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Analogues of 6,8-Dibromoquinazolin-4(3H) Ones: Potential Antimicrobial Agents In Vitro

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ABSTRACT *A new series of some analogues of 6,8-dibromoquinazolin-4(3H)-ones 61-12 have been synthesized by the cyclisation of acrylamide 51-12 with hydrazine hydrate. The overall reaction was based catalysed conventional multistep process. The title compounds have been characterized by spectral data IR, 1H NMR, 13C NMR and elemental analysis. All the synthesized compounds were screened for antibacterial and fungicidal activity In Vitro by disc diffusion method and strength compare with standard drug.*

KEYWORDS : Quinazolin-4(3H) one, Potency, Antibacterial, Antifungal

INTRODUCTION

Chalcones was either natural or synthetic unsaturated well known intermediates for synthesizing various heterocyclic compounds viz. pyranones, quinolines, pyrazolines and quinazolines. The pharmaceutical importance of chalcones lies in the fact that they can be effectively utilized to enhanced the bioactive moieties synthetically[1,2].

Quinazolin-4(3*H*)-one was an important pharmacophore in medicinal chemistry, since a large number of its derivatives possess broad spectrum of pharmacological and therapeutic properties. The large number of synthetic compounds of quinazolin-4(3*H*)-one with pyrazoline used for anti-inflammatory and analgesics agents[3,4]. Quinazolin-4(3H) one with its pyrazoline analogs have extensively used as anti-inflammatory, anticancer [5,6], anti-fungal[7], anti-hyperglycemic[8], HIV-1 integrase inhibitors[9], in medicinal chemistry.

Encourage by the wide spectrum of therapeutic activities exhibited and literature survey of quinazoline derivatives revealed that in this study, we have synthesized some analogues of quinazolin-4(3H) ones incorporating heterocyclic moiety pyrazoline at C-3 and studied its antibacterial and antifungal activities.

METHOD AND MATERIAL

General I nstrumentation

All reagents used were of AR grade. The solvents were distilled prior to use. The melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on Perkin-Elmer 1300 FTIR spectrophotometer using KBr powder. 1H NMR and 13C NMR spectra of the title compounds were recorded in $CDCl_3$ on a Bruker spectrometer at 400 MHz and 75 MHz respectively (chemical shift in d ppm). TMS used as internal standard. The purity of compounds was checked by TLC on silica gel G plates and spot visualization was done by exposing to iodine vapour. The synthesized compounds were analyzed for carbon, hydrogen and nitrogen and the result were varying within ± 0.04 % of the calculated values. The starting compound **1** was prepared according to the reported method[10].

Experimental section

2-(2-phenylamino)phenylmethyl–6,8-dibromo-3,1-benzoxazin-4(*H*)one 2

To solution of 2–(2-phenylamino)phenyl acetyl chloride (2.45 g, 0.01 mol) in pyridine(25ml) kept on an ice bath at 0-5 0C. Add each small portion of 3:5-dibromo anthranilic acid (2.94 g, 0.01 mol) was added portion wise and stirred for 1 h. to maintain temprature 0-5 0C. Further reaction mixture was stirred 1h at room temperature. A pasty mass thus obtained which was washed thoroughly with sodium bicarbonate (5%) to remove unreacted acid. A solid separated was filtered, dried and recrystalised from methanol.

M.P.:161 0C Yeild:69 % IR(KBr): 3407(NH),3073,2861(C=-H)1725,(C-O),1614 (C=N), 1323(C-N),1236(C-O-C), 750(NH wag), 614(C-Br). Anal. (%) for $C_{21}H_{14}N_2O_2Br_2$ Calcd; C, 51.85; H, 2.88; N, 5.76; Found; C, 51.87; H, 2.89; N, 5.77.

3-Amino 2-(2-phenyl)amino)phenyl methyl-6,8–dibromo quinazolin–4(3*H*) one 3

To a mixture of 2-(2-phenyl) amino) phenyl methyl–6,8-dibromo-3,1-benzoxazine-4(*H*)-one (4.86 g, 0.01 mol) and hydrazine hydrate (99 %) (0.50 g, 0.01mol) in 25.0 ml pyridine was heated at 180-200 0C in an oil bath for 5 - 6 h. The oily mass was slowly poured onto crushed acidic (HCl, 25ml) ice cold water with occasional stirring. The product obtained was filtered and washed several times with water. The crushed product was dried and recrystallized from ethanol.

