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Study on Antibacterial Activity for Multidrug Resistance Stain by using Phenyl Pyrazolones Substituted 3-amino 1H-pyrazolon (3,4-b) Quinoline Derivative In Vitro Condition

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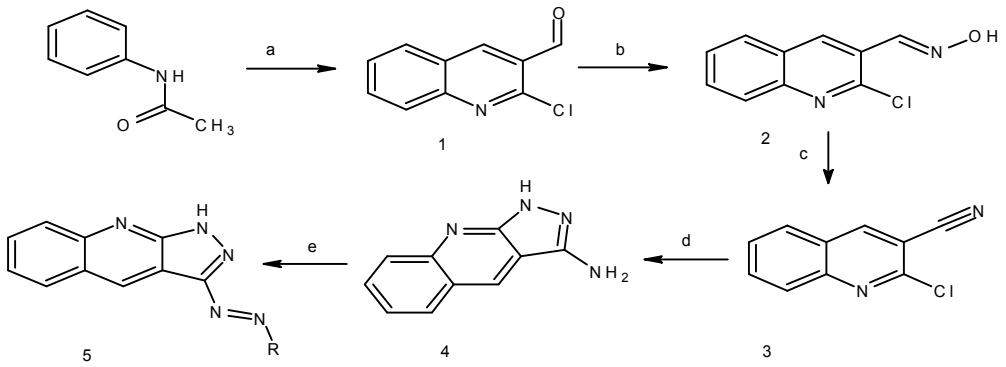
Abstract: 3-methyl-1-phenyl-4-[(E)-2-{1H-pyrazolo [3,4-b] quinolin-3-yl} diazen-1-yl]-4,5-dihydro-1H-pyrazol-5-one derivative have been synthesized by the reaction between substituted phenyl pyrazolones and 3-amino -1 H-pyrazolo [3,4-b] quinoline. The novel compound structure has been established on the basis of their substituted phenyl pyrazolones derivatives. These compounds were tested for in vitro antifungal or antibacterial activity against Gram-Positive and Gram-Negative stain by standard method and synthesized compounds showed moderate to good antibacterial and antifungal activity with respect to standard drugs Ciprofloxacin and Flucanazole.
Keywords: 3-amino-1H-pyrazolo[3,4-b]quinoline, phenyl pyrazolones, antibacterial activity, antifungal activity.

Introduction

The synthesis of quinoline and their derivatives have got considerable attention because of a large number of natural products and drugs comprises of this heterocyclic moiety.[1-3] A variety of biological properties including enzyme inhibition,[4] antibacterial,[4] antifungal,[5] and anticancer,[6] activities have been reported for quinoline and quinoline derivatives. Among quinolines, 2-choloroquinolin-3-carbaldehyde absorb an important position.[6-7]The chemistry and biological activity of quinolines and pyrazolones have been very broadly studied.[8] Quinoline ring combined with five or six member ring in linear approach is found in natural products as well as in synthetic compounds of biological interest. Biological importance of azo compounds is recognized for their use as antineoplastics,[9] antidiabetics,[10] antiseptics,[11] and other useful chemo-therapeutic agents. Pyrazole derivatives are also considered as effective biologically active compounds.[12-13] In the present endeavor, therefore it has been thought of interest to synthesize fused 3-amino-1H-pyrazolo[3,4-b]quinoline through coupling reaction between quinoline and pyrazolones and demonstrate their utility as possible antimicrobial agents. These compounds are gaining increasing attention for developing novel structures.



R= various coupling components

Scheme 1. Reagents: a) DMF, $POCl_3$ (3: 12), reflux, 6 h, 80-90°C. b) NH_2OH, CH_3COONa, stirring RT, 1 h. c) $SOCl_2$, benzene, reflux, 2 h. d) $NH_2NH_2H_2O$, ethanol. F $NaNO_2$, HCl, 0- 5°C, diazotization & coupling

The minimum inhibitory concentration (MIC) was determined using disk diffusion method according to the standard Kirby baur disk diffusion method at concentrations 100 µg/ml, 200 µg/ml. Synthesized compounds were tested activity against Gram-Positive bacteria like Staphylococcus Aureus, Bacillus Subtilis and Gram-Negative bacteria like Escherichia Coli and Pseudomonas Aeruginosa. Antifungal activity has been also assayed in vitro at concentrations 100 µg/ml and 200 µg/ml against Candida Albicans. The increasing clinical importance of the drug resistance and the bacterial pathogen has lent additional urgency to microbial or microbiological research and the development of novel biologically active compounds.[15]

Therefore the present study plans to synthesize some new quinoline derivative which has given a good activity and less effect. A general structure of synthesized compound is given in figure 1.

