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***RESEARCH ARTICLE***

# Synthesis, Characterisation and Antimicrobial Activity of Novel 2-Methyl-3-(2-(substituted ylidene)hydrazinyl)quinazolin-4(3H)-ones

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## ABSTRACT:
Several novel quinazolinones were designed and synthesized from anthranilic acid by a multistep synthesis. Structures of synthesized compounds were well characterized using FT-IR, $^{1}$H-NMR, Mass spectroscopy and bases of elemental analysis. Entire test compounds were screened for their antibacterial and antifungal activities by agar streak dilution test against various pathogenic strains of bacteria and fungi. Antimicrobial studies revealed that all title compounds exhibited mild to good antibacterial activity and mild to moderate antifungal activity. The relationship between the functional group variation and the biological activity of the screened compounds were discussed. Out of thirteen tested analogs, the most active compound was found to be 3-(2-(1-(4-chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene) hydrazinyl)-2-methylquinazolin-4(3H)-one **VIIg**.

**KEYWORDS:** Antimicrobial, Isoxazole, Pyrazole, Pyrimidine, Quinazolinone.

## INTRODUCTION:
Emergence of drug resistance has created a critical and unmet medical requirement for the innovation and development of novel classes of antibacterial agents. The synthesis of a newer class of anti-bacterial and anti-fungal agents is in need of time, especially against drug-resistant bacteria and fungi, such as gram-positive and gram-negative strains, which are responsible for a number of serious infections in the acute and chronic care units in hospitals. Developing novel antimicrobial agents is a top priority in fighting against bacterial resistance.

Heterocyclic species have received much interest in the field of medicinal chemistry because of its synthetic utility and broad spectrum of biological activity [1-10]. The chemical versatility of quinazolinone derivatives has led to their extensive use as synthons for the preparation of many biologically active compounds.

In the field of pharmaceutical and medicinal chemistry, quinazolinone and its analogs are found to be trendy structures employed for discovery of drugs within the vast range of heterocycles. Literature survey indicates that the quinazolinones showed significant antimicrobial activities.

In addition, the biological activities exhibited by compounds containing isoxazole [11-15] / pyrimidine [16-20] / pyrazole [21-25] moiety has prompted chemists to synthesis more and more libraries of these moieties and screen them for potential activities. Particularly isoxazoles, pyrimidines, and pyrazoles[27, 30, 34] are reported to possess diverse antimicrobial activities. In view of biological importance of these heterocyclic moieties, it was planned to synthesize a new series isoxazole/pyrimidine/pyrazole substituted quinazolinones and to evaluate the new compounds for their anti-microbial activities with the hope to obtain more active and less toxic antimicrobial agents.

## MATERIALS AND METHODS:
***General:***
The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. These solvents used were of LR grade and purified before their use. The silica gel G

