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Design, Synthesis and Antifungal Activity of 1,2,4-Triazole and 1,3,4- Oxadiazole-ciprofloxacin hybrids

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Abstract

Due to the rapid development of fungal strains resistance, there is become great demand of newer agents that can withstand these resistant strains. This study involves design of ciprofloxacin-triazole or ciprofloxacin-oxadiazole hybrids as potential antifungal agents for the purpose of synergism. Results showed that combination of 1,2,4-triazole or its bioisoster oxadiazole with ciprofloxacin can enhance antifungal activity against *Candida albicans*. The triazole hybrid **11** experienced comparable antifungal activity with itroconazole (MIC = 10.23 and 11.22 µg/mL, respectively). Molecular docking study showed that hybrid **11** can bind to the active site of lanosterol 14-α-demethylase CYP 51 with reasonable docking scores.

Key words
Fluoroquinolones, Azoles, Hybrids, Antifungal, Molecular docking

1. Introduction

Fluoroquinolones stand among the most important antibacterial agents [1 , 3] used nowadays. They showed other versatile biological activities such as antifungal [4 , 5], antitubercular [6 , 7], antitumor [8 , 9], ant-HIV-1 integrase and anti- HCV-NS3 helicase [10], antimalarial [11] and anti-alzheimer [12] activities. Fluoroquinolones exert their antibacterial activity mainly through inhibition of DNA gyrase enzyme [13], therefore fluoroquinolones may have a similar effect on topoisomerases of yeast and molds so topoisomerase II represents a potential target for manipulation of novel antifungal agents [14]. The fourth generation members including gatifloxacin **1** and moxifloxacin **2** showed better antifungal activity in fungal ocular infection during and after ophthalmic surgery and may reduce fungal contamination of the bottle of ophthalmic solution during patient use [15].

Interestingly, combination of antifungal agents such as amphotericin B **5** or fluconazole **6** with two clinically available quinolones such as ciprofloxacin **3** and trovafloxacin **4** showed better antifungal activity against *Candida albicans* than using quinolones alone [16]. *In vivo* studies indicate treated mice infected with *C. albicans* had more survival time when injected intravenously with combination of quinolone and fluconazole than those treated with fluconazole alone that appeared in higher efficacy of antifungal agents when they are combined with fluoroquinolones against candidiasis and aspergillosis [16 , 18].The mechanism of such combination still unclear [19]. These results can explain that combination of quinolones and other antifungal drugs has synergistic effect [15 , 20]. In addition, some norfloxacin derivatives such as compound **7** were found to possess a significant antifungal activity. Also, some quinolone drugs such as ciprofloxacin **3** and moxifloxacin **2** showed a synergistic activity to antifungal drugs, and was found to be beneficial for treatment of cases with concomitant bacterial and fungal infections [21]. On the other hand, azole system is a structural element of many drugs that have antifungal activity such as fluconazole **6**, itraconazole, voriconazole and posaconazole are widely used for treatment of invasive fungal infections (IFIs) [22]. They exerted their antifungal activity by inhibition of ergosterol synthesis, a major component of fungal membrane, through blocking the P450-dependent enzyme, lanosterol 14-α-demethylase (CYP 51) [22], where the azole ring bonded to the heme iron of CYP 51. Lack of ergosterol and accumulation of 14-α-demethylase lead to loss of the function of several enzymes located in membrane that result in inhibition of fungal growth and DNA replication [23].

Moreover, triazoles are promising heterocyclic compounds that showed various pharmacological activities as antimicrobial [24 , 27], antifungal [28 , 30], anticancer [31 , 32], anticonvulsant [33], anti-HIV [34] and antimycobacterial activities [31 , 35]. SAR study of the different antifungal agents indicates that the triazole ring, the difluorophenyl group, and the hydroxyl group are the important pharmacophores for the antifungal activity. Development in biological and pharmacological activities against resistant fungal strains can be obtained by modification of substituents on their side chain [22]. Similarly, 1,3,4-oxadiazole as a bioisoster of 1,2,4 triazole represents an important motif in medicinal chemistry, some 1,3,4-oxadiazole derivatives experienced good antifungal activity when bonded to fluoroquinolones [36 , 37]. Based on the above findings, the aim of this research work is the incorporation of