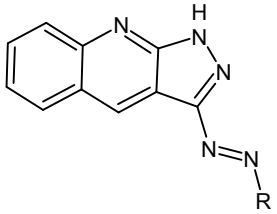
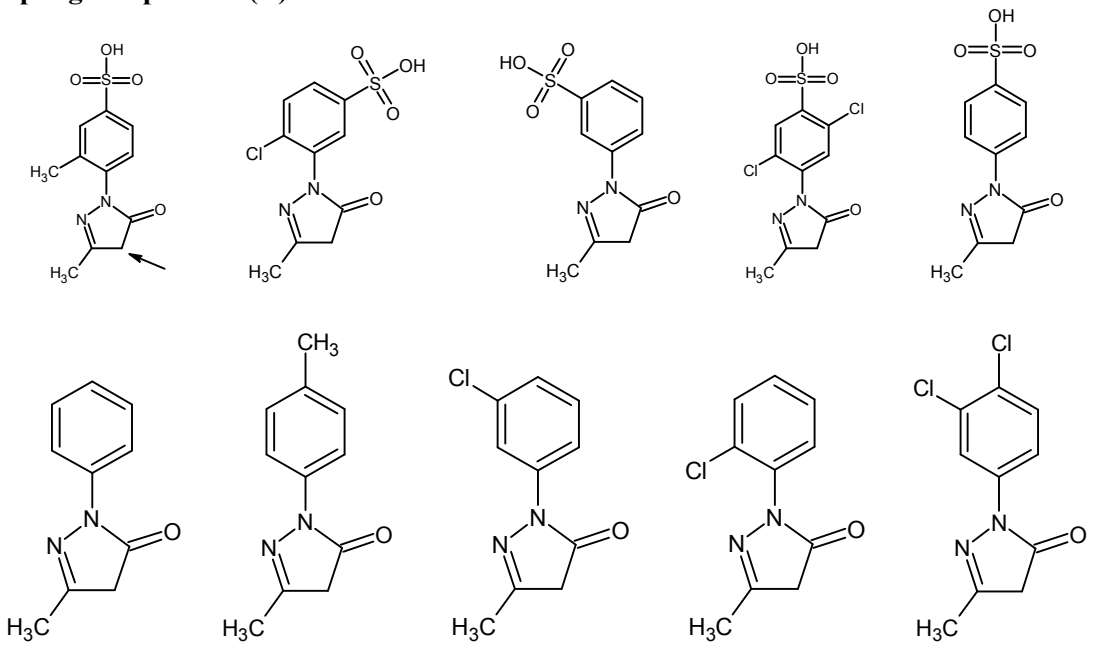


Figure 1:General structure of synthesized compounds

Various coupling components (R)



Experimental Section

Material and methods:

All chemicals were of analytical grade and used directly. Melting points of all compounds were determined using PMP-DM scientific melting point apparatus and are uncorrected. The completion of reaction was monitored by thin-layer chromatography (TLC) using silica gel-G coated Al-plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation. Infrared spectra were recorded on a Shimadzu FT-IR 8400s model using KBr pellets. ^{1}H NMR spectra were recorded on a Varian 400 MHz model spectrophotometer using DMSO as a solvent and TMS as internal reference (chemical shifts in δ, ppm).