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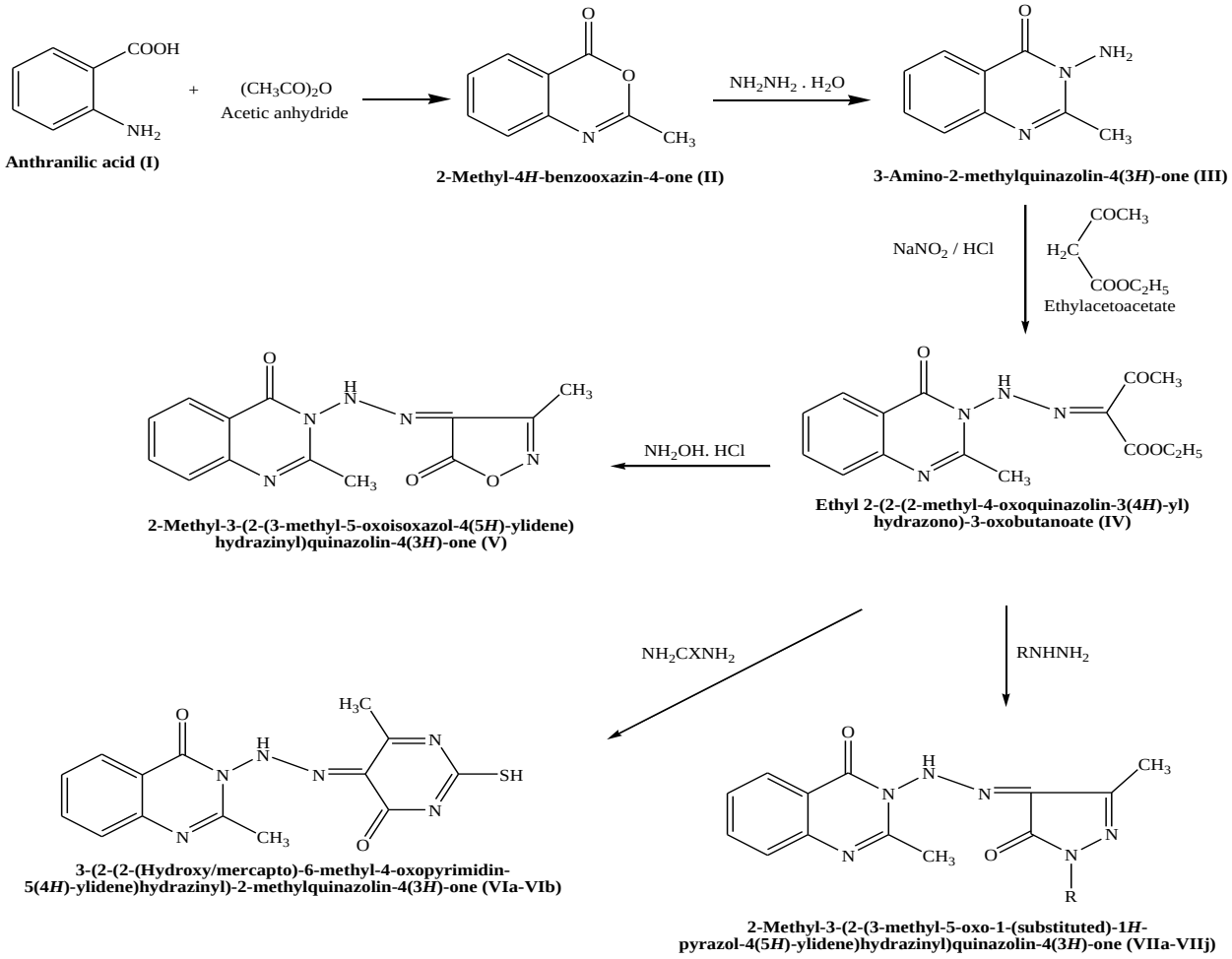
used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. All the melting points were taken in open glass capillary and are uncorrected. $^1$H-NMR spectra were recorded at 300 MHz on Bruker Avance-300 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 2400C analyzer and were within ± 0.4 % of the theoretical values.




**VIa) X = S; VIb) X = O; VIIa) R = $C_5H_4NCO$; VIIb) R = $CSNH_2$; VIIc) R = H;VIId) R = *m*-$FC_6H_4$; VIIe) R = *p*-$FC_6H_4$; VIIf) R = *m*-$ClC_6H_4$; VIIg) R = *p*-$ClC_6H_4$; VIIh) R = *m*-$OCH_3C_6H_4$;VIIi) R = *p*-$OCH_3C_6H_4$; VIIj) R = $C_6H_5$;**

**Scheme 1: Synthesis of novel isoxazole / pyrimidine / pyrazole substituted quinazolinones (V, VIa-VIb & VIIa-VIIj)**

*2.2. Synthesis of 2-methylbenzo-(1,3)-oxazin-4-one (II)*

A mixture of anthranilic acid **I** (1.37 g; 0.01 mol) and acetic anhydride (10.2 ml; 0.1 mol) was refluxed on gentle flame for 1 h. The excess of acetic anhydride was distilled off under reduced pressure and the residue was dissolved in petroleum ether and kept aside for 1 h. The light brown solid **II** which obtained was filtered and dried. Yield = 73 %, m.p. 181-183 °C. IR (KBr) $cm^{-1}$: 3071 (Ar-CH), 2916 ($CH_3$-CH), 1730 (C=O), 1644 (C=N), 1619 (C=C), 1032 (Cyclic C-O-C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.10-7.63 (m, 4H, Ar-H), 2.09 (s, 3H, $CH_3$). EI-MS *m/z*: 161 ($M^+$). *Anal*. Calcd for $C_9H_7NO_2$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.29; H, 4.40; N, 8.66.

*2.3. Synthesis of 3-amino-2-methylquinazolin-4(3H)-one (III)*

2-Methylbenzo-(1,3)-oxazin-4-one**II** (1.61 g; 0.01 mol) and hydrazine hydrate (0.50 g; 0.01 mol) was dissolved in anhydrous pyridine (30 ml) and heated on oil bath for 5 h. The resulting solution was cooled in ice bath, and treated with dilute hydrochloric acid (60 ml). The product separated **III** was filtered, washed with water, and crystallized from ethanol. Yield = 77 %, m.p. 150-

152 °C. IR (KBr) $cm^{-1}$: 3345 ($NH_2$), 3039 (Ar-CH), 2921 ($CH_3$-CH), 1716 (C=O), 1634 (C=N), 1608 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.03-7.68 (m, 4H, Ar-H), 5.30 (s, 2H, $NH_2$), 1.89 (s, 3H, $CH_3$). EI-MS *m/z*: 175 ($M^+$). *Anal*. Calcd for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.86; H, 5.16; N, 23.92.