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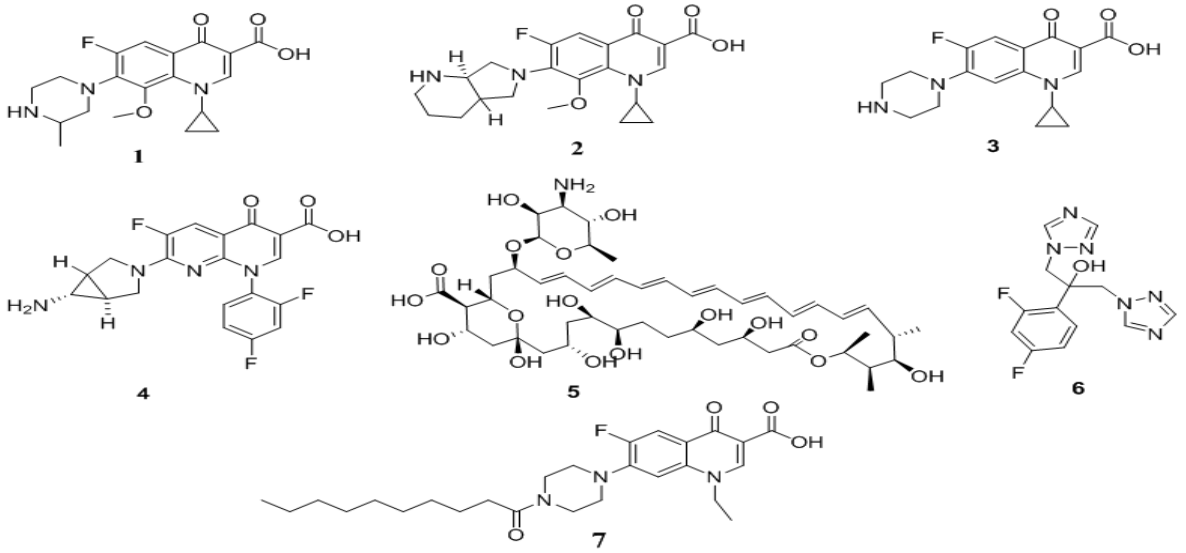


Figure (1): Chemical structure of gatifloxacin **1**, moxifloxacin **2**, ciprofloxacin **3**, trovafloxacin **4**, amphotericin B **5,** fluconazole **6** and compound **7.**

fluoroquinolones and triazole ring or oxadiazole ring in one compact structure for the purpose of synergism. Both antifungal screening and molecular docking study have been carried out to investigate the antifungal activity of the target hybrids.

2. Experimental

2.1. Chemistry

2.1.1. 1-cyclopropyl-7-(4-(2-ethoxy-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4 dihydroquinoline-3-carboxylic acid 8 [38]

A mixture of ethylbromoacetate (0.256mL, 2.4 mmol) and ciprofloxacin HCl (0.30 g, 0.8 mmol) in acetonitrile (50 mL) and TEA (0.17 g, 1.6 mmol) was heated at reflux for 24 h. Solvent was removed under reduced pressure. The residue obtained was crystallized from acetonitrile to afford the target compound **8**. White crystals; 88 % yield; mp: 190-191 °C (reported m.p 189-190 °C).

2.1.2. 1-cyclopropyl-6-fluoro-7-(4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 9 [38]

A mixture of compound **8** (0.5 g, 1.19 mmol) and hydrazine hydrate (100%) (0.37 mL, 119 mmol) in absolute ethanol (50 mL) was heated at reflux for 24 h. Solvent was removed under reduced pressure. The residue obtained was crystallized from absolute ethanol to afford the target compound **9**. Pale yellow crystal; 70.39 % yield; mp: 245-250 °C (reported m.p 246-247 °C).

2.1.3. 1-cyclopropyl-7-(4-(2-(2-(ethylcarbamothioyl)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 10 [38]

An equimolar mixture of compound **9** (0.2, 0.49 mmole), Ethyl isothiocynate (0.042g (0.043mL), 0.00049 mole) and absolute ethanol was heated at reflux for 18 h. Solvent was removed under reduced pressure. The residue obtained was crystallized from absolute ethanol to afford compound **10**. Yellowish white powder; 62.50% yield; m.p : 226 °C (reported m.p 224-225).