2-chloroquinoline-3-carbaldehyde (1)

The title compound was synthesized following a reaction according to a procedure described in the literature. Yield 70%, m.p.146-152°C.[16]

2-chloro-3-quinoline-car-boxaldehyde oxime (2)

The title compound was synthesized following a reaction according to a procedure described in the literature. Yield 78%, m.p.238-242°C.[17]

2-chloro-3-quinoline carbonitrile (3)

The title compound was synthesized following a reaction according to a procedure described in the literature. Yield 84%, m.p.190-205°C.[18]

1H-pyrazolo[3,4-b]quinolin-3-amine (4)

The title compound was synthesized following a reaction according to a procedure described in the literature. Yield 79%, m.p.290-300°C.[18]

Diazotization and Coupling (5)

Diazotization and coupling was carried out by usual procedure.[19]

Result and Discussion

Chemistry

The derivatives of 3-methyl-1-phenyl-4-[(E)-2-{1H-pyrazolo [3,4-b] quinolin-3-yl} diazen-1-yl]-4,5-dihydro-1H-pyrazol-5-one were synthesized using following stage. 2-choloroquinolin-3-carbaldehyde (1) has been synthesized using the Vilsmeier-Haack reagent as per the literature [14]. This on further reaction with hydroxyl amine hydrochloride by using sodium acetate as catalyst gave oxime (2) in high yield and purity. Compound (3) was obtained in high yield and purity by condensation of compound (2) and thionyl chloride in benzene. The product (3) was then treated with hydrazine hydrate in ethanol to produce compound (4). Compound (4) on diazotization and coupling with phenyl pyrazolones derivative gave scheme 1. All the compounds were fully characterized by IR, ^{1}H-NMR spectroscopy and elemental analysis.

Table-1: Molecular Formula of Synthesized Compound with elemental analysis.

MOL ID	Formula	% C (Found)	% H (Found)	% N (Found)	% Yield
R_1	$C_{21}H_{17}N_7O_4S$	54.48	3.80	20.95	85
R_2	$C_{20}H_{14}ClN_7O_4S$	49.70	2.96	20.26	79
R_3	$C_{20}H_{15}N_7O_4S$	53.41	3.32	21.72	88
R_4	$C_{20}H_{13}Cl_2N_7O_4S$	46.30	2.64	18.79	90
R_5	$C_{14}H_{11}N_7O$	53.52	3.39	21.88	82
R_6	$C_{20}H_{15}N_7O_4S$	65.40	4.90	25.40	77
R_7	$C_{20}H_{14}ClN_7O$	65.38	4.95	25.37	81
R_8	$C_{20}H_{13}Cl_2N_7O$	65.32	4.85	25.39	89
R_9	$C_{21}H_{17}N_7O$	54.79	3.00	22.30	79
R_{10}	$C_{20}H_{14}ClN_7O$	57.31	3.72	33.40	87

Compound (R_1): Yield 85%, brown; mp >300°C; Anal. Calcd for $C_{21}H_{17}N_7O_4S$: C, 54.42%; H, 3.70%; N, 21.15%. found: C, 54.48%; H,3.80%; N,20.95%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.69 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.91 (m, 8H, Ar-H), 13.51(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3198 cm^{-1} (-NH-), 1491-1579 cm^{-1} (-N=N-), 3032-3059 cm^{-1} (-C-H) stretching of aromatic rings, 2821-2997 cm^{-1} (-C-H) stretching of methyl group.

Compound (R_2): Yield 79%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{14}ClN_7O_4S$: C, 49.64%; H, 2.92%; N, 20.26%. found: C, 49.70%; H,2.96%; N, 20.30%; **NMR DMSO**: 2.42 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.90 (m, 8H, Ar-H), 13.70(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3178 cm^{-1} (-NH-), 1595 cm^{-1} (-N=N-), 3032-3057 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group, 723 cm^{-1} (-C-Cl-).

Compound (R_3): Yield 88%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{15}N_7O_4S$: C, 53.45%; H, 3.36%; N, 21.82%. found: C, 53.41%; H, 3.32%; N, 21.72%; **NMR DMSO**: 2.35 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.91 (m, 9H, Ar-H), 13.65 (s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120 cm^{-1} (-NH-), 1475-1618 cm^{-1} (-N=N-), 3032-3057 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group.

Compound (R_4): Yield 90%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{13}Cl_2N_7O_4S$: C, 46.34%; H, 2.53%; N, 18.92%. found: C, 46.30%; H, 2.64%; N, 18.79%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.91 (m, 7H, Ar-H), 13.51(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1489-1585 cm^{-1} (-N=N-), 3055-3084 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group, 742 cm^{-1} (-C-Cl-).