*2.4. Synthesis of ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-3-oxobutanoate (IV)*

3-Amino-2-methylquinazolin-4(3*H*)-one **(III)** (1.75 g; 0.01 mol) was dissolved in a mixture of concentrated hydrochloric acid (10 ml) and water (10 ml). The solution is cooled to 5° C by immersing the flask in a mixture of ice and water. The solution of powdered sodium nitrite (1.38 g; 0.02 mol) dissolved in water (10 ml) was drop wise added to the solution of 3-amino-2-methylquinazolin-4(3*H*)-one hydrochloride with stirring. The stirring was continued for 30 min after complete addition of sodium nitrite. The obtained diazonium salt was added to a solution of ethylacetoacetate (1.30 g; 0.01 mol) in ethanol (30 ml) with stirring. Then the reaction mixture was stirred for 4 h magnetically at room temperature and kept aside at room temperature for further 2 h. The solid product **IV**, so formed, was collected by filtration and recrystallised from ethanol. Yield = 74 %, m.p. 196-198 °C. IR (KBr) $cm^{-1}$: 3360 (NH), 3031 (Ar-CH), 2958 ($CH_3$-CH), 1703 (C=O), 1647 (C=N), 1609 (C=C); 1046 (C-O-C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.09-7.48 (m, 4H, Ar-CH), 6.39 (s, 1H, NHof hydrazone), 4.36-4.64 (t, 2H, $CH_2$), 2.31 (s, 3H, $CH_3$), 2.06 (s, 3H, $CH_3$), 1.52-1.79 (t, 3H, $CH_3$). EI-MS *m/z*: 316 ($M^+$). *Anal*. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.79; H, 5.12; N, 17.75.

*2.5. Synthesis of 2-methyl-3-(2-(3-methyl-5-oxoisoxazol-4(5H)-ylidene)hydrazinyl) quinazolin-4(3H)-one (V)*

A mixture of ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-3-oxobutanoate **IV** (3.16 g; 0.01 mol) and hydroxylamine hydrochloride (1.04 g; 0.015 mol) in ethanol (25 ml) was refluxed for 12 h in water bath. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water and stirred well. The product obtained **V** was filtered, washed with water twice, dried and recrystallised using ethanol. Yield = 79 %, m.p. 139-141 °C. IR (KBr) $cm^{-1}$: 3346 (NH), 3075 (Ar-CH), 2928 ($CH_3$-CH), 1702 (C=O), 1640 (C=N), 1619 (C=C). $^1$H-NMR ($CDCl_3$,500 MHz) δ ppm: 6.68-7.39 (m, 4H, Ar-CH), 6.38 (s, 1H, NH of hydrazone), 2.54 (s, 3H, $CH_3$), 2.20 (s, 3H, $CH_3$). EI-MS *m/z*: 285 ($M^+$). *Anal*. Calcd for $C_{13}H_{11}N_5O_3$: C, 54.74; H, 3.89; N, 24.55. Found: C, 54.91; H, 3.90; N, 24.47.

*2.6. Synthesis of 3-(2-(2-(hydroxy/mercapto)-6-methyl-4-oxopyrimidin-5(4H)-ylidene) hydrazinyl)-2-methylquinazolin-4(3H)-one (VIa-VIb)*

A mixture of ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-3-oxobutanoate **IV** (3.16 g; 0.01 mol), urea/thiourea (0.015 mol) and potassium carbonate (0.2 g) in ethanol (30 ml) was refluxed for 18 h on a water bath. On cooling the solid separated out was filtered. The residue was dissolved in hot water and filtered when hot. The filtrate was neutralized with acetic acid and the solid precipitated out **VIa-VIb** was filtered and recrystallised from ethanol.

*2.6.1. 3-(2-(2-Mercapto-6-methyl-4-oxopyrimidin-5(4H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIa)*

Yield = 72 %, m.p. 174-177 °C. IR (KBr) $cm^{-1}$: 3370 (NH), 3028 (Ar-CH), 2915 ($CH_3$-CH), 2526 (SH), 1725 (C=O), 1639 (C=N), 1612 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.23-7.95 (m, 4H, Ar-CH), 6.58 (s, 1H, NH of hydrazone), 2.40 (s, 1H, SH), 1.93 (s, 3H, $CH_3$), 1.77 (s, 3H, $CH_3$). EI-MS *m/z*: 328 ($M^+$). *Anal*. Calcd for $C_{14}H_{12}N_6O_2S$: C, 51.21; H, 3.68; N, 25.59. Found: C, 51.05; H, 3.69; N, 25.68.