M.P. :118-119 0C Yeild : 64 % IR(KBr) : 3403(NH), 3068, 2869(C-H), 1721(C=O), 1612(C=N), 1321(C-N), 611(C-Br).1HNMR($CDCl_3$)**:** 9.79(s,1H, -NH-), 2.1(s, 2H, -N-NH_2), 6.42-7.96(m, 11H, Ar-H), 2.71(s, 2H, -CH_2). Anal. (%) for $C_{21}H_{16}N_4OBr_2$ Calcd; C, 50.40; H, 3.20; N, 11.20; Found; C, 50.42; H, 3.21; N, 11.21.

2-(2-phenylamino)phtenyl methyl-3-acetamido-6,8-dibromo quinazolin-4(3*H*)-one 4

To solution of 3-amino2-(2-phenylamino)phenyl methyl-6,8–dibromo quinazolin–4(3*H*)–one **(**5.00 g, 0.01 mol) in dry benzene(50 ml), acetyl chloride(0.785g, 0.01mol) was added drop by drop at 0-5 0C, for 1 h with constant stirring after completion of addition the reaction mixture was kept overnight. The excess of solvent was distilled off under reduced pressure and then poured onto ice, the solid thus obtained was recrystalized from methanol.

M.P**. :**173-174 0C Yeild **:** 69 % IR(KBr): 3407(NH), 3062,2859(C-H),1727(C=O), 1645 (C=O of –$COCH_3$),1320(C-N), 613(C-Br). 1H-NMR($CDCl_3$) **:** 9.78(s, 1H, -NH-), 2.12(s, 1H, -N-NH-), 6.42-7.96(m, 11H, Ar-H), 2.70(s, 3H, -CH_3), 2.71(s, 2H, -CH_2). Anal. (%) for $C_{23}H_{18}N_4O_2Br_2$ Calcd;C,50.92; H, 3.32; N, 10.33; Found; C, 50.93; H, 3.34; N, 10.34.

2-(2-phenyl)amino)phenyl methyl-3-(phenyl acryl amido)-6,8-dibromo quinazolin-4(3*H*)-one 5_1

A solution of 2-(2-phenylamino)phenyl methyl-3-acetamido-6,8-dibromo quinazolin-4(3*H*)-one **(**5.42g, 0.01 mol) in absolute ethanol (50 ml) and added benzaldehyde (0.01 mol) in 2 % NaOH was refluxed for 10-12 h., cooled and poured into ice cold water. The solid obtained was filtered, washed with water and recrystalized from methanol.

M.P**. :**162-163 0C Yeild **:** 71 % IR(KBr) : 3411(NH), 3061, 2852(C-H), 1719(C =O), 1653(C=O of –$COCH_3$), 1576 (CH=CH), 1316(C-N), 611(C-Br).1H-NMR($CDCl_3$) **:** 9.78(s, 1H, -NH-), 2.11(s, 1H, -N-NH), 6.42-7.96(m, 16H, Ar-H), 2.61 (s, 2H, -CH_2), 6.80(d, 1H, COCH=), 8.62(d, 1H, =CH-Ar). Anal. (%) for $C_{30}H_{22}N_4O_2Br_2$ Calcd; C, 57.14; H, 3.49; N, 8.89; Found; C, 57.15; H, 3.51; N, 8.90.

The remaining $5_{2\text{-}12}$ compounds were prepared by the above mention similar method.

2-(2-phenylamino)phenyl methyl-3–[(5-phenyl)-1,5-dihydro-1*H*-pyrazol-3-yl amino] -6,8-dibromoquinazolin-4(3*H*)-one 6_1

To a solution of 2-[(2-phenyl) amino] phenyl methyl-3– (5-phenyl chromen amido)-6,8-dibromoquinazolin-4(3*H*) one (6.30g, 0.01 mole)

in methanol, add hydrazine hydrate (99 %) (1.00 g, 0.02 mole) and few drop of glacial acetic acid. The reaction mixture was refluxed for 8-10 h. After reaction mixture were distilled and cooled. The separated solid was filtered, washed with water and recrystallized from methanol.