Compound (R_5): Yield 82%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{15}N_7O_4S$: C, 53.45%; H, 3.36%; N, 21.82%. found: C, 53.52%; H, 3.39%; N, 21.88%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.91 (m, 9H, Ar-H), 13.70 (s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1494 cm^{-1} (-N=N-), 3032-3055 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group.

Compound (R_6): Yield 77%, brown; mp >300°C; Anal. Calcd for $C_{21}H_{17}N_7O$: C, 65.44%; H, 4.97%; N, 25.44%, found: C, 65.40%; H, 4.90%; N, 25.40% **NMR DMSO**: 2.44 (s, 3H, Me), 2.69 (s, 3H, Me), 2.85 (s, 1H, -CH), 7.43-8.91 (m, 9H, Ar-H), 13.51(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 31230-3188 cm^{-1} (-NH-), 1510-1572 cm^{-1} (-N=N-), 3032-3043 cm^{-1} (-C-H) stretching of aromatic rings, 2810-2920 cm^{-1} C-H stretching of methyl group.

Compound (R_7): Yield 81%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{14}ClN_7O$: C, 65.44%; H, 4.97%; N, 25.44%. found: C, 65.38%; H, 4.95%; N, 25.37%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.85(s, 1H, -CH), 7.43-8.91 (m, 9H, Ar-H), 13.65 (s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1541-1589 cm^{-1} (-N=N-), 3032-3053 cm^{-1} (-C-H) stretching of aromatic rings, 2818-2920 cm^{-1} C-H stretching of methyl group, 781 cm^{-1} (-C-Cl-).

Compound (R_8): Yield 89%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{14}ClN_7O_4S$: C, 65.44%; H, 4.97%; N, 25.44%. found: C, 65.32%; H, 4.85%; N, 25.39%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.85 (s, 1H, -CH), 7.43-8.91 (m, 9H, Ar-H), 13.70 (s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1489-1572 cm^{-1} (-N=N-), 3032-3057 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group, 748 cm^{-1} (-C-Cl-).

Compound (R_9): Yield 79%, brown; mp >300°C; Anal. Calcd for $C_{20}H_{13}Cl_2N_7O$: C, 54.81%; H, 2.99%; N, 22.37%. found: C, 54.79%; H, 3.00%; N, 22.30%; **NMR DMSO**: 2.46 (s, 3H, Me), 2.85 (s, 1H, -CH), 7.43-8.32 (m, 4H, Ar-H), 13.51(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1479-1570 cm^{-1} (-N=N-), 3032-3057 cm^{-1} (-C-H) stretching of aromatic rings, 2873-2920 cm^{-1} C-H stretching of methyl group, 750 cm^{-1} (-C-Cl-).

Compound (R_{10}): Yield 87%, brown; mp >300°C; Anal. Calcd for $C_{14}H_{11}N_7O$: C, 57.33%; H, 3.78%; N, 33.43%. found: C, 57.31%; H, 3.72%; N, 33.40% **NMR DMSO**: 2.46 (s, 3H, Me), 2.85 (s, 1H, -CH), 7.43-8.91 (m, 8H, Ar-H), 11.28 (s, 1H, -NH), 13.51(s, 1H, -NH) **IR (KBr)/cm^{-1}** : 3120-3178 cm^{-1} (-NH-), 1543-1572 cm^{-1} (-N=N-), 3032-3057 cm^{-1} (-C-H) stretching of aromatic rings, 2810-2955 cm^{-1} C-H stretching of methyl group.

Spectral properties

All the compounds showed a characteristic band at 1450-1590 cm^{-1} for the (-N=N-) group. The band at 2810-2995 cm^{-1} is due to the C-H stretching of methyl groups. The characteristic band 3120-3178 cm^{-1} is attributed to N-H stretching of secondary amine group. The band appears at 3032-3057 cm^{-1} corres. ponding to C-H stretching of aromatic rings. The band at 740-785 cm^{-1} is due to a C-Cl stretching. The ^{1}H-NMR spectra of all the synthesized compounds based on 1H-pyrazolo[3,4-b]quinolin-3-amine shows important signals at their respective positions, confirming the structures.