2.1.4. 1-cyclopropyl-7-(4-((4-ethyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 11 [38]

A mixture of compound **10** (0.20 g, 0.41 mmole) and KOH (0.0457 g, 0.82 mmole) in distilled water (10 mL) was heated at reflux for 24 h, then acidification with 1 M HCl drop wise until reach pH = 2, the resulted precipitate obtained is filtrated and washed several times with distilled water then recrystalized from absolute ethanol to afford compound **11**. Pale yellow powder; 67.35% yield; m.p : >300 °C (reported m.p : 320 °C).

2.1.5. 1-cyclopropyl-6-fluoro-4-oxo-7-(4-((5-thioxo-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 12[38]

An equimolar mixture of compound **9** (0.2 g, 0.49 mmole) , carbon disulfide (0.037 g (0.03 mL), 0.49 mmole), KOH (0.06 g, 1.1 mmole) and absolute ethanol was heated at reflux for 12 h. Solvent was removed under reduced pressure. The residue obtained was dissolved in about 100 mL distilled H_2O Then add 1 M HCl drop wise until reaching PH=2, the obtained precipitate is filtrated and washed several times with distilled H_2O to afford the target compound **12**. Yellow powder; 81.81% yield; m.p : 257 °C (reported m.p : 256-257 °C).

2.2. Screening of antifungal activity

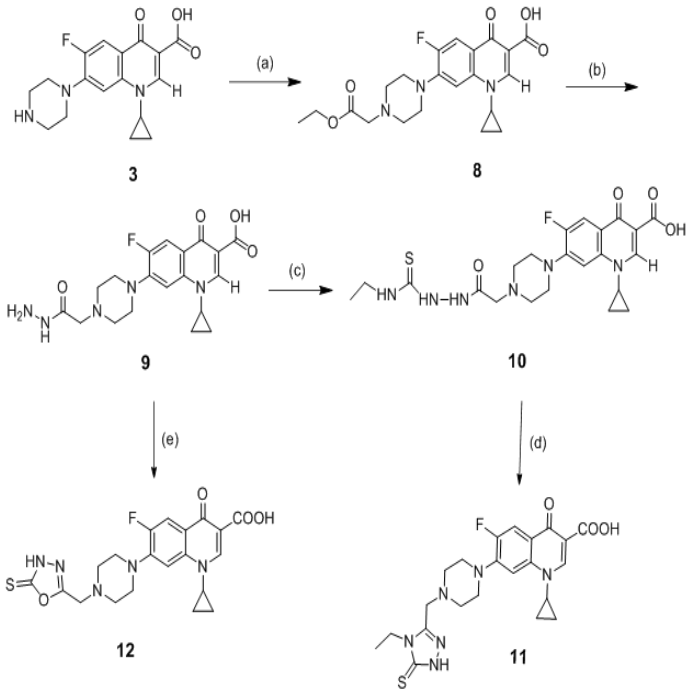
From all the tested fungi 0.5 mL of 1×108 CFU/mL (0.5 McFarland turbidity) were plated in sterile petri dishes, then 20 mL of Mueller Hinton Agar media (Oxoid) was added to each petri dish. The plates were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork bore. Two fold serial dilutions of the tested

compounds using DMSO were performed. An equal volume of 100µL of each dilution was applied separately to each well in three replicates using a micropipette. All plates were incubated at 37 °C for 24 h. The inhibition zones were measured and their average was calculated. The MIC was calculated by plotting the natural logarithm of the concentration of each dilution of the tested compounds against the square of zones of inhibition and a regression line was drawn through the points then the antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value [39].