M.P.: 121-122 0C YieId: 72%.IR(KBr):3369(N-H), 3063, 2857(C-H),1716(-C=O),1614 (C=N), 1315(C-N),608(C-Br). 1H NMR($CDCl_3$): 2.17(s,1H,=N-NH),3.06(d, 1Ha), 3.42 (d,1Hb), 6.43(t, 1Hx), 3.62 (s, 2H, Ar- CH_2 –), 8.18(s, 1H, -N –NH), 9.86 (s, 1H, - NH-), 6.42-7.96(m,16H, Ar- H). 13C NMR: 30.4(Methylene-C), 35.6, 41.4, 161.1(pyrazole-3C), 109.21-143.20(aromatic-24C), 162.32 (C = O), 173.6 (immine Aromatic- C). Anal. (%) for $C_{30}H_{24}N_6OBr_2$ Calcd; C, 55.90; H, 3.72; N, 13.04; Found; C, 55.91; H, 3.74; N, 13.05.

The remaining $6_{2\text{-}12}$ compounds were prepared by the above mention similar method.

2-(2-phenyl amino) phenyl methyl -3-[5-(2-hydroxy phenyl)-1, 5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_2

M.P.: 130-131 0C Yeild:75 % IR(KBr):3548(O-H),3381(N-H),3072,2858 (C-H), 1721(C=O),1614(C=N),1319 (C-N), 608(C-Br). 1H NMR($CDCl_3$): 2.14(s,1H,=N-NH), 3.07(d,1Ha),3.45(d,1Hb),6.43(t,1Hx), 3.61 (s, 2H, Ar- CH_2 –), 8.18(s, 1H, -N –NH –), 9.84 (s, 1H, - NH-), 6.42-7.94(m,15H, Ar- H), 10.3(s,1H, -OH) 13C NMR: 30.5 (–CH_2), 35.7, 40.8, 161.1 (immine pyrazole–3C), 109.21-143.20 (aromatic-24C), 162.3 (> C = O), 173.0 (immine aromatic- C). $C_{30}H_{24}N_6O_2Br_2$ anal % calcd : C 54.54, H 3.63, N 12.72 found C 54.55 H 3.65 N 12.73.

2-(2-phenylamino) phenyl methyl -3-[5-(3-hydroxy phenyl)-1,5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazoline-4(3*H*)-one 6_3

M.P.:142-143 0C Yeild: 69% IR(KBr): 3549(O-H), 3370(N-H), 3062, 2861(C-H), 1720 (C=O),1614(C=N),1325(C-N), 615(C-Br). 1H NMR($CDCl_3$): 2.16(s,1H,=N-NH), 3.09(d,1Ha),3.45(d,1Hb),6.43(t,1Hx), 3.63 (s, 2H, Ar- CH_2 –), 8.17(s, 1H, -N –NH –), 9.86 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H), 10.27(s,1H, -OH). 13C NMR: 30.5(–CH_2), 35.7, 41.4, 161.1(immine pyrazole-3C), 109.21-143.20 (aromatic-24C), 162.3 (> C = O), 173.6 (immine aromatic-C). $C_{30}H_{24}N_6O_2Br_2$ anal % calcd : C 54.54, H 3.63, N 12.72 found C 54.56 H 3.64 N 12.74.

2-(2-phenyl amino) phenyl methyl -3-[5-(4-hydroxy phenyl)-1,5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_4

M.P.: 148-149 0C YeiId : 71 % IR(KBr):3543(O-H),3389(N-H),3058,2862 (C-H), 1723(C=O), 1613(C=N),1321(C-N), 610(C-Br). 1H NMR($CDCl_3$): 2.17(s,1H,=N-NH), 3.06(d,1Ha),3.41(d,1Hb),6.45(t,1Hx), 3.60 (s, 2H, Ar-CH_2 –), 8.19(s, 1H, -N –NH –), 9.87 (s, 1H, - NH-), 6.42-7.94(m,15H, Ar-H), 10.27(s,1H, -OH). 13C NMR: 30.4(–CH_2), 34.8, 41.6, 160.9(immine pyrazole-3C), 109.21-143.20(aromatic-24C), 162.1 (> C = O), 173.4 (immine aromatic- C). $C_{30}H_{24}N_6O_2Br_2$ anal % calcd : C 54.54, H 3.63, N 12.72 found C 54.55 H 3.64 N 12.73.

