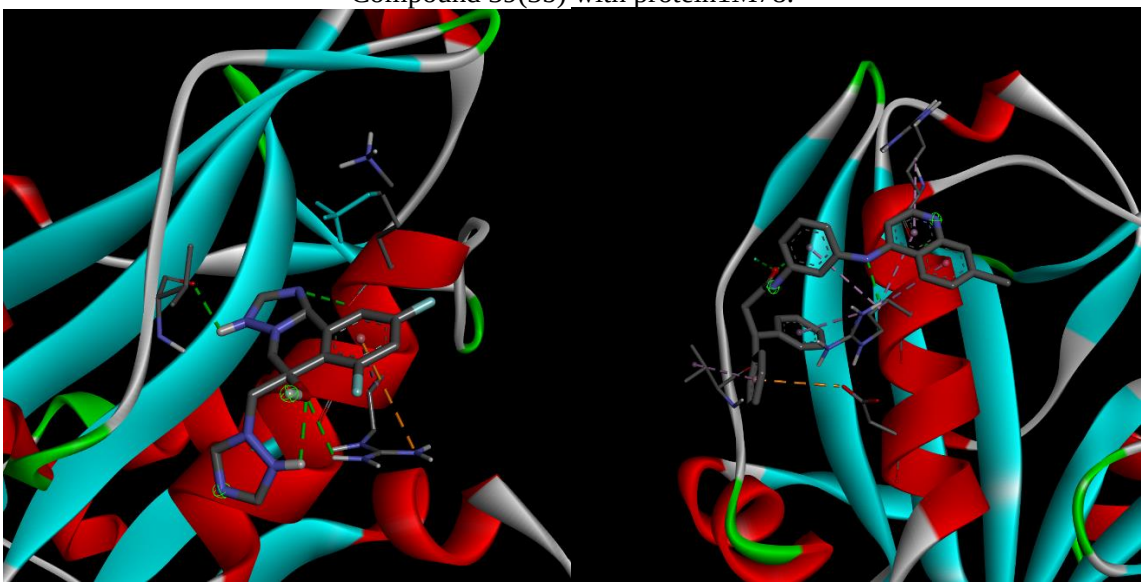
(C) **(D)**

Figure 2. Ligand interaction in 3D:(C)-co-crystallized ligand withprotein1M78 and (D)-compound 39(3b) with protein1M78.



(A) (B)

Figure 3. Ligand interaction diagram (2D): (A)-The standard Fluconazole with protein1M78 (B) Compound 39(3b) with protein1M78.