*2.6.2. 3-(2-(2-Hydroxy-6-methyl-4-oxopyrimidin-5(4H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIb)*

Yield = 77 %, m.p. 130-132 °C. IR (KBr) $cm^{-1}$: 3542 (OH), 3326 (NH), 3094 (Ar-CH), 2937 ($CH_3$-CH), 1725 (C=O), 1652 (C=N), 1618 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.09-7.81 (m, 4H, Ar-CH), 6.60 (s, 1H, NH of hydrazone), 2.87 (s, 1H, OH), 1.82 (s, 3H, $CH_3$), 1.45 (s, 3H, $CH_3$). EI-MS *m/z*: 312 ($M^+$). *Anal*. Calcd for $C_{14}H_{12}N_6O_3$: C, 53.85; H, 3.87; N, 26.91. Found: C, 53.68; H, 3.88; N, 27.00.

*2.7. Synthesis of 2-methyl-3-(2-(3-methyl-5-oxo-1-(substituted)-1H-pyrazol-4(5H)-ylidene)hydrazinyl) quinazolin-4(3H)-one (VIIa-VIIj)*

A mixture of ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-3-oxobutanoate **IV** (3.16 g; 0.01 mol) and various hydrazine hydrochloride (0.015 mol) in ethanol (30 ml) was refluxed for 24 h in water bath. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water and stirred well. The product separated **VIIa-VIIj** was filtered, dried and recrystallised.

*2.7.1. 3-(2-(1-Isonicotinoyl-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIa)*

Yield = 76 %, m.p. 237-238 °C. IR (KBr) $cm^{-1}$: 3362 (NH), 3057 (Ar-CH), 2924 ($CH_3$-CH), 1729 (C=O), 1646 (C=N), 1609 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.16-8.29 (m, 8H, Ar-CH), 6.73 (s, 1H, NH of hydrazone), 2.05 (s, 3H, $CH_3$), 1.71 (s, 3H, $CH_3$). EI-MS *m/z*: 389 ($M^+$). *Anal*. Calcd for $C_{19}H_{15}N_7O_3$: C, 58.61; H, 3.88; N, 25.18. Found: C, 58.79; H, 3.86; N, 25.11.

*2.7.2. 3-Methyl-4-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-5-oxo-4,5-dihydro pyrazole-1-carbothioamide (VIIb)*
Yield = 80 %, m.p. 259-261 °C. IR (KBr) $cm^{-1}$: 3353 (NH), 3018 (Ar-CH), 2972 ($CH_3$-CH), 2547 (SH), 1718 (C=O), 1654 (C=N), 1621 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.09-7.82 (m, 4H, Ar-CH), 6.43 (s, 1H, NH of hydrazone), 8.36 (s, 2H, $CSNH_2$), 2.10 (s, 3H, $CH_3$), 1.65 (s, 3H, $CH_3$). EI-MS *m/z*: 343 ($M^+$). *Anal*. Calcd for $C_{14}H_{13}N_7O_2S$: C, 48.97; H, 3.82; N, 28.55. Found: C, 49.15; H, 3.81; N, 28.46.

*2.7.3. 2-Methyl-3-(2-(3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)quinazolin-4(3H)-one (VIIc)*
Yield = 75 %, m.p. 213-215 °C. IR (KBr) $cm^{-1}$: 3350 and 3308 (NH), 3046 (Ar-CH), 2921 ($CH_3$-CH), 1719 (C=O), 1635 (C=N), 1602 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.03-7.68 (m, 4H, Ar-CH), 6.56 (s, 1H, NH of hydrazone), 6.18 (s, 1H, NH of pyrazole), 1.74 (s, 3H, $CH_3$), 1.32 (s, 3H, $CH_3$). EI-MS *m/z*: 284 ($M^+$). *Anal*. Calcd for $C_{13}H_{12}N_6O_2$: C, 54.93; H, 4.25; N, 29.56. Found: C, 54.76; H, 4.27; N, 29.64.

*2.7.4. 3-(2-(1-(3-Fluorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIId)*
Yield = 78 %, m.p. 264-266 °C. IR (KBr) $cm^{-1}$: 3316 (NH), 3043 (Ar-CH), 2937 ($CH_3$-CH), 1719 (C=O), 1640 (C=N), 1615 (C=C), 1062 (C-F). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.17-8.05 (m, 8H, Ar-CH), 6.72 (s, 1H, NH of hydrazone), 2.08 (s, 3H, $CH_3$), 1.86 (s, 3H, $CH_3$). EI-MS *m/z*: 378 ($M^+$). *Anal*. Calcd for $C_{19}H_{15}FN_6O_2$: C, 60.31; H, 4.00; N, 22.21. Found: C, 60.51; H, 3.99; N, 22.13.