3. Result and discussion

3.1. Chemistry

The target compounds **10**, **11**, and **12** were prepared as described by Mentese *et al.*, [38]. Equimolar mixture of ciprofloxacin. HCl, ethylbromoacetate and TEA as catalyst were heated at reflux to give ester **8**. Hydrazinolsis of ester **8** in ethanol for 24 h gave the hydrazide **9**. Reaction of the hydrazide **9** with ethyl isothiocyanate in ethnol afforded the intermediate **10**. Cyclization of compound **10** with equimolar amount of aqueous solution of KOH gave the triazole **11**. Cyclization of **9** with CS_2/KOH in ethanole afforded the oxadiazole **12** (**scheme 1**).



Scheme (1): Synthesis of compounds **10**, **11**, and **12**.

Reagents and reaction condition as the following: (a) Ethyl bromooacetate / (TEA) , reflux, 24 h ; (b) NH_2NH_2. H_2O, EtOH, reflux, 24 h; (c) Ethyl isothiocynate, EtOH, reflux, 24 h; (d) KOH pellets, H_2O, reflux, 6 h; (e) ethyl isothiocynate, EtOH, reflux, 24 h; (d) KOH pellets, CS_2, EtOH, reflux, 24 h.

3.2. Screening of antifungal activity

The antifungal activity of compounds **10**, **11** and **12** was screened against *Candida albicans* using itraconazole as reference antifungal drug using Muller Hinton as media for fungal growth. The results were illustrated in **(Table 1)**. From the results, it is clear that the ciprofloxacin-azole hybrid **11** showed promising antifungal activity with MIC value = 10.23µg/mL, which is comparable in activity with the reference itraconazole (MIC = 11.22 µg/mL). Compound **10** showed weak antifungal activity with MIC value = 199.52 µg/mL. Also, compound **12** showed a moderate antifungal activity (MIC = 64.56 µg/mL). From the above results, it is obvious that the incorporation of 1,2,4-triazole with ciprofloxacin improved the antifungal activity. Replacement of 1,2,4-triazole with the bioisoster, 1,3,4-oxadiazole ring decreased the antifungal activity of such hybrids.

Table 1: MIC (µg/mL) of the tested compounds (**10**, **11**, and **12**) and itraconazole.

Compound	MIC(µg/mL) *Candida albicans*
10	199.52
11	10.23
12	64.56
Itraconazole	11.22

3.3. Molecular Docking Studies

In order to investigate the binding mode and binding energies of the target final hybrids **10-12**, docking study has been carried out using MOE 2014 Dock program. The tested compounds were docked into the binding pocket of the active site of lanosterol 14-α-demethylase CYP 51 (PDB: 5esg). The docking reliability was validated using the known X-ray structure of lanosterol 14-α-demethylase in complex with itraconazole. Conformation of each compound was selected on the basis of docking score. The docking scores of the studied compounds are shown in **(Table 2)**.

Table 2: Docking Scores of the tested compounds

Compound	Docking Score/Ki
10	-4.91
11	-8.29
12	-6.94
Itraconazole	-12.44

In the CYP 51 model, the heme group was bound into the active site through hydrophobic, Van der Waals, and hydrogen-bonding interactions. Itraconazole forms hydrophobic interactions with active site of the enzyme where heme group presents and also with Cys 470 amino acid residue as shown in **Figures (1 and 2)**. In these two figures, itraconazole appear to form hydrogen bonding by its two carboxylate moieties, the first one forms one hydrogen bond with Tyr 126 amino acid residue, two hydrogen bonds with Arg 385 amino acid residue and one water mediated hydrogen bond with Gly 465 amino acid residue. The other carboxylate moiety forms one hydrogen bond with Tyr 140 amino acid residue, one hydrogen bond with Lys 151 amino acid residue and two hydrogen bonds with His 468 amino acid residue. Also itraconazole forms three Van der Waalls interactions with Thr 318, Gly 472, Ile 471 amino acid residues.