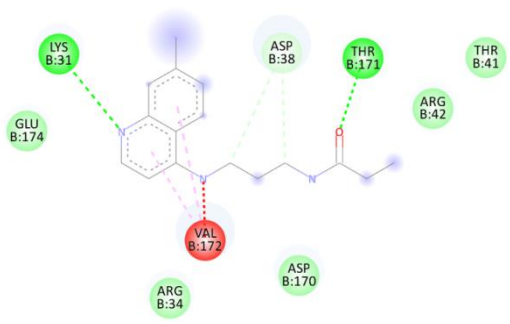
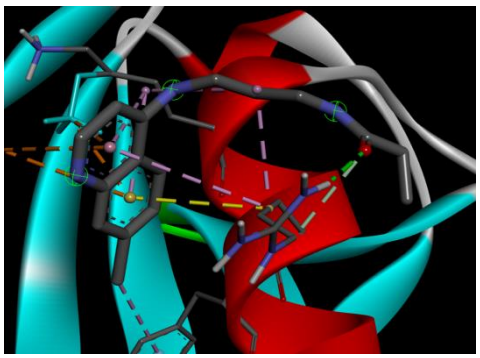
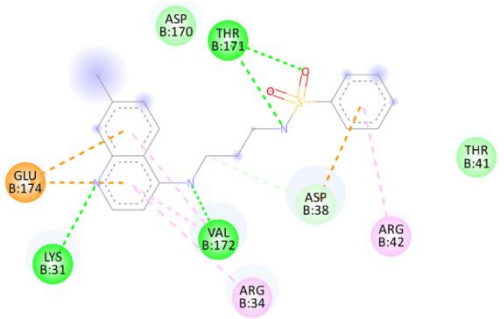
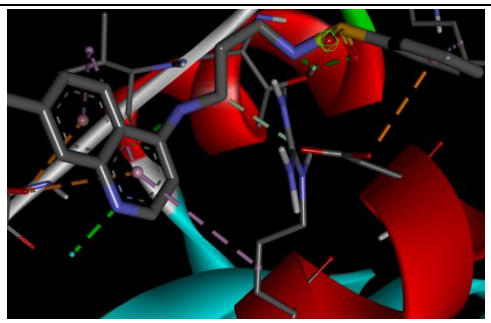
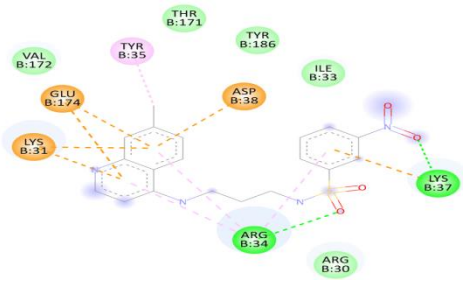
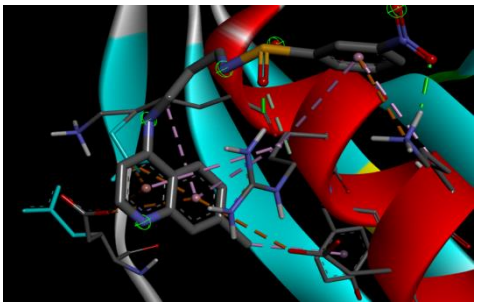
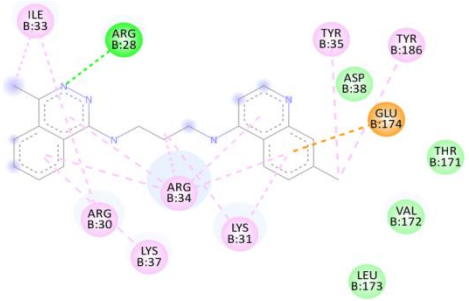
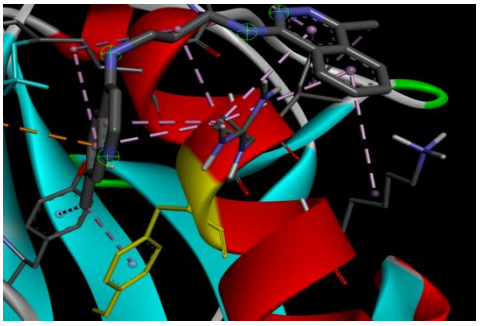
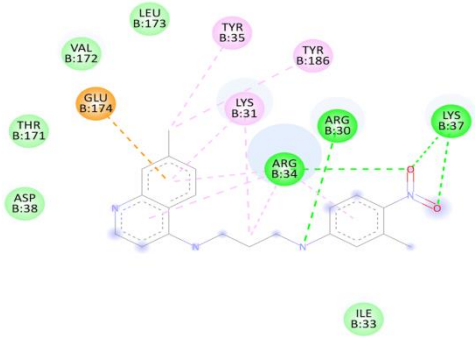
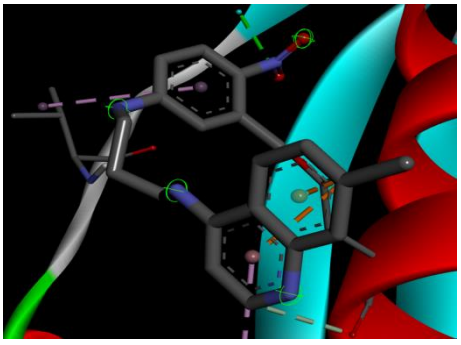