*2.7.5. 3-(2-(1-(4-Fluorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIe)*
Yield = 81 %, m.p. 227-229 °C. IR (KBr) $cm^{-1}$: 3335 (NH), 3062 (Ar-CH), 2914 ($CH_3$-CH), 1729 (C=O), 1635 (C=N), 1601 (C=C), 1056 (C-F). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 6.93 -7.86 (m, 8H, Ar-CH), 6.49 (s, 1H, NH of hydrazone), 2.20 (s, 3H, $CH_3$), 1.74 (s, 3H, $CH_3$). EI-MS *m/z*: 378 ($M^+$). *Anal*. Calcd for $C_{19}H_{15}FN_6O_2$: C, 60.31; H, 4.00; N, 22.21. Found: C, 60.14; H, 4.02; N, 22.30.

*2.7.6. 3-(2-(1-(3-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIf)*
Yield = 74 %, m.p. 243-245 °C. IR (KBr) $cm^{-1}$: 3325 (NH), 3031 (Ar-CH), 2979 ($CH_3$-CH), 1747 (C=O), 1642 (C=N), 1618 (C=C), 860 (C-Cl). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.16-8.09 (m, 8H, Ar-CH), 6.55 (s, 1H, NH of hydrazone), 2.37 (s, 3H, $CH_3$), 2.02 (s, 3H, $CH_3$). EI-MS *m/z*: 396 ($M^{+2}$), 394 ($M^+$). *Anal*. Calcd for $C_{19}H_{15}ClN_6O_2$: C, 57.80; H, 3.83; N, 21.29. Found: C, 58.01; H, 3.82; N, 21.25.

*2.7.7. 3-(2-(1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIg)*
Yield = 79 %, m.p. 209-211 °C. IR (KBr) $cm^{-1}$: 3353 (NH), 3049 (Ar-CH), 2945 ($CH_3$-CH), 1732 (C=O), 1640 (C=N), 1605 (C=C), 871 (C-Cl). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.12-7.78 (m, 8H, Ar-CH), 6.62 (s, 1H, NH of hydrazone), 2.19 (s, 3H, $CH_3$), 1.86 (s, 3H, $CH_3$). EI-MS *m/z*: 396 ($M^{+2}$), 394 ($M^+$). *Anal*. Calcd for $C_{19}H_{15}ClN_6O_2$: C, 57.80; H, 3.83; N, 21.29. Found: C, 57.96; H, 3.84; N, 21.22.

*2.7.8. 3-(2-(1-(3-Methoxyphenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIh)*
Yield = 72 %, m.p. 220-222 °C. IR (KBr) $cm^{-1}$: 3326 (NH), 3042 (Ar-CH), 2975 ($CH_3$-CH), 1724 (C=O), 1638 (C=N), 1601 (C=C), 1050 (C-O-C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.15-8.33 (m, 8H, Ar-CH), 6.79 (s, 1H, NH of hydrazone), 3.34 (s, 3H, $OCH_3$), 1.96 (s, 3H, $CH_3$), 1.61 (s, 3H, $CH_3$). EI-MS *m/z*: 390 ($M^+$). *Anal*. Calcd for $C_{20}H_{18}N_6O_3$: C, 61.53; H, 4.65; N, 21.53. Found: C, 61.36; H, 4.67; N, 21.60.

*2.7.9. 3-(2-(1-(4-Methoxyphenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methyl quinazolin-4(3H)-one (VIIi)*
Yield = 76 %, m.p. 275-277 °C. IR (KBr) $cm^{-1}$: 3358 (NH), 3083 (Ar-CH), 2967 ($CH_3$-CH), 1730 (C=O), 1649 (C=N), 1614 (C=C), 1062 (C-O-C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 6.86-7.95 (m, 8H, Ar-CH), 6.41 (s, 1H, NH of hydrazone), 3.69 (s, 3H, $OCH_3$), 2.04 (s, 3H, $CH_3$), 1.71 (s, 3H, $CH_3$). EI-MS *m/z*: 390 ($M^+$). *Anal*. Calcd for $C_{20}H_{18}N_6O_3$: C, 61.53; H, 4.65; N, 21.53. Found: C, 61.70; H, 4.63; N, 21.46.

