EXPLORING SYNTHESIS OF SOME QUINAZOLINE-INDAZOLE ANALOGUES AND EVALUATION OF THEIR ANTIBIOFILM AND ANTICANDIDAL POTENTIALS

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Abstract

Candidiasis a pathologically important condition imposing huge impact on mankind as it is mainly found in the immunocompromised patients. Emergence of resistance against existing antifungals makes them ineffective causing high incidence and accompanying mortality due to fungal infections. Generation of biofilms by various species of candida is the most frequent underlying mechanism in the emergence of resistance. Development of newer antifungal with lower resistance remains a challenging task for the researchers.

In this report, we have developed a facile and environment friendly synthesis of quinazoline-indazole scaffold and investigated their anti-biofilm, antifungal potential. Among the synthesized compounds, **14i and 14m** have shown potent inhibitory activity against *C. albicans* than Fluconazole (standard antifungal agent). Compound **14j 14k and 14n** also exhibited significant antibiofilm and antifungal activity as compared to fluconazole against *C. albicans*. The above results could serve as important lead in the discovery of effective antifungal agent to overcome the resistance problem associated with the existing antifungal agents.

Keywords: Quinazoline; Indazole; Antifungal activity; Antibiofilm.

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1. Introduction

Clinical studies have found invasive fungal infections emerged as major co-morbid and co-mortal conditions especially in immune-suppressed patients such as patients suffering with AIDS, organ transplant and cancer.[1–3] Candidiasis is the major contributing infection in the immunocompromised patients.[4,5] The key virulent characteristics of *C. albicans* is host tissue invasion achieved through morphogenetic [Yeast-to-Hypha (Y-H)] transitions. Besides this, the formation of biofilms by candida is the other contributing factor in *Candida* infections on host tissues or abiotic devices.[6-8] Biofilms are defined as microbial colonization which is encapsulated and suspended in extracellular polymeric substances (EPS) matrix which contains nucleic acid responsible for maintaining and protecting biofilm structure. The EPS also provides increased tolerance to antifungal drugs. Biofilms formed by *Candida* turns them more resistant to antifungal agents than planktonic cells.[9] The traditional treatment for *Candida* infections as shown in fig. 1 includes azoles (fluconazole, itraconazole, and voriconazole), echinocandins (anidulafungin, caspofungin, and micafungin), amphotericin B (deoxycholate and various lipid formulations) and flucytosine (5-FC).[10-13] Fluconazole is currently the choice of drug for mucosal and invasive candidiasis because the drug has fewer side effects than amphotericin B.[5,14]

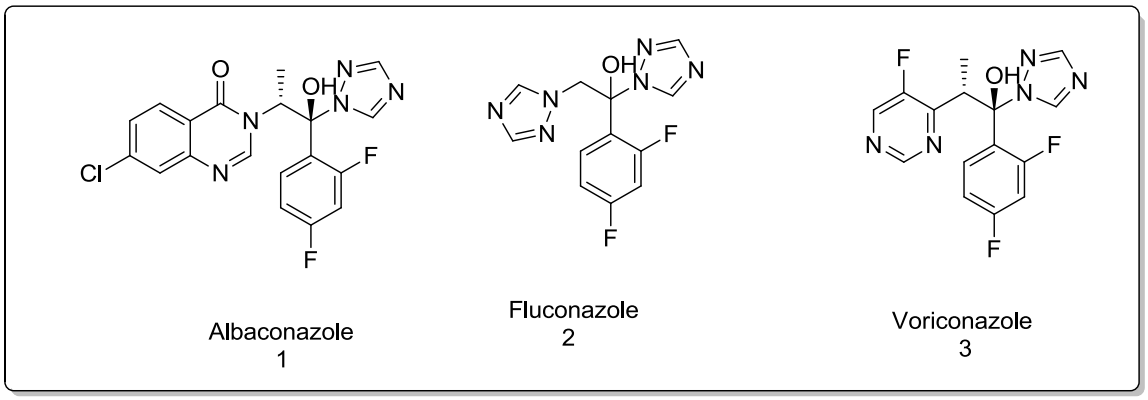


Fig.-1. Marketed antifungal drugs

However, fluconazole has little effect against different strains of *Candida* due to increased number of fluconazole-resistant strains. Additionally, most antifungals were vulnerable to resistance and cause toxicity to patients making them least preferable for the use. Moreover, research findings has shown that these existing antifungal agents also kills probiotics essential for human immune systems.[15] These limitations led scientists to develop newer antifungal agents with enhanced antibiofilm potential.