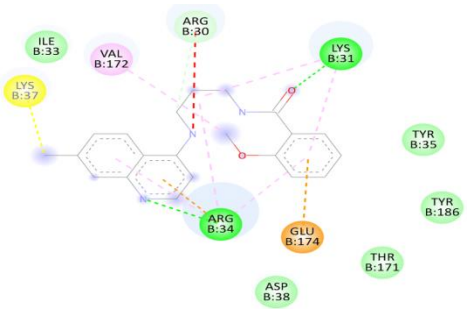
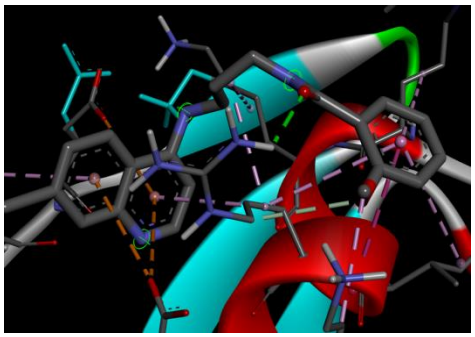
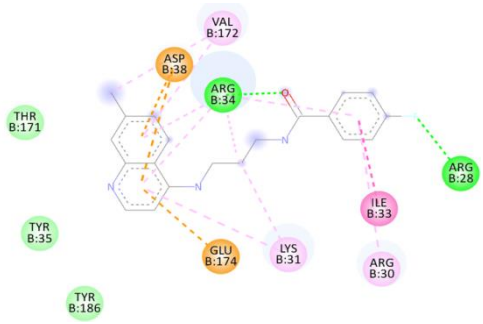
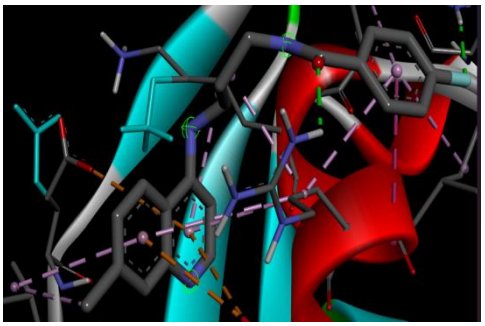
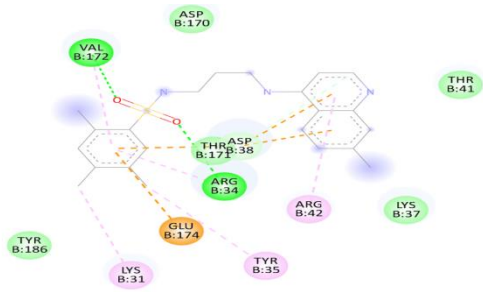
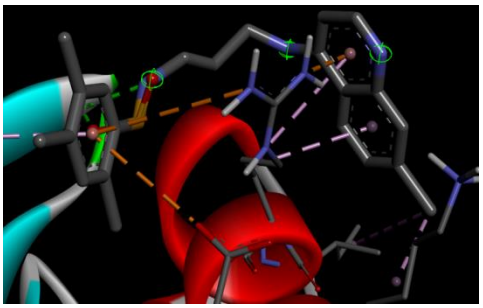
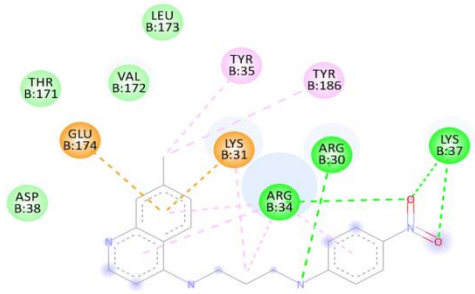
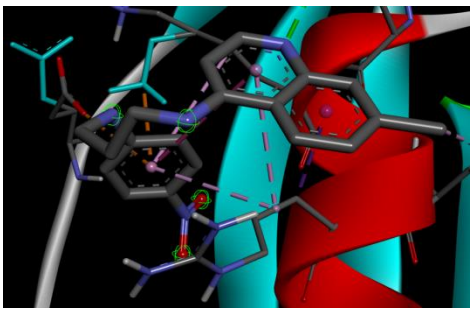
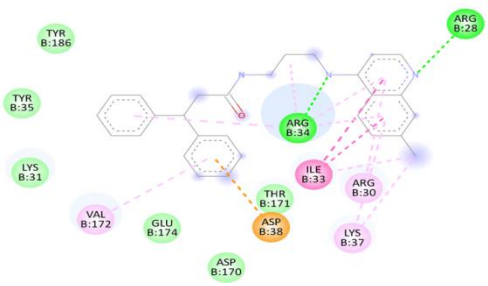
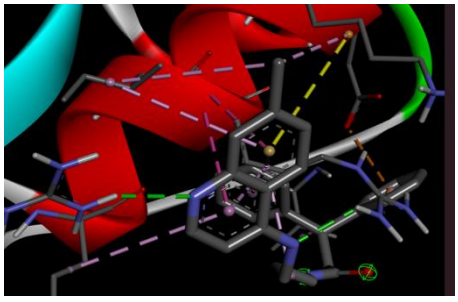
(C) **(D)**