*2.7.10. 2-Methyl-3-(2-(3-methyl-5-oxo-1-phenyl-1H-pyrazol-4(5H)-ylidene)hydrazinyl) quinazolin-4(3H)-one (VIIj)*
Yield = 73 %, m.p. 233-235 °C. IR (KBr) $cm^{-1}$: 3321 (NH), 3087 (Ar-CH), 2943 ($CH_3$-CH), 1729 (C=O), 1645 (C=N), 1616 (C=C). $^1$H-NMR ($CDCl_3$,300 MHz) δ ppm: 7.03-8.25 (m, 9H, Ar-CH), 6.58 (s, 1H, NH of hydrazone), 1.71 (s, 3H, $CH_3$), 1.36 (s, 3H, $CH_3$). EI-MS *m/z*: 360 ($M^+$). *Anal*. Calcd for $C_{19}H_{16}N_6O_2$: C, 63.32; H, 4.48; N, 23.32. Found: C, 63.49; H, 4.46; N, 23.24.

***2.8. Antimicrobial Activity:***
In this study, all the synthesized compounds were screened for antimicrobial activity by agar streak dilution method.[31-33] The antibacterial activity of the compounds was evaluated against four Gram-positive bacteria *Bacillus cereus* ATCC 11778, *Staphylococcus*

*aureus* ATCC 9144, *Micrococcus luteus* ATCC 4698 and *Staphylococcus epidermidis* ATCC 155, and three Gram-negative bacteria *Klebsiella pneumoniae* ATCC 11298, *Pseudomonas aeruginosa* ATCC 2853,and *Escherichia coli* ATCC 25922. The antifungal activities of the synthesized compounds were evaluated against two fungi *Aspergillus fumigatus* ATCC 46645 and *Aspergillus niger* ATCC 9029. [35-36] Bacterial strains were cultured over night at 37°C in Mueller–Hinton broth and the yeast were cultured overnight at 30°C in YEPDE agar for antibacterial and antifungal activity tests. Test strains were suspended in nutrient agar to give a final density of 5 x $10^{-5}$ cfu/ml.

### *2.9. Minimum Inhibitory Concentration (MIC):*

MIC of the compound was determined by agar streak dilution method [26, 28, 29]. A stock solution of the synthesized compound (100 µg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabouraud's dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50ºC) containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately 5 x $10^{-5}$ cfu/ml and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37ºC for 24 h and 48 h for bacteria and fungi respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

## RESULTS AND DISCUSSION:

*Chemistry:*

Various novel isoxazole / pyrimidine / pyrazole substituted quinazolinones (**V, VIa-VIb and VIIa-VIIj**) were synthesized from anthranilic acid and acetic anhydride by a multistep synthesis as per the protocol shown in Scheme 1. By substituting different groups at imine nitrogen, a sequence of novel quinazoline derivatives were synthesized in the present study. Initially, using anthranilic acid and acetic anhydride by simple acetylation followed by ring closure reaction 2-methylbenzo-[1,3]-oxazin-4-one (**II)** was synthesized. In the succeeding step, 3-amino-2-methylquinazolin-4(3*H*)-one **(III)** was synthesized through simple condensation reaction by treating compound **II** with hydrazine hydrate with the elimination of water molecules. In the next step, obtained amino derivative **III** was diazotized using sodium nitrite and hydrochloric acid. Subsequently the diazotized salt was treated with ethyl acetoacetateto produce ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)hydrazono)-3-oxobutanoate (**IV**) through intramolecular rearrangement reaction. In the final step, the obtained keto ester **IV** undergoesdehydrative cyclisation with various amine derivatives such as hydroxylamine hydrochloride, urea/thiourea, and different hydrazine hydrochloride analogues and produces corresponding isoxazole **V**, pyrimidine **Via-VIb**, and pyrazole derivatives **VIIa-VIIj**, respectively. To optimize the reactions for purity and completion, TLC was performed throughout the reactions.