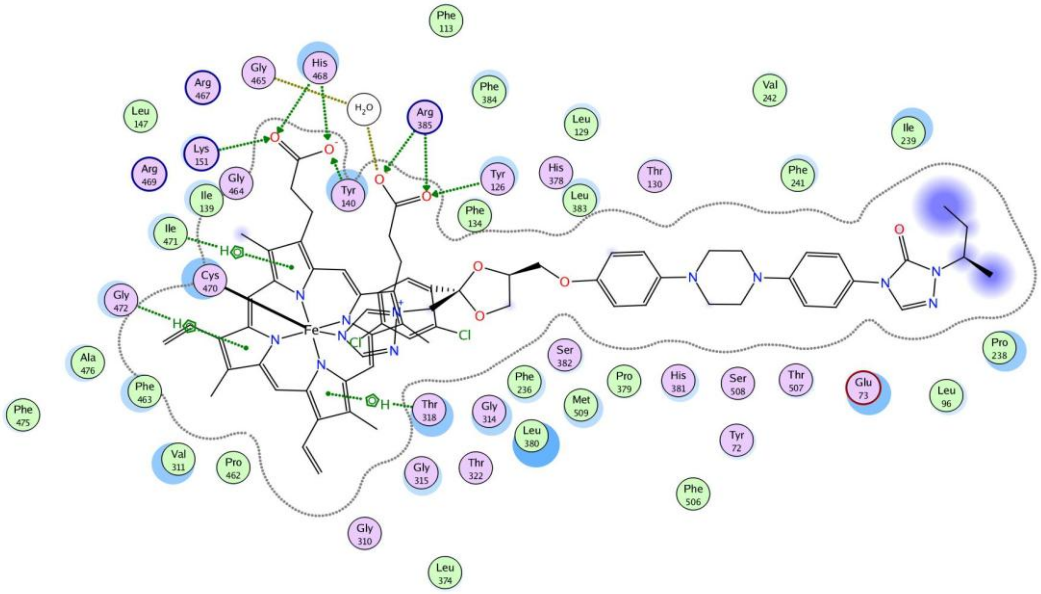


Figure 2: 2D model of binding of itraconazole with active site of lanosterol 14-α-demethylase enzyme.

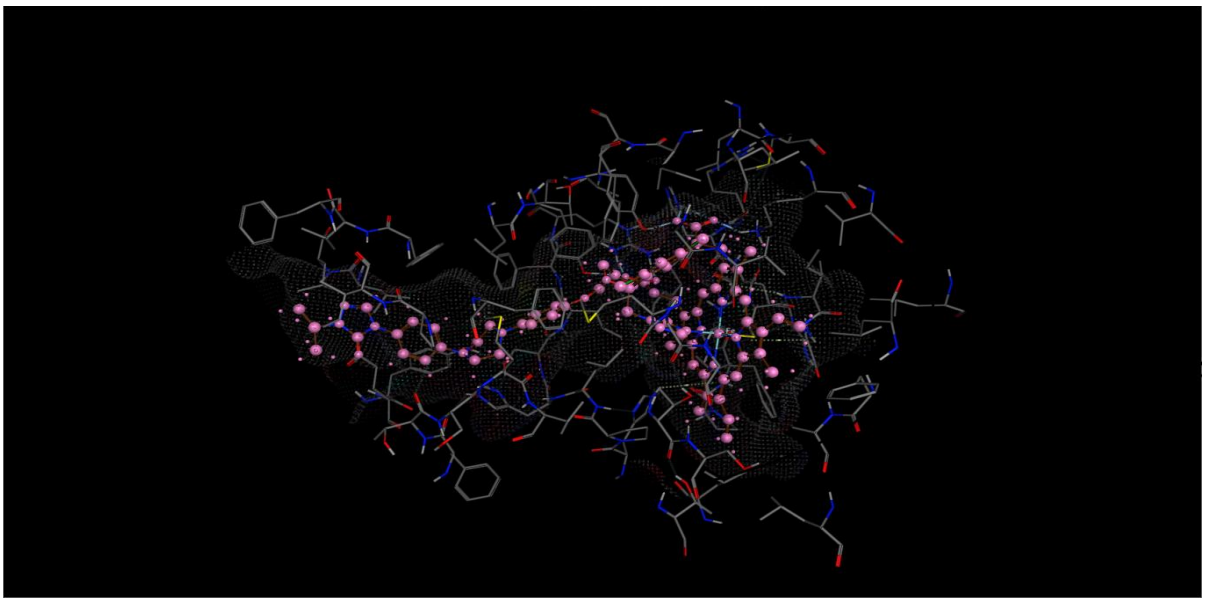


Figure3: 3D model of binding of itraconazole(appear as pink balls) with active site of lanosterol 14-α-demethylase enzyme.