2-(2-phenyl amino) phenyl methyl -3-[5-(2-chloro phenyl)-1,5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_5

M.P.: 118-119 0C YeiId : 78 % IR(KBr):3398(N-H),3062, 2859(C-H),1725 (C=O), 1614 (C=N), 1320(C-N), 778(C-Cl) 622(C-Br). 1H NMR($CDCl_3$): 2.17(s,1H,=N-NH), 3.09(d,1Ha), 3.41 (d,1Hb),6.45(t,1Hx), 3.63 (s, 2H, Ar- CH_2 –), 8.17(s, 1H, -N –NH –), 9.86 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H). 13C NMR: 30.4 (–CH_2), 35.6, 40.3, 160.1(immine pyrazole-3C), 109.21-143.20(aromatic-24C), 162.1 (> C = O), 173.6(immine aromatic-C). $C_{30}H_{23}N_6OBr_2Cl$ anal % calcd : C 53.05, H 3.38, N 12.38 found C 53.07 H 3.40 N 12.39.

2-(2-phenyl amino) phenyl methyl -3-[5-(3-chlorophenyl)-1, 5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_6

M.P.: 126-127 0C Yeild:74 % IR(KBr): 3409 (N-H), 3064, 2856 (C-H),1727(C=O), 1617(C=N),1322(C-N), 781(C-Cl), 611(C-Br). 1H NMR($CDCl_3$): 2.16(s,1H,=N-NH), 3.06 (d,1Ha), 3.42(d,1Hb),6.49(t,1Hx), 3.61 (s, 2H, Ar- CH_2 –), 8.16(s, 1H, -N –NH –), 9.84 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H).13C NMR: 30.4 (–CH_2), 35.6, 40.4, 161.1(immine pyrazole-3C), 109.21-143.20(aromatic-24C), 162.2 (> C = O), 173.0 (immine aromatic- C). $C_{30}H_{23}N_6OBr_2Cl$ anal % calcd : C 53.05, H 3.38, N 12.38 found C 53.06 H 3.39 N 12.40.

2-(2-phenyl amino) phenyl methyl -3-[5-(4-chloro phenyl)-1, 5-dihydro-1*H*-pyrazol-3-yl-amino-6,8-dibromoquinazolin-4(3*H*)-one 6_7

M.P.: 139-140 0C YeiId: 68 % IR(KBr):3408(N-H),3066,2880(C-H), 1730 (C=O), 1609(C=N),1319(C-N),768(C-Cl), 622(C-Br). 1H NMR($CDCl_3$): 2.17(s,1H,=N-NH), 3.09(d,1Ha), 3.44 (d,1Hb),6.45(t,1Hx), 3.62 (s, 2H, Ar- CH_2 –), 8.17(s, 1H, -N –NH –), 9.89 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H). 13C NMR: 30.5(–CH_2), 35.5, 40.4, 161.1 (immine pyrazole-3C), 109.21-143.20(aromatic-24C), 162.2 (> C = O), 173.2 (immine aromatic- C). $C_{30}H_{23}N_6OBr_2Cl$ anal % calcd : C 53.05, H 3.38, N 12.38 found C 53.06 H 3.39 N 12.39.

2-(2-phenyl amino) phenyl methyl -3-[5-(2-nitrophenyl)-1,5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one6_8

M.P.: 151-152 0C Yeild : 69 % IR(KBr):3370(N-H),3065,2861(C-H),1727(C=O), 1613(C=N),1543,1361(-NO_2), 1321(C-N), 610(C-Br).1H NMR($CDCl_3$): 2.15(s,1H,=N-NH), 3.10(d,1Ha),3.45(d,1Hb), 6.47(t,1Hx), 3.60 (s, 2H, Ar- CH_2 –),8.19(s, 1H, -N –NH –), 9.85 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H). 13C NMR: 30.4 (–CH_2), 35.7, 41.4, 160.9 (immine pyrazole–3C), 109.21-143.20 (aromatic-24C), 162.0(> C = O), 173.5 (immine aromatic- C). $C_{30}H_{23}N_7O_3Br_2$ anal % calcd : C 52.24, H 3.33, N 12.38 found C 52.26 H 3.34 N 12.40.