Assigned structures of the test compound were confirmed using various spectral studies (IR, NMR, mass spectra, and elemental analysis). Presence of particular groups was identified from IR spectra by means of some characteristic absorption peaks. Formation of the benzoxazine ring in compound II was confirmed by the presence of absorption peak at 1730 and 1032 $cm^{-1}$ in IR due to presence of C=O and C-O-C stretching, respectively. The formations of compound III were confirmed by the appearance of absorption peak around 3345 $cm^{-1}$ in IR corresponds to N-H stretching of $NH_2$ and appearance of singlet at δ 5.30 ppm for two protons in its $^1$H-NMR spectra which might be assigned to $NH_2$ group.The formations of keto ester IV were confirmed from the appearance of sharp peak at 1046 $cm^{-1}$ in IR corresponds to C-O-C stretching and appearance of three singlet peaks at δ 6.39, 2.31 and 2.06 ppm for one, three and three protons which might be assigned to NH of hydrazone, $CH_3$ of quinazolinone and $COCH_3$ proton, respectively. This is further confirmed from NMR spectroscopy by the appearance of quartet peak at δ 4.36–4.58 ppm for two protons which might be assigned to $CH_2$ of $COOC_2H_5$ and triplet peak at δ 1.55-1.64 ppm for three protons which might be assigned to $CH_3$ of $COOC_2H_5$.The formation of novel heterocyclic substituted quinazolinone analogs (V, VIa-VIb and VIIa-VIIj) from ethyl 2-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)hydrazono)-3-oxobutanoate (IV)can be recognized from the disappearance of quartet and triplet peaks in its $^1$H-NMR spectra corresponds to the $CH_2$ and $CH_3$ of $COOC_2H_5$. The formation of compounds V, VIa, and VIb are confirmed by the appearance of singlet peak in its $^1$H-NMR spectra at δ 2.54 ppm, 2.40 ppm, and 2.87 ppm corresponds to the 3 protons of $CH_3$, 1 proton of SH, and 1 proton of OH, respectively. The formation of compound VIIa was confirmed by appearance of multiplet at δ 7.16-8.29 ppm for 8 protons. Meanwhile, appearance of singlet for two protons of thioamide group at δ 8.36 ppm confirms the formation of compound VIIb. Appearance of singlet for one proton of pyrazole NH at δ 6.18 ppm confirms the formation of compound VIIc. Appearance of sharp peak at 1062 $cm^{-1}$, and 1056 $cm^{-1}$, in IR corresponds to fluorine confirms the presence of fluorine in compounds VIId and VIIe, respectively. Similarly, appearance of

sharp peak at 860 $cm^{-1}$, and 871 $cm^{-1}$, in IR corresponds to chlorine confirms the presence of chlorine in compounds VIIf and VIIg, respectively. Appearance of singlet for three protons of methoxy group at δ 3.34 and 3.69 ppm confirms the formation of compound VIIh and VIIi. Multiplet for 9 protons observed at δ 7.03-8.25 ppm confirms the structure of compound VIIj. The structure of title compounds V, VIa-VIb and VIIa-VIIj were further confirmed by appearance of various other peaks in NMR spectroscopy corresponds to assigned structure. Mass spectrum further confirmed their molecular weight and purity.

Antimicrobial Activity

Agar streak dilution method was used for screening in vitro antibacterial and antifungal activity of title derivatives. Simultaneously MICs of Ciprofloxacin and Ketoconazole were determined in order to control the sensitivity of the test organisms.

**Table. 1 MIC (Minimum inhibitory concentration in µg/ml) of synthesized compounds 3-16**

| Compound | Antibacterial activity | | | | | | | Antifungal activity | |
|---|---|---|---|---|---|---|---|---|---|
| | B. cereus | S. aures | M. luteus | S. epidermidis | K. pneumoniae | P. aeruginosa | E. coli | A. fumigatus | A. niger |
| **V** | 125 | 125 | > 125 | 62.5 | 125 | > 125 | 62.5 | > 125 | > 125 |
| **VIa** | 62.5 | 62.5 | 125 | 62.5 | 62.5 | 125 | 62.5 | >125 | 125 |
| **VIb** | 125 | 62.5 | 125 | 125 | 62.5 | 62.5 | 62.5 | 125 | >125 |
| **VIIa** | 31.25 | 31.25 | 31.25 | 62.5 | 31.25 | 31.25 | 31.25 | 62.5 | 62.5 |
| **VIIb** | 62.5 | 31.25 | 62.5 | 62.5 | 31.25 | 62.5 | 31.25 | 62.5 | 62.5 |
| **VIIc** | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 31.25 | 31.25 | 62.5 | 62.5 |
| **VIId** | 15.62 | 15.62 | 31.25 | 15.62 | 31.25 | 7.81 | 15.62 | 31.25 | 31.25 |
| **VIIe** | 3.9 | 15.62 | 7.81 | 7.81 | 7.81 | 3.9 | 7.81 | 15.62 | 15.62 |
| **VIIf** | *15.62* | *31.25* | *7.81* | *15.62* | *15.62* | *7.81* | *15.62* | *31.25* | *15.62* |
| **VIIg** | 7.81 | 7.81 | 3.9 | 3.9 | 7.81 | 3.9 | 7.81 | 15.62 | 15.62 |
| **VIIh** | 15.62 | 31.25 | 15.62 | 31.25 | 15.62 | 15.62 | 15.62 | 31.25 | 31.25 |
| **VIIi** | *7.81* | *7.81* | *3.9* | *7.81* | *7.81* | *7.81* | *15.62* | *15.62* | *15.62* |
| **VIIj** | 31.25 | 31.25 | 31.25 | 62.5 | 31.25 | 15.62 | 31.25 | 62.5 | 62.5 |
| **Standard*** | *3.9* | *7.81* | *1.95* | *3.9* | *7.81* | *1.95* | *7.81* | *7.81* | *3.9* |