Biological Evaluation

All newly synthesized 3-methyl-1-phenyl-4-[(E)-2-{1H-pyrazolo [3,4-b] quinolin-3-yl}diazen-1-yl]-4,5-dihydro-1H-pyrazol-5-one derivative were assayed in vitro for antibacterial activity against Gram Positive bacteria, Gram Negative bacteria and antifungal stain. The obtained result of all compounds of related activity to Gram Negative bacteria which show almost approximately average value for the Pseudomonas Aeruginosa, Escherichia Coli (100 µg/ml) and (200 µg/ml) [Figure-2 and 3]. All compounds related to Gram Positive bacteria Staphylococcus Aureus, Bacillus Subtilis for (100 µg/ml) and (200 µg/ml) which show approximately moderate to good activity [Figure-4 and 5].

Antibacterial and Antifungal activity

Antibacterial activity of synthesized compounds was examined in vitro by known Kirby-Bauer disk diffusion method. All the compounds were tested for activity against Gram-Positive bacteria like Staphylococcus Aureus, Bacillus Subtilis and Gram-Negative bacteria like Escherichia coli and Pseudomonas Aeruginosa (Table 2 and Fig. 2 to 7). All the compounds were dissolved in DMF (< 500ppm concentration). Ciprofloxacin was employed as the standard drug. The minimum inhibitory concentration (MIC) was determine for the synthesized compounds $R_1,R_4,R_6,R_8,R_9,R_{10}$ against Gram Negative Bacteria with respect to standard drug Ciprofloxacin and also the antifungal agent Flucanazole for Candida Albicans which show approximately average activity [Figure 6]. Whereas the MIC of synthesized compounds $R_1,R_2,R_4,R_7,R_8,$ R_9,R_{10} show approximately moderate activity against Gram Positive bacteria and show moderate to good antifungal activity against Candida Albicans [Figure 7].

Table-2: MIC Determination of Antibacterial agents and Antifungal agent

MIC Determination of Antibacterial agents and Antifungal agent					
	Gram Negative Bacteria	Gram Negative Bacteria	Gram Positive Bacteria	Gram Positive Bacteria	Antifungal Agent
	PSEUDOMONAS AERUGINOSA	**ESCHERICHIA COLI**	**STAPHYLOCOCCUS AUREUS**	**BACILLUS SUBTILIS**	**CANDIDA ALBICANS**
Compd.	**MIC µg**	**MIC µg**	**MIC µg**	**MIC µg**	**MIC µg**
R_1	>500	500	400	100	200
R_2	>500	200	100	50	100
R_3	50	50	50	50	50
R_4	400	150	150	100	100
R_5	>500	100	50	50	50
R_6	400	300	50	100	50
R_7	>500	>500	50	50	150
R_8	300	200	150	50	150
R_9	>500	500	50	50	100
R_{10}	>500	400	100	100	100
Ciprofloxacin	10	20	10	10	
Flucanazole					10

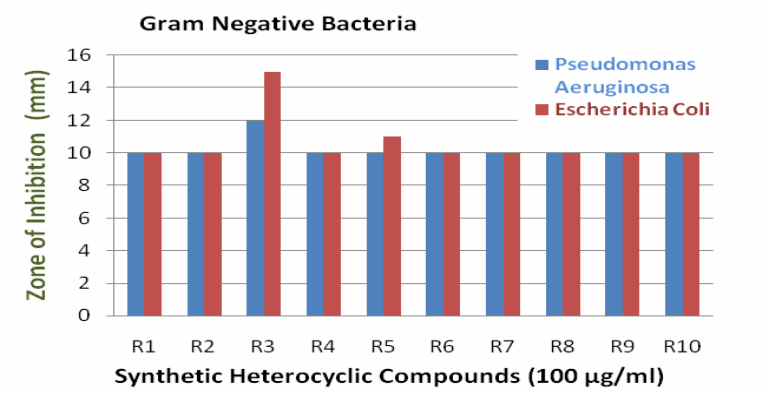


Figure 2: Antibacterial Activity against Gram Negative Bacteria [Pseudomonas Aeruginosa, Escherichia Coli (100 µg/ml)]