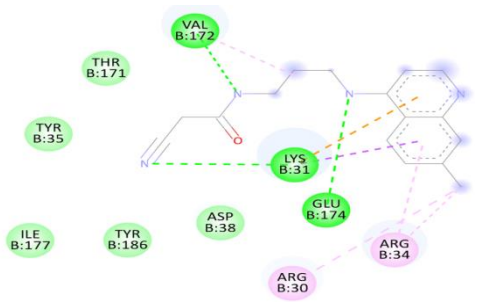
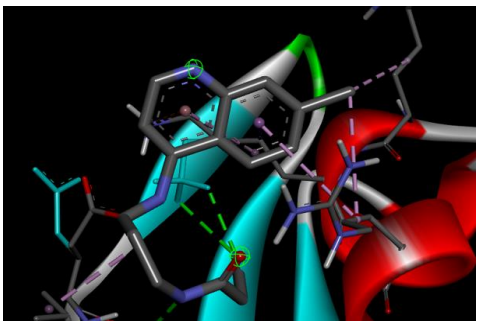
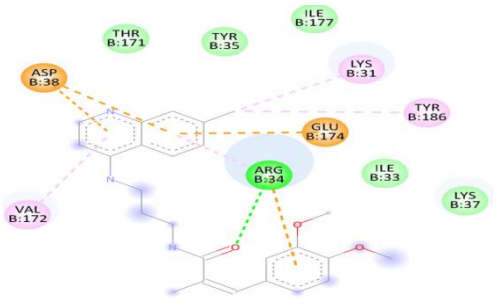
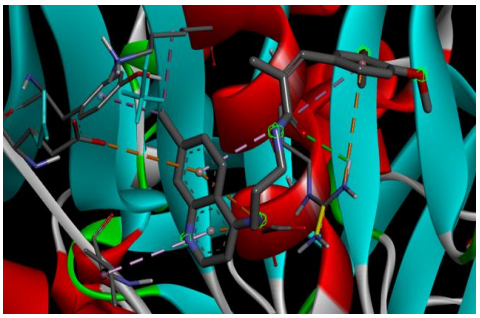
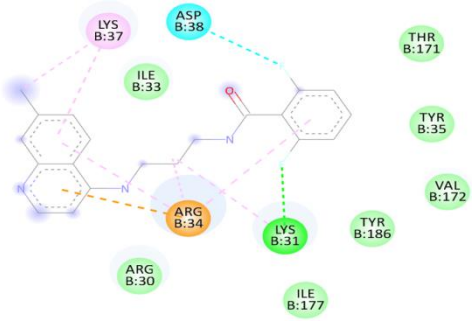
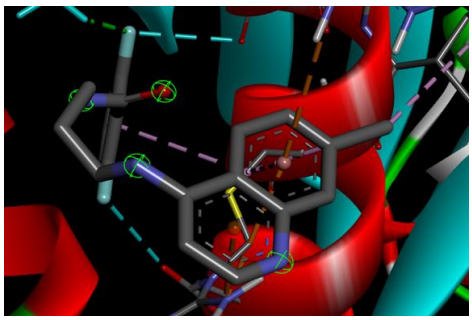
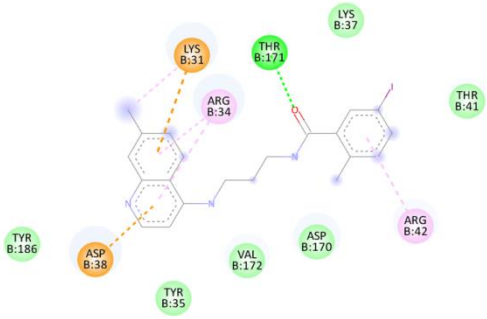