Majority of therapeutic agents have found that nitrogen-containing heterocycle as an important scaffold for their therapeutic action. Typically, recent reports suggest derivatives of heterocyclic quinazoline-2,4-dione have antifungal and antibacterial actions.[16-20] Marketed drugs containing quinazoline as a core structure has shown in Fig. 2.

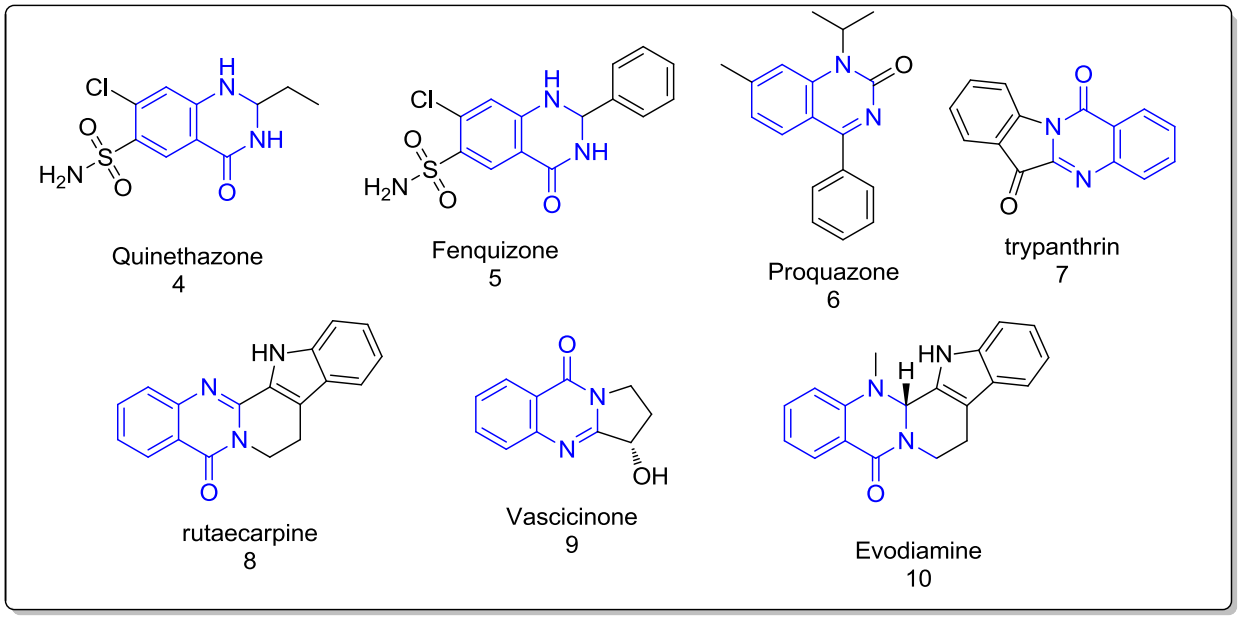


Fig.-2 Therapeutically active drug containing quinazoline as a core structure

Another most important classes of nitrogen-containing heterocyclic compounds is the indazole, which consists of two rings, a pyrazole ring and a benzene ring. The indazole scaffold is also an important component of medicinal chemistry because it has several biological functions, including anti-inflammatory, anti-protozoal, and anti-cancer, anti-mutagenic, anti-hypertensive, anti-fungal, anti-bacterial, anti-HIV, anti-platelet.[21-29] Diverse indazole-containing compounds with different pharmacologically significant functional groups serve as structural motifs in drug molecules. Based on the above findings, in this report we propose the conjugation of indazole with the quinazoline moiety in order to achieve efficient antifungal and anti-biofilm activity.