2-(2-phenyl amino) phenyl methyl -3-[5-(3-nitro phenyl)-1, 5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_9

M.P.: 168-169 0C YeiId: 73 % IR (KBr):3377(NH),3066,2861(-C-H),1730(C=O), 1614(C=N),1541,1359(-NO_2),1319(C-N), 615(C-Br). 1H NMR($CDCl_3$): 2.16(s,1H,=N-NH), 3.06(d,1Ha),3.45(d,1Hb), 6.47(t,1Hx), 3.62 (s, 2H, Ar- CH_2 –),8.17(s, 1H, -N –NH –), 9.84 (s, 1H, - NH-), 6.42-7.96(m,15H, Ar- H).13C NMR: 31.4 (–CH_2), 34.7, 41.3, 161.1 (immine pyrazole–3C), 109.21-143.20 (aromatic-24C), 162.1(> C = O), 173.5 (immine aromatic- C). $C_{30}H_{23}N_7O_3Br_2$ anal % calcd : C 52.24, H 3.33, N 12.38 found C 52.25 H 3.34 N 12.39.

2-(2-phenyl amino) phenyl methyl -3-[5-(4-nitrophenyl)-1,5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_{10}

M.P.: 183-184 0C YeiId: 70 % IR(KBr): 3375(NH), 3067, 2857(C-H),1731(-C=O), 1615(C=N),1539,1363(-NO_2),1318(C-N), 611(C-Br). 1H NMR(C-DCl_3): 2.15(s,1H,=N-NH), 3.07(d,1Ha),3.43(d,1Hb), 6.45 (t,1Hx), 3.63 (s, 2H, Ar- CH_2),8.16(s, 1H, N –NH), 9.86 (s, 1H, - NH-), 6.42-7.96 (m,15H, Ar- H). 13C NMR: 30.5(–CH_2), 35.6, 41.3, 161.1 (immine pyrazole–3C), 109.21-143.20 (aromatic-24C), 162.0 (> C = O), 173.5 (Immine Aromatic- C). $C_{30}H_{23}N_7O_3Br_2$ anal % calcd : C 52.24, H 3.33, N 12.38 found C 52.26 H 3.35 N 12.40.

2-(2-phen83ryl amino) phenyl methyl -3-[5-(2-methoxy phenyl)-1,5-dihydro-1H-pyrazol-3-yl-amino]-6,8-dibromoquinazoline-4(3*H*)-one 6_{11}

M.P.: 114-115 0C YeiId : 76 % IR(KBr):3389 (N-H),3057,2861 (C-H), 1719 (C=O), 1613 (C=N),1319 (C-N), 1243, 1107(C-O-C), 616(C-Br). 1H NMR($CDCl_3$): 2.16(s,1H,=N-NH), 3.07(d,1Ha),3.41(d,1Hb), 6.49(t,1Hx), 3.62 (s, 2H, Ar- CH_2 –), 3.81(s, 3H, -OCH_3), 8.19(s, 1H, -N –NH), 9.86 (s, 1H, - NH), 6.42-7.96(m,15H, Ar- H). 13C NMR: 30.4(–CH_2), 35.6, 40.3, 160.9(immine pyrazole–3C), 62.3(Methoxy-C) 109.21-143.20 (aromatic-24C), 162.1(> C = O), 173.5 (immine aromatic- C). $C_{31}H_{26}N_6O_2Br_2$ anal % calcd : C 55.19, H 3.85, N 12.46 found C 55.20 H 3.86 N 12.48.