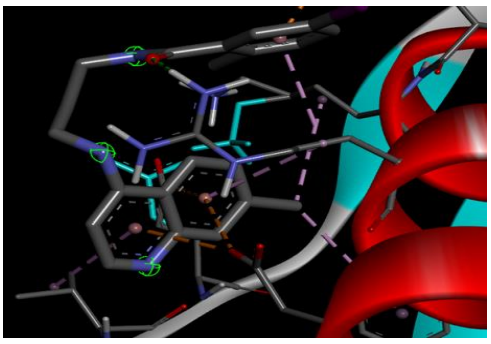
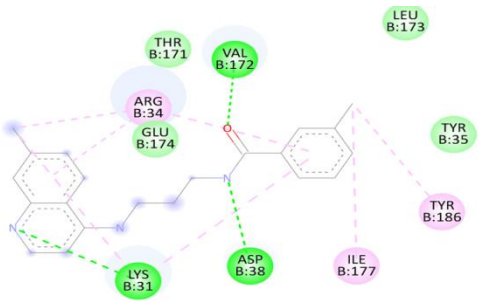
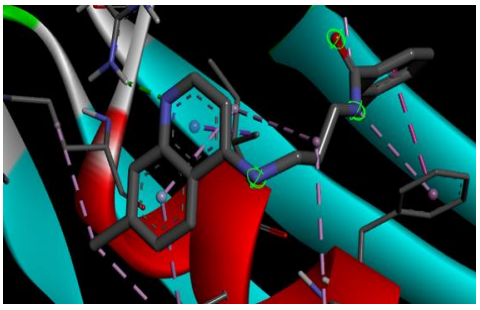
Figure 4. Ligand interaction in 3D:(C)-The standard Fluconazole with protein1M78 and (D)-Compound 39(3b) with protein1M78.