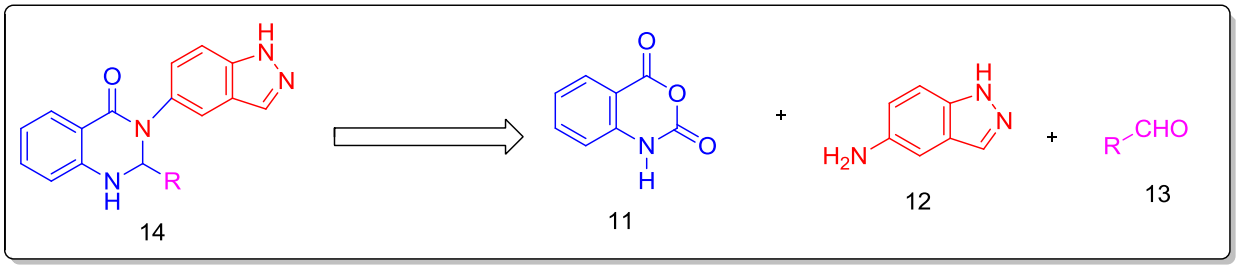
2. EXPERIMENTAL

Chemical methods

^{1}H NMR spectra recorded in solvent dimethyl sulfoxide (DMSO)-d6 using BRUKER AV 500MHz.Chemical shifts were reported in δ ppm with Tetramethylsilane as an internal standard (TMS, δ = 0.0). Mass spectra were recorded on LC-QTOF MS/MS. Melting points were recorded using 'ThermoScientific' apparatus and are uncorrected.

*General procedure for the synthesis of 3-(1H-indazol-5-yl)-2-substitutedphenyl-2,3-dihydroquinazolin-4(1H)-one (**14a-o**)*

In a round bottom flask (10 mL) a mixture of isatoic anhydride **1** (1 mmol), indazole amine **2** (1 mMol), substituted aldehydes (1 mmol) were taken in a 5 mL ethanol. The resulting reaction mixture was refluxed for 120 minute. Reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled and poured on ice cold water. The solid product obtained was filtered and recrystallized in hot ethanol to obtain the pure product.



Scheme-1: RetroSynthesis of titled compounds **14 a-o**

2-(4-chlorophenyl)-3-(1H-indazol-5-yl)-2,3-dihydroquinazolin-4(1H)-one (14a)
Yield: 91%; m.p. 218°C; 1H-NMR (500 MHz, DMSO-d6): δ 5.38 (s, 2H, phenyl -OH), 5.78 (s, 1H, C_1-NH), 6.12 (s, 1H, C_2-H), 6.90 (t, 1H, C_6-H), 7.10 (d, 1H, -C_8H), 7.28 (s, 1H, phenyl -CH), 7.58 (s, 1H, phenyl-CH), 7.72 (t, 1H, -C_7H), 7.85 (d, 1H, -C_5H), 7.91 (d, 1H, indazolyl–C_2H), 7.95 (s, 1H, indazolyl–C_6H), 8.01 (s, 1H, indazolyl–C_7H), 8.09 (d, 1H, indazolyl–C_3H) 10.4 (s, 1H, indazolyl–NH) MS (LC-QTOF) $C_{21}H_{15}ClN_4O$ $[M+1]^+$ Std: 423.85 Found: 424.84.

2-(3,4-dihydroxyphenyl)-3-(1H-indazol-5-yl)-2,3-dihydroquinazolin-4(1H)-one (14d)
Yield: 91%; m.p. 218°C; 1H-NMR (500 MHz, DMSO-d6): δ 5.78 (s, 1H, C_1-NH), 6.12 (s, 1H, C_2-H), 6.90 (t, 1H, C_6-H), 6.98 (s, 1H, phenyl-CH), 7.10 (d, 1H, -C_8H), 7.28 (d, 1H, phenyl -CH), 7.58 (d, 1H, phenyl-CH), 7.72 (t, 1H, -C_7H), 7.85 (d, 1H, -C_5H), 7.91 (d, 1H, indazolyl–C_2H), 7.95 (s, 1H, indazolyl–C_6H), 8.01 (s, 1H, indazolyl–C_7H), 8.09 (d, 1H, indazolyl–C_3H) 13.01 (s, 1H, indazolyl–NH) MS (LC-QTOF) $C_{21}H_{16}N_4O_3$ $[M+1]^+$ Std: 372.38 Found: 373.36.