2-(2-phenyl amino) phenyl methyl -3-[5-(4-methoxy phenyl)-1, 5-dihydro-1*H*-pyrazol-3-yl-amino]-6,8-dibromoquinazolin-4(3*H*)-one 6_{12}

M.P.: 132-133 0C Yeild: 73 % IR(KBr): 3398(N-H), 3057, 2865(C-H),1718(C=O), 1610(C=N),1318(C-N),1239,1105 (C-O-C), 609(C-Br). 1H NMR($CDCl_3$): 2.16(s,1H,=N-NH), 3.09(d,1Ha),3.45(-d,1Hb), 6.43 (t,1Hx), 3.63 (s, 2H, Ar- CH_2 –), 3.80(s, 3H, -OCH_3), 8.17(s, 1H, -N –NH –), 9.86 (s, 1H, - NH-), 6.42-7.96 (m,15H, Ar- H).13C NMR: 30.4(–CH_2), 35.7, 40.3, 161.1 (immine pyrazole–3C), 62.3(Methoxy-C) 109.21-143.20(aromatic-24C), 162.3 (> C = O), 173.6(immine aromatic- C). $C_{31}H_{26}N_6O_2Br_2$ anal % calcd : C 55.19, H 3.85, N 12.46 found C 55.21 H 3.86 N 12.47.

Determinatio5n of Antimicrobial Activity
Disc Diffusion Method

The *in vitro* antimicrobial activity of synthesized compounds was carried out by disc diffusion method 11,12]. The cup was bore in to the inoculated Petri dish. The cups were made (equidistance) by punching in to the agar surface with sterile cup borer and scooping out the punch part of the agar. After punching a bore, in to these cups were added 0. 01 ml portion of the test compound (0. 01 g dissolved in 10 ml DMF solvent) in solvent with the help of sterile syringe. The solution was allowed to defuse for about an hour in to the medium.

Measurement of the zone of Inhibition

After 2 h, for the diffusion of the substance in the agar medium and the plates were incubated at 37 0C for 24 h. After incubation period observed the plate for zone of inhibition around the cups. Measure the diameter of each zone in mm.

A solvent control was also run to know the activity of the blank . This was carried out in DMF at concentration of 0. 05 ml in similar manner and the zone of the inhibition of the bacterial growth were measured in diameter and it was 0.0 mm. The standard drugs were also screened under similar condition.

The zone of inhibition measured for anti bacterial activity at two different concentrations 100 and 50 µg/ml, Penicillin-G was used as standard, where as zone of inhibition measured for anti fungal activity also at two different concentrations 20 and 10 µg/ml and Fluconazole was used as a standard.

RESULT AND DISCUSSION

The title compound some analogus of quinazolin-4(3H) one incorporating pyrazoline moiety $\mathbf{6_{1\text{-}12}}$ were synthesized and structure was confirmed by the spectral results. The IR spectra showing strong stretching vibration at 1729 and 1646 cm-1 indicates the presence of C=O group of quinazolinone and acetamide respectively. This was further confirming by 1H NMR spectra which showed singlet at δ 2.73 ppm equivalent to three protons of acetamide group**(4)**. The acrylamide $\mathbf{5_{1\text{-}12}}$ which showed CH=CH stretching at1578 cm-1 in IR spectrum while 1H NMR spectra showed doublet of these protons at δ 6.81 and δ 8.61 ppm with coupling constant *J* = 16.0-16.6 Hz. The IR spectra of compounds $\mathbf{6_{1\text{-}12}}$ showed C=O and C=N stretching of quinazolinone at 1725 and 1616 cm-1 respectively. The 1H NMR spectra of compounds 6a-l indicates that the $-CH_2$ protons of the pyrazoline ring resonated as a pair of doublet of doublets (Ha and Hb) because of geminal and vicinal coupling. The CH proton appeared as a doublet of doublet (Hx) because of vicinal coupling with the two magnetically nonequivalent protons of methylene group at C-4 of pyrazolin ring. The Ha proton which is cis to Hx resonates up field in the range δ 3.01-3.08 ppm as a doublet of doublet while Hb, the other proton which is trans to Hx resonates downfield in the range of δ 3.45-3.51 ppm as a doublet of doublet. The Hx proton which is vicinal to two methylene protons (Ha and Hb) resonates as a doublet of doublet in the range of δ 6.45-6.53 ppm. In 13C NMR spectra, signals at δ 36.4 ppm, δ 41.1 ppm and δ 161.3 ppm confirms the presence of CH_2, CH and C=N of pyrazoline ring respectively, whereas C=O and C=N signals of quinazolinone ring are appear at around δ 162.2 and δ 173.1 ppm respectively[13,14].