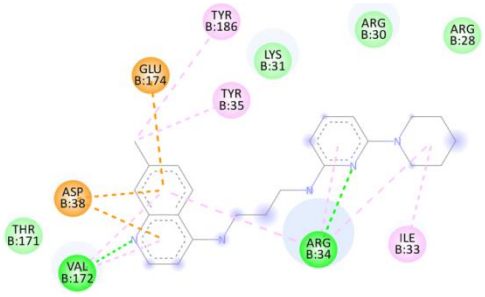
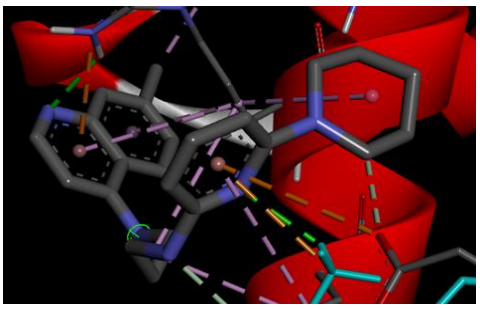
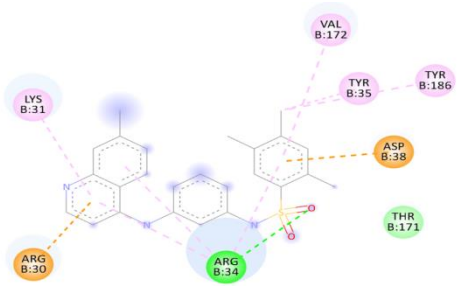
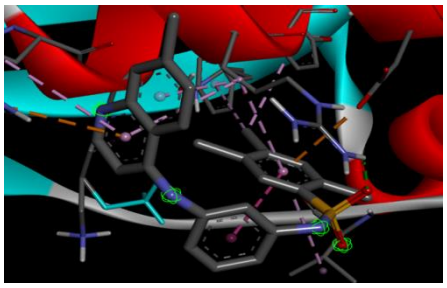
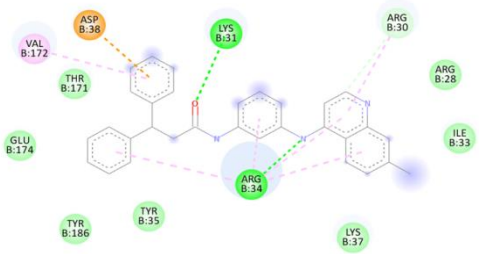
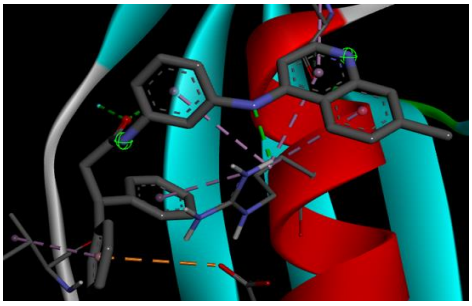
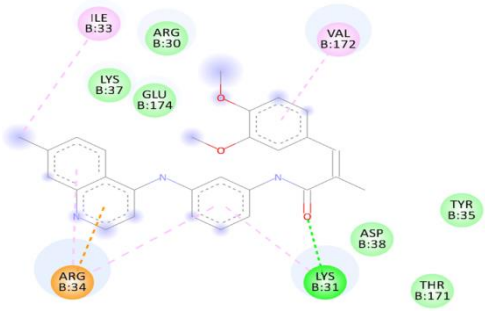
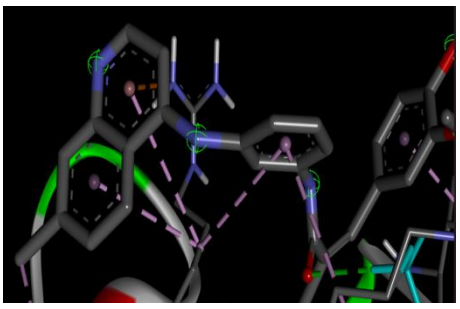
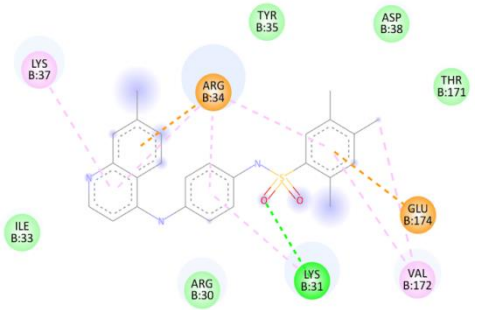
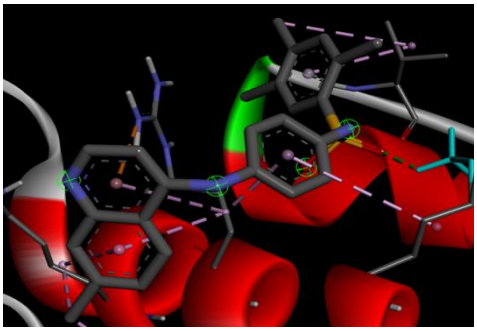
Table 1: 2D and 3D interaction of all synthesized molecules with protein and docking score.