2-(2,6-dichlorophenyl)-3-(1H-indazol-5-yl)-2,3-dihydroquinazolin-4(1H)-one (14f)
Yield: 91%; m.p. 218°C; 1H-NMR (500 MHz, DMSO-d6): δ 5.78 (s, 1H, C_1-NH), 6.12 (s, 1H, C_2-H), 6.90 (t, 1H, C_6-H), 7.10 (d, 1H, -C_8H), 7.28 (d, 1H, phenyl -CH), 7.46 (t, 1H, phenyl -CH),7.58 (t, 1H, phenyl-CH), 7.65 (d, 1H, phenyl -CH), 7.72 (t, 1H, -C_7H), 7.85 (d, 1H, -C_5H), 7.91 (d, 1H, indazolyl–C_2H), 7.95 (s, 1H, indazolyl–C_6H), 8.01 (s, 1H, indazolyl–C_7H), 8.09 (d, 1H, indazolyl–C_3H) 11.76 (s, 1H, O=C-OH), 12.4 (s, 1H, indazolyl–NH) MS (LC-QTOF) $C_{22}H_{16}N_4O_3$ $[M+1]^+$ Std: 384.39 Found: 385.41.

3-(1H-indazol-5-yl)-2-(4-nitrophenyl)-2,3-dihydroquinazolin-4(1H)-one (14j)
Yield: 91%; m.p. 218°C; 1H-NMR (500 MHz, DMSO-d6): δ 5.38 (s, 1H, phenyl-OH),5.78 (s, 1H, C_1-NH), 6.12 (s, 1H, C_2-H), 6.90 (t, 1H, C_6-H), 7.10 (d, 1H, -C_8H), 6.28 (d, 2H, phenyl -CH), 6.58 (d, 1H, phenyl-CH), 7.58 (t, 1H, phenyl-CH), 7.72 (t, 1H, -C_7H), 7.85 (d, 1H, -C_5H), 7.91 (d, 1H, indazolyl–C_2H), 7.95 (s, 1H, indazolyl–C_6H), 8.01 (s, 1H, indazolyl–C_7H), 8.09 (d, 1H, indazolyl–C_3H) 10.4 (s, 1H, indazolyl–NH) MS (LC-QTOF) $C_{21}H_{16}N_4O_2$ $[M+1]^+$ Std: 356.38 Found: 357.33.

3-(1H-indazol-5-yl)-2-(3,4,5-trimethoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (14n)
Yield: 91%; m.p. 218°C; 1H-NMR (500 MHz, DMSO-d6): δ 3.80 (s, 6H, O-CH_3), 5.78 (s, 1H, C_1-NH), 6.12 (s, 1H, C_2-H), 6.90 (t, 1H, C_6-H), 7.10 (d, 1H, -C_8H), 7.28 (d, 1H, phenyl -CH), 7.34 (d, 1H, phenyl -CH), 7.58 (s, 1H, phenyl-CH), 7.72 (d, 1H, -C_7H), 7.85 (d, 1H, -C_5H), 7.91 (d, 1H, indazolyl–C_2H), 7.95 (s, 1H, indazolyl–

C_6H), 8.01 (s, 1H, indazolyl–C_7H), 8.09 (d, 1H, indazolyl–C_3H) 10.4 (s, 1H, indazolyl–NH) MS (LC-QTOF) $C_{23}H_{20}N_4O_3$ $[M+1]^+$ Std: 400.43 Found: 401.41.