Antimicrobial Assay

The *in vitro* antimicrobial screening results of synthesized compounds were recorded in the table **1** and **2**. Potency [15] was calculated from the screening results and compares the strength of synthesized compounds with standard drug.

Table: 1 Anti-bacterial activity of compound $\mathbf{6_{1\text{-}12}}$

Compd	R_1	Zone of inhibition in (mm)											
		S. aureus ATCC12228			***B. subtilis*** ATCC6633			**E.coli** ATCC11778			**Certium** ATCC27957		
		C_H	C_L	Pot%	C_H	C_L	Pot%	C_H	C_L	Pot%	C_H	C_L	Pot %
6_1	H	14	11	50.47	14	12	50.10	12	10	40.38	11	09	41.85
6_2	2-OH	15	12	52.83	14	11	52.61	15	13	47.11	15	13	52.40
6_3	3-OH	13	10	48.23	13	11	47.05	14	12	44.64	13	11	46.83
6_4	4-OH	15	12	52.83	15	13	53.35	15	13	47.11	14	12	49.63
6_5	2-Cl	19	16	63.39	18	15	**65.85**	16	13	52.46	15	13	52.40
6_6	3-Cl	16	12	57.87	16	13	58.83	17	14	55.02	15	12	55.43
6_7	4-Cl	18	14	62.76	17	13	**63.61**	16	13	52.46	15	13	52.40
6_8	2-NO_2	13	10	48.23	13	11	47.05	20	16	65.04	19	15	**68.97**
6_9	3-NO_2	13	10	48.23	14	12	50.10	20	17	63.51	18	15	**64.31**
6_{10}	4-NO_2	15	12	52.83	15	12	55.69	22	19	69.87	21	18	**74.50**
6_{11}	2-OCH_3	15	12	52.83	14	11	52.61	16	13	52.46	15	13	52.40
6_{12}	4-OCH_3	16	13	55.29	15	12	55.69	16	13	52.46	15	13	52.40
PenicillinG		30	25	100	27	21	100	31	25	100	28	23	100

C_H Zone of inhibition at concentration 100 µg/ml, C_L Zone of inhibition at concentration 50 µg/ml, potency of compound(%) as compared to penicillin-G.

Table: 2 Antifungal activity of compound $\mathbf{6_{1\text{-}12}}$

Compd No.	R_1	Zone of inhibition in (mm)					
		C.albicans ATCC 10231			A.niger ATCC 16404		
		C_H	C_L	Pot%	C_H	C_L	Pot %
6_1	H	18	15	68.82	19	16	**66.90**
6_2	2-OH	11	09	43.89	12	10	43.08
6_3	3-OH	12	10	46.68	14	12	48.51
6_4	4-OH	10	08	41.30	12	10	43.08
6_5	2-Cl	10	08	41.30	09	07	35.88
6_6	3-Cl	11	09	43.89	11	09	40.53
6_7	4-Cl	12	10	46.68	11	09	40.53
6_8	2-NO_2	12	10	46.68	13	11	45.75
6_9	3-NO_2	14	12	52.71	14	12	48.59
6_{10}	4-NO_2	12	10	46.68	12	10	43.08
6_{11}	2-OCH_3	16	13	61.86	17	14	**60.10**
6_{12}	4-OCH_3	18	16	67.26	18	15	63.41
Fluconazole		26	21	100	28	22	100

CH Zone of inhibition at concentration 20 µg/ml, CL Zone of inhibition at concentration 10 µg/ml, potency of compound(%) as compared to fluconazole.

CONCLUSION

The title compounds analogues of 6, 8-dibromoquinazolin-4(3H) ones derivatives $\mathbf{6}_{1\text{-}12}$ were synthesized by well organized method. The active pharmacophore pyrazoline and quinazolin-4(3H) one present in a newly synthesized compounds possessed good antibacterial and antifungal activity *In Vitro*. The chloro group in phenyl nucleus on *ortho,* and *para* position showed very good activity against gram positive bacteria while nitro analogues displayed very good activity against gram negative bacteria compared to standard. More over phenyl nucleus, *ortho* and *para* methoxy substituted phenyl compounds showed very good antifungal activity. From these work, we were able to identify a few active molecules which are capable to inhibiting the growth of some bacteria and fungus species *In Vitro*.