The tested compounds were found to bind strongly to CYP 51 as inferred by binding energy values where the binding scores range from -6.94 to -8.29 Kcal/mol **(Figure 4-6, table 2)**.

(Figure 4) showed that the thiosemicarbazide **10** forms hydrophobic interactions with active site of the enzyme and the naphthyridone moiety of drug **10** forms Van der Waals interaction with Gly 472.

It is obvious that, the triazole hybrid **11** forms hydrophobic interactions with active site of the enzyme Also, carboxylate moiety of compound **11** forms water mediated hydrogen bond with Gly 465 amino acid residue. In addition, compound **11** forms hydrogen bond with each of the following amino acid residues: Cys 470, Val 311and Leu 312 **(Figure 5)**.

(Figure 6) shows that the oxadiazole hybrid **12** forms hydrophobic interactions with active site of the enzyme .One hydrogen bond is formed between carboxylate moiety and Arg 385 amino acid residue. In addition, compound **12** forms Van der Waals interaction with Val 311.

Depending on the previous data obtained from this docking study of compounds **10**, **11** and **12** in comparison with reference itraconazole, it was found that formation of two hydrogen bonds with Arg 385 amino acid residue is essential to show antifungal activity. It is obvious that the most active derivative **11** has good fitting ability to the suggested active site. It can form a hydrophobic interaction and water mediated hydrogen bonding with Gly 465. In addition it can form hydrogen bonding with Cys 470, Val 311 and Leu 312 amino acid residues. The triazole ring nitrogen can form hydrogen bond with Val 311. On the other hand, the less active oxadiazole derivative 12 can form hydrogen bonding only with Arg 385. These findings explain an important and essential role for the triazole ring nitrogen with the suggested target.

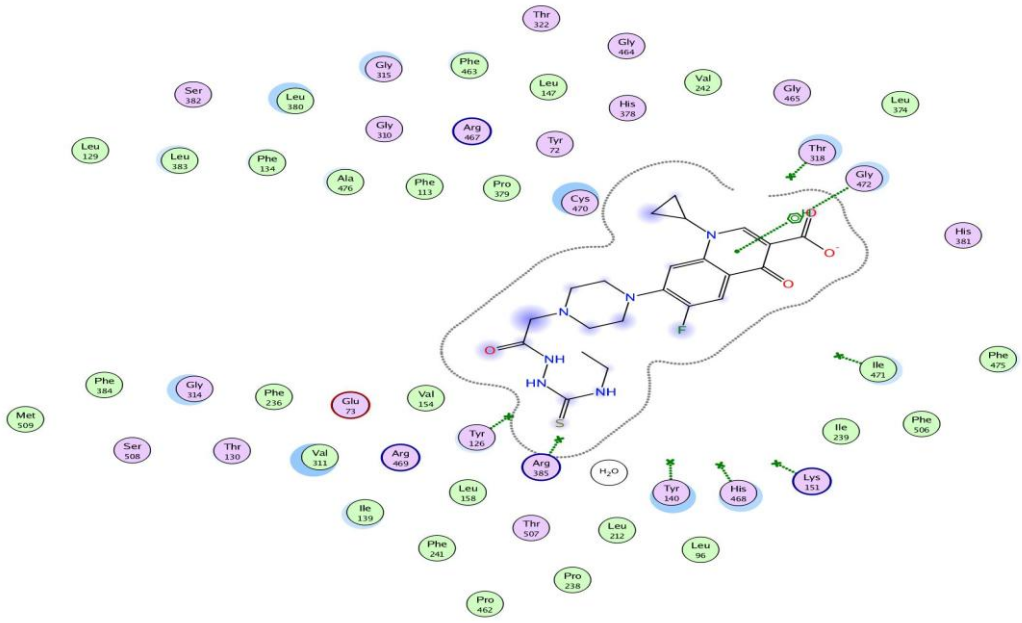


Figure 4: 2D model of binding of compound **10** with active site of lanosterol 14-α-demethylase enzyme.