Biological evaluation

Antimicrobial activity

Newly synthesized derivatives were evaluated using agar method for antifungal and anti-biofilm activity by performing assay against *C. albicans*. Results were obtained in terms of respective IC_{50} and MIC conc. In order to precise the results, all the experiments were performed in triplicates.*

3. RESULT DISCUSSION

Chemistry

Our efforts to synthesize conjugated quinazoline-indazole scaffold by exploring one-pot multi component synthesis approach by reacting isatoic anhydride **1** (1 mmol), indazole amine **2** (1 mmol), substituted aldehydes (1 mmol) in a 5 mL water. Compound **14a** was obtained under the catalytic environment of Citric acid, Molecular Iodine and Acetic acid. Citric acid was emerged as most efficient catalyst amongst them with the yield of 88%. The study was continued in order to screen the effect of solvent on the yield of **14a** using various solvents such as ethanol, methanol, DMF, dioxane, EtOAc, acetonitrile using citric acid as a catalyst. Polar aprotic solvents failed to provide the desired product (Entries 7, 8, 9, 10 in table 1).

Table-1: Optimization of reaction conditions and quantity of catalysts for the synthesis of **14a**[a]

Entry	Catalyst	Solvent	Catalyst loading (Mol %)	Time (minutes)	Yields[b] (%)
1	Citric acid	Water	5	40	84
2	Molecular Iodine	Water	10	25	70
3	Acetic acid	Ethanol	10	35	74
4	Citric acid	Methanol	5	20	87
5	Citric acid	Ethanol	5	20	91
6	Citric acid	Ethanol	10	20	91
7	Citric acid	DMF	10	60	-
8	Citric acid	Dioxane	10	60	-
9	Citric acid	EtOAc	10	60	-
10	Citric acid	Acetonitrile	10	60	-

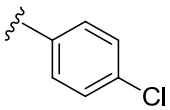
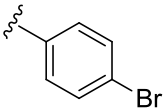
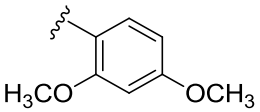
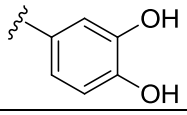
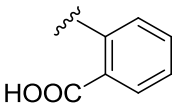
[a]All reactions were carried out with 1 mmol of isatoic anhydride, 1 mmol of indazole amine, and 1 mmol of 4-chlorobenzaldehyde in the presence of catalyst in solvent (5 mL) at refluxing temperature.

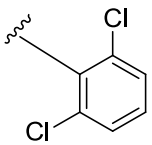
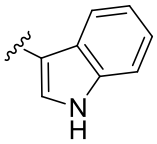
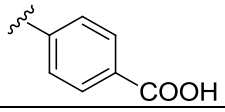
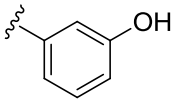
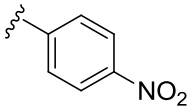
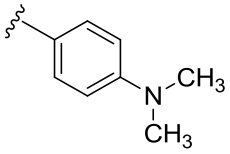
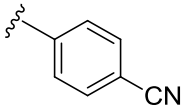
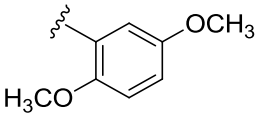
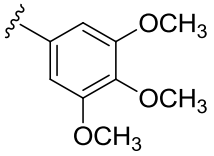
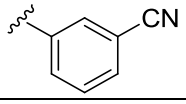
[b]Isolated yields.

On contrary, Methanol and Ethanol provided the yields of 87% and 91% respectively. We also investigated the effect of amount of catalyst over the yield and found that yield was increased upto 91% using 5 mol% of citric acid. Beyond this concentration, no significant increase in the yield was observed. With the optimized conditions in hand i.e. 5 mol % of citric acid in ethanol, we synthesized library of **14 a-o** compounds as shown in Table 2. Both the electron withdrawing groups as well as electron donating groups were found well tolerated in this reaction condition.