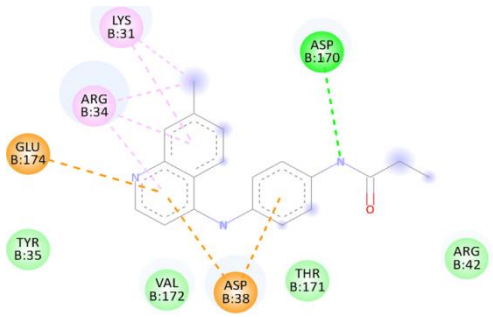
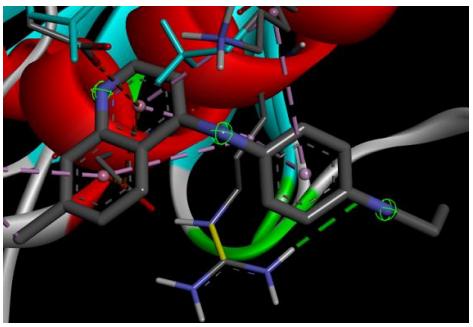
Compound code	**2D Docking pose**	**3D Docking pose**	**Docking score**

(16)2a			-5.8
(17)2b			-6.7
(24)2c			-6.8
(25)2d			-7.3
(27)2e			-6.8

(28)2f			-6.6
(29)2g			-7.1
(30)2h			-6.4
(31)2i			-6.6
(35)2j			-6.9

(36)2k			-6
(37)2l			-6.2
(38)2m			-6.9
(41)2n			-6.6
(42)2o			-6.9

(5)2p			-6.6
(32)3a			-7.1
(39)3b			-7.4
(40)3c			-7.2
(26)4a			-7.1

(34)4b			6.6

4.2 Antifungal Activity

Three series of 21, 4-aminoquinoline derivatives bearing a quinoline moiety (2a-b, 3a-c, and 4a-b) have been evaluated as an antifungal agent against the potent marketed drug like Fluconazole. Compound (29)2g and (38)2mwere found to be the most potent of all the compounds tested, with an MIC value of 1µg/mLagainst *Candida albicans*NICM-3102,ATCC-2091.

Table-2: Antifungal properties in terms of Zone of inhibition(mm) ofsynthesized compounds

Sr. No.	Compound	ZOI (mm) with SEM
1	Control Negative	0.00±0.00
2	Control Positive (Fluconazole)	19.33±0.33
3	(16)2a	8.67±0.33
4	(17)2b	8.00±0.58
5	(24)2c	5.67±0.33
6	(25)2d	10.33±0.33
7	(27)2e	8.67±0.33
8	(28)2f	3.67±0.33
9	(30)2h	13.33±0.33
10	(29)2g	19.37±0.32
11	(31)2i	9.67±0.33
12	(35)2j	15.33±0.33
13	(36)2k	17.33±0.33
14	(37)2l	12.00±0.00
15	(38)2m	19.13±0.13
16	(41)2n	14.37±0.32
17	(42)2o	10.67±0.33
18	(5)2p	9.00±0.58
19	(32)3a	9.00±0.58
20	(39)3b	11.00±0.58
21	(40)3c	14.33±0.33