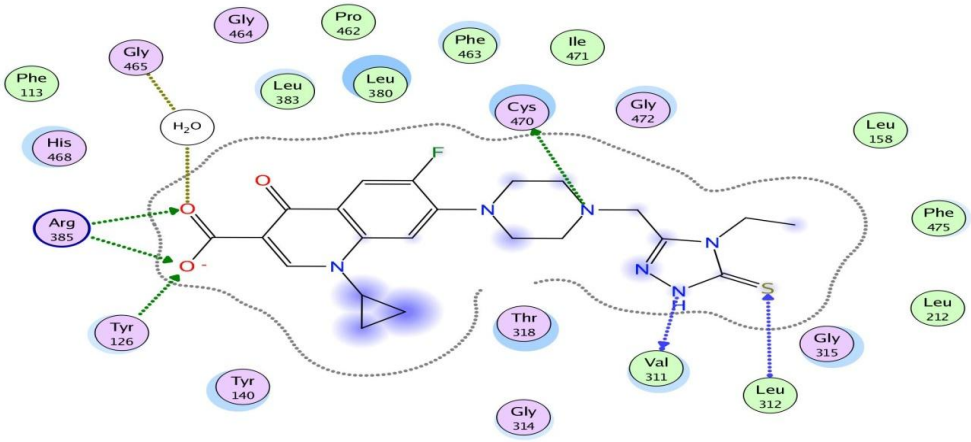


Figure 5: 2D model of binding of compound **11** with active site of lanosterol 14-α- demethylase enzyme.

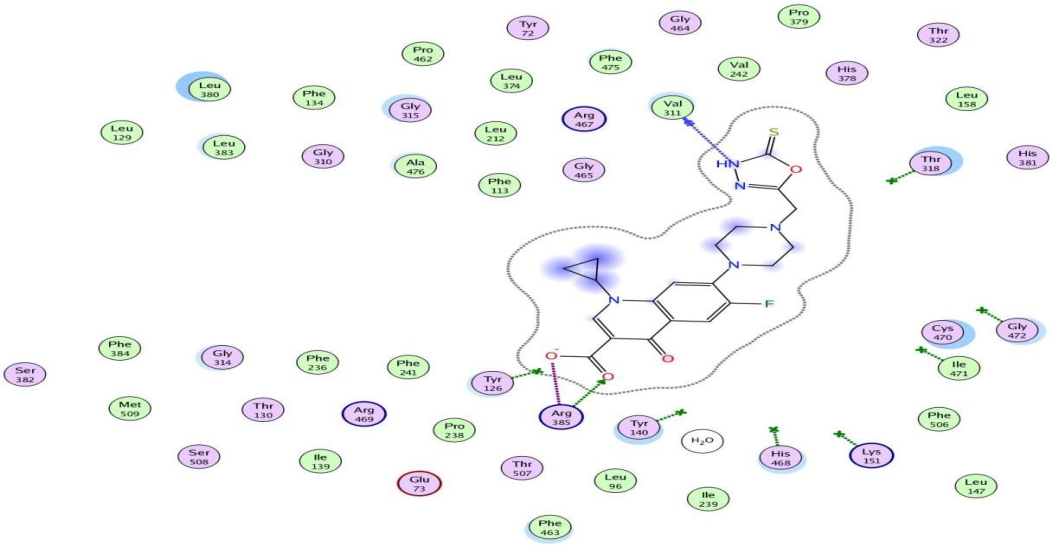


Figure 6: 2D model of binding of compound **12** with active site of lanosterol 14-α-demethylase enzyme.

Conclusion

Fluoroquinolone-triazole hybrid **11** and its corresponding bioisoster oxadiazole were prepared and tested for their antifungal activity. The fluoroquinolone-triazole hybrid showed comparable activity with the reference itraconazole. From docking results, it is worth to note that the active antifungal hybrid **11** can bind with the active site of lanosterol 14-α-demethylase CYP 51 with reasonable docking scores, there is no complete binding was observed for the inactive thiosemicarbazide **10**. The fluoroquinolone-triazole hybrids are considered promising new antifungal agent that requires further investigation for their mechanism of action.

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