The formations of titled compounds were confirmed using 1H-NMR and 13C-NMR data. The characteristic =N-NH peak signal of formed schiff base appeared at δ 10.4 ppm. All the aromatic protons of substituted aldehyde **14 a-o** were appeared in the range of δ 6.95 – 8.30 ppm. On the other hand, -C-NH peak of quinazoline appeared at δ 5.28 ppm in the ^{1}HNMR spectra of all the synthesized compounds **14 a-o**. The protons of the synthesized compounds **14 a-o** resonated in the expected regions confirming the successful formation of desired compounds. The characteristic M+1 peak also appeared in recorded mass spectra of the almost all the compounds again confirming the successful synthesis of compounds **14 a-o**.

Table-2: Synthesis of different substituted indazole-quinazoline scaffold **14 a-o** and their antibiofilm and antifungal activity

Code	R	% Yield	Melting point (^{0} C)	Anti-biofilm activity (IC_{50}μM)	Antifungal activity (MIC Values in μg/mL)
				C. albicans	*C. albicans*
14a		91	218	101.4	89.4
14b		90	214	78.34	69.2
14c		92	216	55.47	74.1
14d		89	218	76.22	94.7
14e		86	220	88.1	102.1

14f		88	222	169.1	177.3
14g		87	226	142.5	165.3
14h		89	218	137.4	190.1
14i		90	212	44.2	50.3
14j		92	234	39.3	59.0
14k		90	226	48.1	56.5
14l		93	224	68.7	75.5
14m		95	208	32.1	51.1
14n		91	210	43.4	55.6
14o		89	220	55.4	73.2
Fluconazole	-	-	-	40	50

Antibiofilm activity

Compound **14m** exhibited excellent antibiofilm activity with IC_{50} value of 32.1 µM suggesting excellent antibiofilm as well as antifungal potential as shown in Fig. 3. Also compounds **14i, 14j, 14k** also showed good biofilm inhibitory potential during assay with IC_{50} value of 44.2, 39.3 and 43.4 µM, respectively.