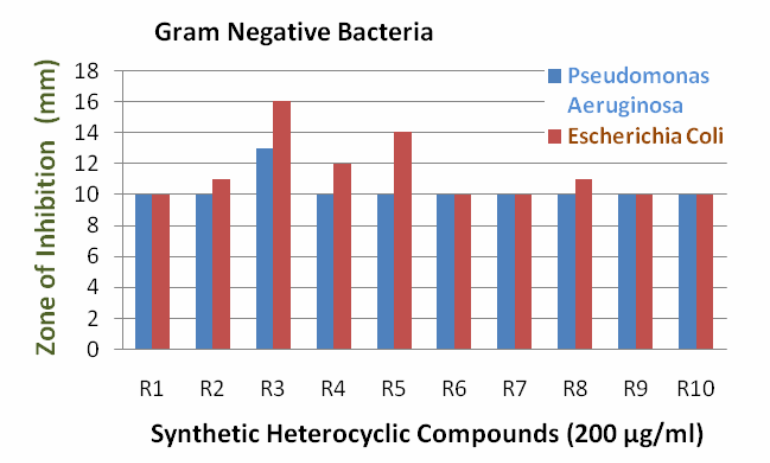


Figure 3: Antibacterial Activity against Gram Negative Bacteria [Pseudomonas Aeruginosa, Escherichia Coli (200 µg/ml)]

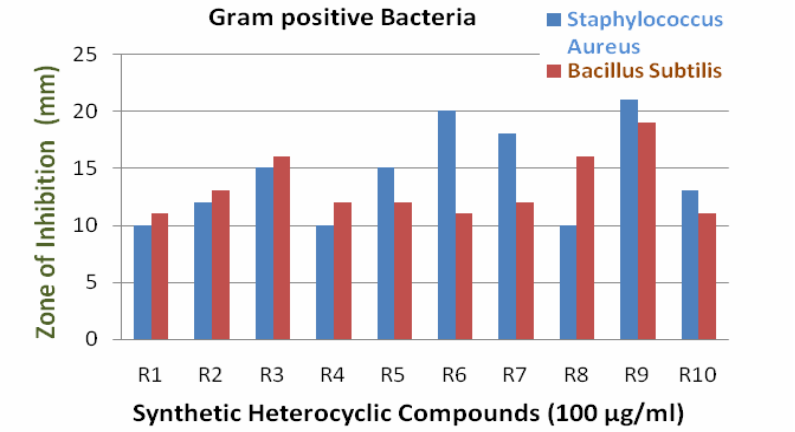


Figure 4: Antibacterial Activity against Gram Positive Bacteria [Staphylococcus Aureus, Bacillus Subtilis (100 µg/ml)]

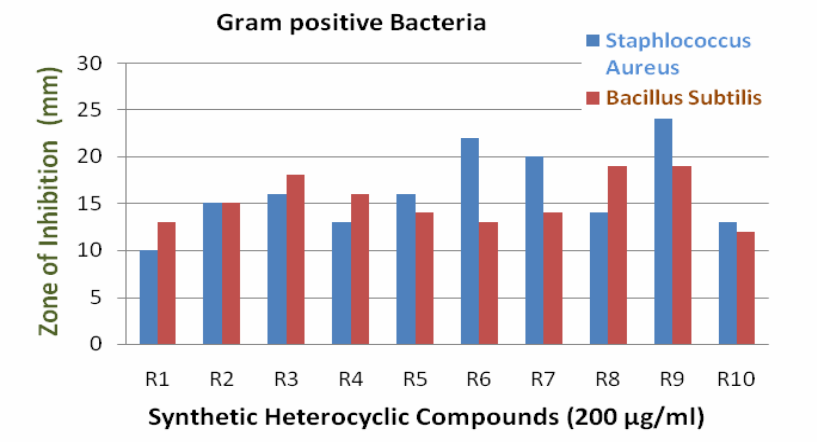


Figure 5: Antibacterial Activity against Gram Positive Bacteria [Staphylococcus Aureus, Bacillus Subtilis (200 µg/ml)]