22	(26)4a	14.33±0.33
23	(34)4b	3.67±0.88

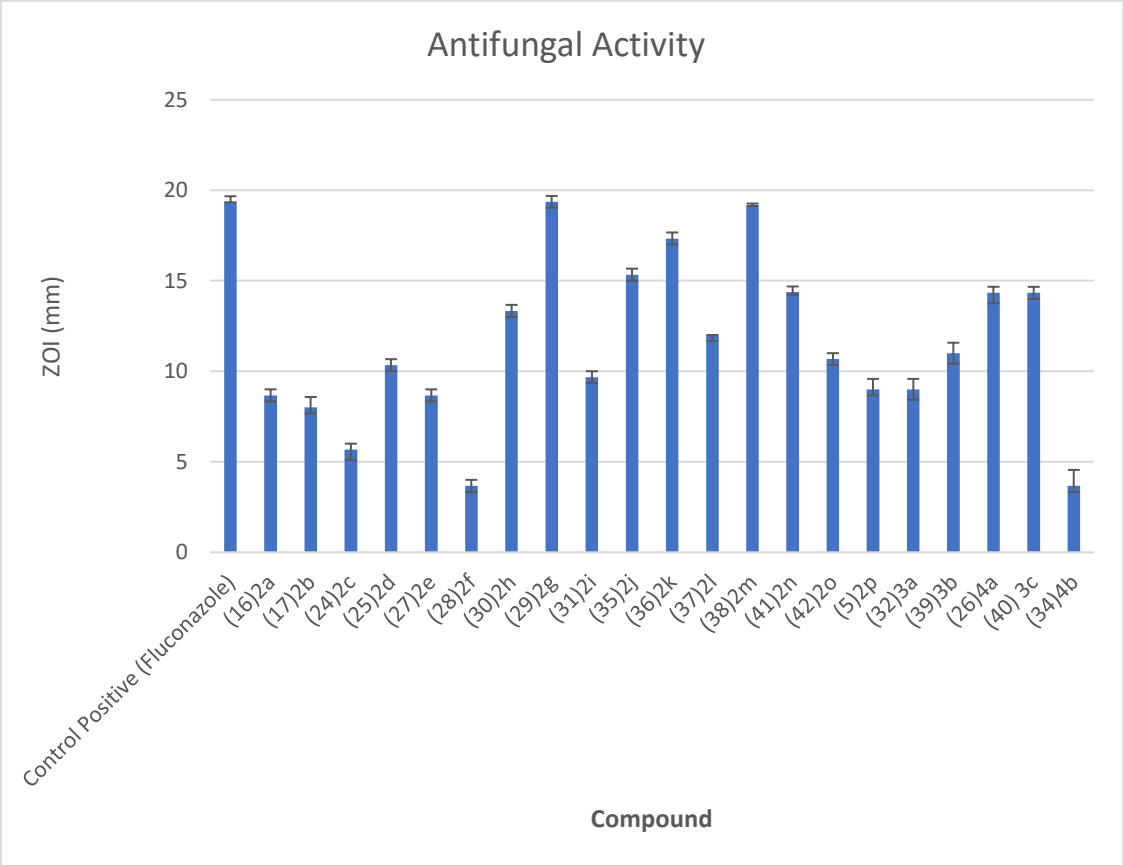


Figure 5: Graphical representation of antifungal activity in terms of zone of inhibition in mm with SEM.

Table 3. Antifungal data as MIC(µg/mL) for prepared compounds.

Compound	*Candida albicans* NICM-3102, ATCC-2091
Fluconazole	2
(16)2a	256
(17)2b	256
(24)2c	512
(25)2d	256
(27)2e	256
(28)2f	512
(29)2g	1
(30)2h	64
(31)2i	256
(35)2j	64
(36)2k	4
(37)2l	256

(38)2m	1
(41)2n	64
(42)2o	256
(5)2p	256
(32)3a	256
(39)3b	256
(40)3c	64
(26)4a	64
(34)4b	512

5. Conclusion

In conclusion, we present a series of synthesised 4-amino-7-chloroquinoline analogues that are easily accessible and exhibit good activity against multidrug-resistant *Candida albicans* NICM-3102, ATCC-2091. In addition, compared to fluconazole, (29)2g and (38)2mwere found to be strong inhibitory action (MIC=1µg/mL) against the *Candida albicans* NICM-3102, ATCC-2091. When compared to fluconazole, these compounds are easier to synthesise and more cost-effective, making them a desirable family of anti-infective agents that can fight drug-resistant fungal strains in the future.

Conflict of Interest

None

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