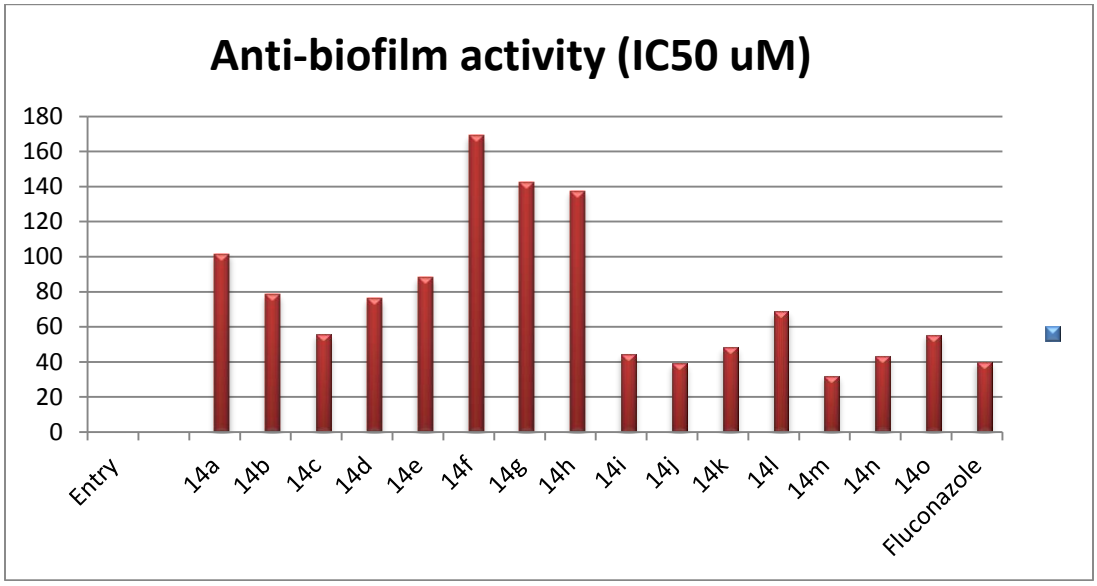


Fig.-3 Graphical representation of anti-biofilm activity of **14 a-o**

Antifungal activity

The synthesized compounds **14 a-p** was evaluated for their *in vitro* antifungal activity by standard agar dilution method. Antifungal assay results shown in table 2 depict good to excellent inhibition against *C. albicans.* Compounds **14i** and **14m** as shown in Fig.1 displayed good inhibitory profile against *C. albicans* with MIC values of 50.3 and 51.1 µg/mL, respectively. Additionally, **14j, 14k** and **14n** also displayed inhibition against *C. albicans* comparable to Fluconazole (MIC = 50µg/mL) with MIC values of 59, 56.5 and 55.6 µg/mL, respectively.

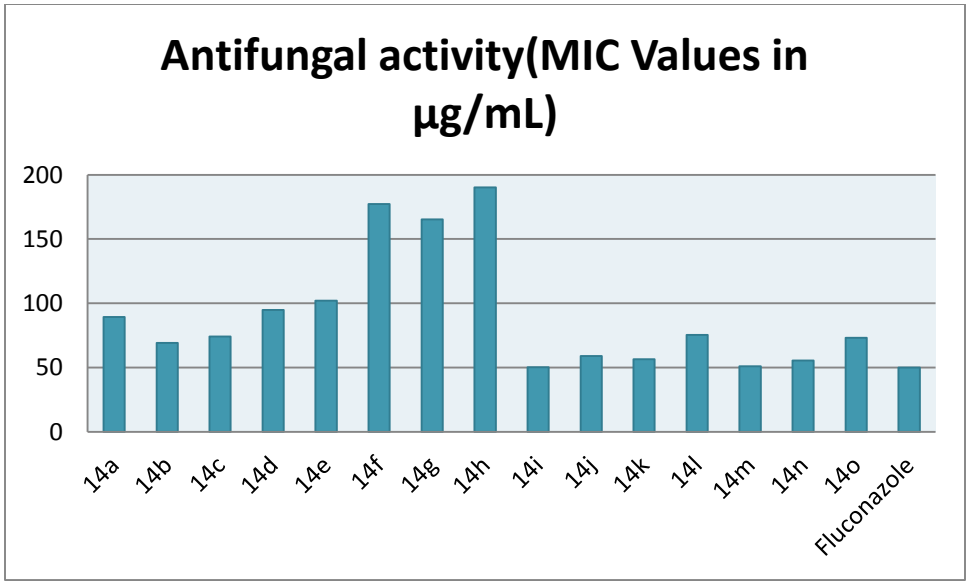


Fig.-4 Graphical representation of antifungal activity of **14 a-o**

Amongst the synthesized compounds, compound **14m** with dimethoxy substitution on 2nd and 5th position of the side ring exhibited the most potent biofilm as well as fungal inhibition with IC_{50} 36.4 □M and MIC 51.1 µg/mL against C. *Albicans*. The compound **14i** with hydroxy substitution on 2nd position also delivered potent anti-biofilm activity with IC_{50} of 44.2 □M and antifungal activity with MIC 50.3 µg/mL. Interestingly, the compounds **14j** and **14k** also delivered significant antibiofilm and antifungal activity compared to fluconazole against *C. albicans* suggesting electron donating group substitution at para position required to have antifungal and antibiofilm activity. On contrary, more than one substitution of electron donating group at ortho or meta position has highest activity against *C. albicans*.

4. CONCLUSION

In conclusion, a facile and environment friendly synthesis and investigation anti-biofilm, antifungal potential of quinazoline-indazole scaffold was done. Among the synthesized compounds, **14i and 14m** have shown potent inhibitory activity against *C. albicans* than Fluconazole (standard antifungal agent). Compound **14j 14k and 14n** also exhibited significant antibiofilm and antifungal activity as compared to fluconazole against *C. albicans*. The above results could serve as important lead in the discovery of effective antifungal agent to overcome the resistance problem associated with the existing antifungal agents.

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Conflict of Interest: No conflict of interest was declared by theauthors.

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