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REFERENCES

(1) Hery Suwito., Jumina., Mustofa., Alfinda, N.K., Ni Nyoman, T.P.,(2014), " Chalcone: synthesis, structure diversity and pharmacological aspects", Journal of Chemical and Pharmaceutical Research., 6(5), 1076-1088.

(2) Patel, N.B., Barat,G.G., (2010), " Synthesis of chalcone containing pyrazolyl quinazolin -4(3H) ones and their In Vitro microbial studies", Int. J. Chem. Sci., 8(2), 1287-1300.

(3) Gokhan-Kelekci, N., Yabanoglu, S., Kupeli, E., Salgin, U., Ozgen, O., Ucar, G., (2007), "A new therapeutic approach in alzeimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and anti-inflammatory, analgesics", Bioorganic and Medical Chemistry, 15(1), 5775- 5786.

(4) Kumar, A., Sharma, S., Bajaj, K., Sharma, S., Panwar, H., Singh, T.,(2003), " Some new 2,3,6- trisubstituted quinazolinones as potential anti-inflammatory, analgesics and COX-II inhibitors", Bioorganic and Medical Chemistry, 11, 5292-5299.

(5) Chandrika, P.M., Yakaiah, T., Rao,A.R., Narsaiah, B., Reddy, N.C., Sridhar, V., Rao, J.V., (2008), "Synthesis of novel 4,6- disubstituted quinazolin derivatives, their anti-inflammatory and anticancer activity against U 937 Leukemia cell lines", Eur. J. Med. Chem., 43, 846-852.

(6) Sureshkumar, Bawa, S., Gupta,H.,(2009), "Biological activity of quinazoline derivatives", Mini review in Medicinal Chemistry, 9(14), 1648-1654.

(7) Bartroli, J., Turmo, E.,Elguero,M.,Boncompte,E., Vericat, M.L., Conte,L.,Ramis,J., Merlos, M., Garcia-Rafanell, J., Forn, J.,(1998), " Synthesis and antifungal activity of 3-substituted 4(3H)-quinazolinones", J. Med. Chem.,41, 1869-1882.

(8) Ram, V.J., Farhanullah, Tripathi, B.K., Srivastava, A.K.,(2003), "Synthesis and anti-hyperglycemic activity of suitably functionalized -3H-quinazolin-4-ones", Bioorg. Med. Chem., 11(11), 2439-2444.

(9) Liming, H., Song, Y., Zaigang, L., Xiao, H., Yujie, W., Zhanyang, W., Changchu, Z.,(2012), " Design practical synthesis and biological evaluation novel 6-(pyrazolyl methyl)-4-quinazolin-3-carboxylic acid derivatives as HIV-1 integrase inhibitors", Molecules, 17, 10652-10666.

(10) Furniss, B.S., Hannaford, A.J., Smith, P.W., Tatchell, A.R.,(1989), In Vogel textbook of practical organic chemistry, 5th ed. New York John Wiley and Sons, p 692.

(11) Bonev, B., Hooper, J., Parisot, J., (2008), " Principles of assessing bacterial susceptibility to antibiotics using agar diffusion method", J. Antimicrob Chemother, 61(6),1295-1301.

(12) Barry, A.L., (1976), The microbial susceptibility test, principles and practices, Philadelphia Pa, USA Illus Le and Febiger, p 180.

(13) Kalsi, P.S.,(1999), Spectroscopy of organic compounds, 4th ed. New Age International Publishers.

(14) Patel, N.B., Shaikh, A.R., Barat, G.G., (2012), "Synthesis and *In Vitro* antimicrobial evaluation of pyrazolylquinazolin-4(3H) ones", Acta Polonia Pharmaceutica Drug Research, 69, 1067-1075.

(15) Edwin, J.D., Marion, B.S., (1945), Assay of Antibiotic Substance, p 459.