*Ciprofloxacin and Ketoconazole used as a reference standard for bacteria and fungi, respectively

Table 1 summarizes the MICs of synthesized compounds and standard drugs. Varying degree of antimicrobial activity is exhibited by test compounds. The results indicate that entire test compounds exhibited good antibacterial activity than antifungal activity. Almost all test compounds exhibited weak antifungal activity against the tested fungi. Out of thirteen examine derivatives, compounds VIIe, VIIg and VIIi exhibited potent antibacterial activity; whereas compounds VIId, VIIf and VIIh displayed moderate antibacterial activity. Rest of compounds V, Via, VIb, VIIa-VIIc and VIIj showed weaker antimicrobial activity. Compound 3-(2-(1-(4-chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methylquinazolin-4(3H)-oneVIIg, displayed equal activity like standard Ciprofloxacin against S. aureus (MIC: 7.81 µg/ml), S. epidermidis (MIC: 3.9 µg/ml), K. pneumoniae (MIC: 7.81 µg/ml) and E. coli (MIC: 7.81 µg/ml). Similarly against B. cereus (MIC: 3.9 µg/ml), K. pneumoniae (MIC: 7.81 µg/ml) and E. coli (MIC: 7.81 µg/ml), compound 3-(2-(1-(4-fluorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methylquinazolin-4(3H)-one VIIe, showed equipotent activity with Ciprofloxacin. In addition, compound 3-(2-(1-(4-methoxyphenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methylquinazolin-4(3H)-one VIIi displayed equal activity like standard Ciprofloxacin against S. aureus (MIC: 7.81 µg/ml), and K. pneumoniae (MIC: 7.81 µg/ml).

The potent antibacterial activity exhibited by compounds VIIe, VIIg and VIIi might be due to the presence of substituent like chloro, fluoro and methoxy group in para position of the phenyl ring attached to pyrazole nucleus. In general, it was found that pyrazole analogs VIIa-VIIj exhibited better activity than isoxazole V and pyrimidine analogs VIa-VIb. Within pyrazole derivatives, phenyl substituted compounds VIId-VIIj exhibited higher antimicrobial activities than pyridine- VIIa / thiocarbamide- VIIb / un-substituted VIIc compounds. In addition, introduction of substituents in phenyl ring increased the antimicrobial potency. From the study it was found that unsubstituted phenyl ring containing pyrazole compound VIIj exhibited less activity than substituted phenyl ring containing pyrazole compounds VIId-VIIi. Moreover, presence of substituent at para position of phenyl ring (VIIe, VIIg and VIIi) produced potent compounds compared to meta position of phenyl ring (VIId, VIIf and VIIh).

## CONCLUSION:

From anthranilic acid, various novel quinazolinone analogs were synthesized by multistep synthesis. FT-IR, $^{1}$H-NMR, Mass spectroscopy and elemental analysis were used employed for characterizing the synthesized

title compounds. Antibacterial and antifungal activity of entire title compounds were examined against several pathogenic bacteria and fungi using agar streak dilution method. Test analogs displayed varying degree of antimicrobial activity. Results of antimicrobial activity reveals that entire test compounds exhibits weak antifungal activity; whereas these derivatives showed better antibacterial activity. From the SAR studies it was found that, nature of heterocyclic nucleus attached to quinazolinone played major role in determining antimicrobial activity. In general, it was found that pyrazole analogs VIIa-VIIj exhibited better activity than isoxazole V and pyrimidine analogs VIa-VIb. Within pyrazole derivatives, phenyl substituted compounds VIId-VIIj exhibited higher antimicrobial activities than pyridine- VIIa / thiocarbamide- VIIb/ un-substituted VIIc compounds. In addition, para substituted phenyl ring possessing compounds (VIIe, VIIg and VIIi) showed higher activities than corresponding meta substituted phenyl ring possessing compounds (VIId, VIIf and VIIh). Out of thirteen tested derivatives, compound 3-(2-(1-(4-chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2-methylquinazolin-4(3H)-one VIIg displayedbetter antibacterial activity which is comparable to reference standard Ciprofloxacin. In addition, compounds VIIe and VIIi also showed some excellent antibacterial activity against some pathogenic strains of microorganism.Hence, these derivatives could be developed as a new class of antibacterial agents. Though, to improve the antibacterial activity further structural modification is planned.

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