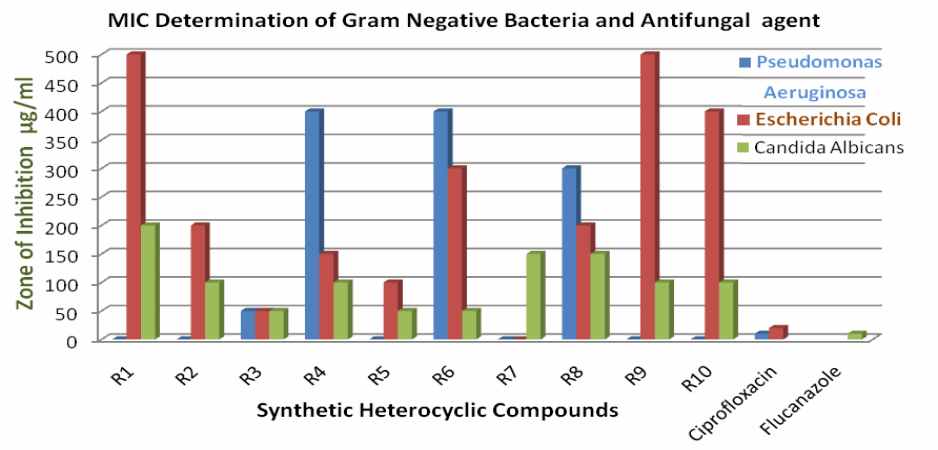


Figure 6: MIC Determination against Gram Negative Bacteria and Antifungal with respect to standard Drug

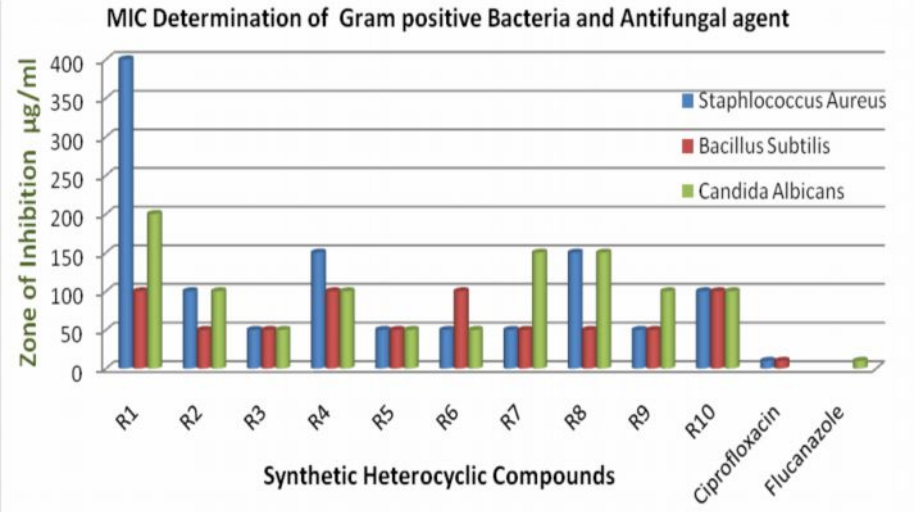


Figure 7: MIC Determination against Gram Positive Bacteria and Antifungal with respect to standard Drug

Conclusion

The results of study of microbial analysis (Table 2 and figures [2-7]) revealed that the synthesized compounds are promisingly significant, good antimicrobial and anti-fungal agents. Here we have synthesized some novel quinoline analogues combining with different substituted phenyl pyrazolones derivative ring system with a view to get a good antimicrobial and antifungal agent with less toxic effects.

As per the results of screening it is clearly indicated that the compounds of the scheme have shown good antibacterial and antifungal equipotent with the standard drugs. This is because of the presence of phenyl pyrazolones derivatives having electron donating and withdrawing groups at the different positions of phenyl nucleus and heterocyclic system attached to quinazoline nucleus. Moreover, phenyl pyrazolones as coupling component in all compounds increased antimicrobial activity. In conclusion, the compounds having pyrazolones as coupling components could be useful for derivatization to develop more effective microbial agents.

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