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Research Article

SYNTHESIS AND ANTIBACTERIAL ACTIVITY SCREENING OF SOME NOVEL PYRAZOLE DERIVATIVES

PRATAP KUMAR PATRA*[1], CH NIRANJAN PATRA[2], SUBASINI PATTNAIK[3]

*[1]Sree Dattha Institute of Pharmacy, Ibrahimpatnam, Hyderabad 501510, [2]Roland Institute of Pharmaceutical sciences, Khodashing, [3]Berhampur University, Bhanjavihar, Berhampur. Email: pratappatra83@gmail.com

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ABSTRACT

Objective: To synthesise a series of quinazolinone clubbed pyrazole derivatives and evaluate for their antibacterial activity.

Method: Various Quinazolinone clubbed Pyrazole derivative compounds were synthesized as per standard chemical procedure and characterized by physical and spectral analysis. The antimicrobial activities of all the synthesized compounds were evaluated separately for their possible antimicrobial activity against common pathogenic bacteria. Bacteria used in the present study were *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), as gram positive and Escherichia coli (E. Coli), Pseudomonas aeruginosa (P. aeruginosa)* as gram negative bacteria. The study was conducted by cup-plate method.

Result: The antibacterial data's of the newly synthesized compounds indicate that some of them show better antibacterial activity than compared to their reference drug Ampicilin.

Conclusion: Four (7a1, 7a2, 7b1, 7b2) new biologically active pyrazole were synthesized for the first time. Synthesized compounds exhibited good antibacterial activity against the tested microorganism.

Keywords: Pyrazole, Quinazolinone, Antibacterial, Zone of inhibition, Ampiciline.

INTRODUCTION

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are also called as Azoles [1]. Recently Pyrazole derivatives have been found in nature [1], β- [1-pyrazolyl] alanine was isolated from the seeds of water melons [Citurllus lanatus]. The best described property of almost every group of pyrazoles is in the treatment of inflammation and inflammation associated disorders, such as arthritis [2]. Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial[3], antiviral[4], antitumor[5,6], antihistaminic[7], antidepressant[8], insecticides[9] and fungicides[9].

Several pyrazole derivatives have been found to possess significant activities such as 5-α-red-uctase inhibitor [10], anti-proliferative [11], anti-parasitic [12], herbicides [13]. A good number of pyrazoles have also been reported to have interesting biological activities like anti-inflammatory [14] and antiprotozoal [15-16] which render them valuable active ingredients of medicine and plant protecting agents. Further, current literature indicates 1, 2-pyrazole derivatives to possess various biological activities [17].

In this present study some novel pyrazole clubbed with quinazolinone compounds were synthesized and were evaluated for antimicrobial activity by cup-plate method.

MATERIALS AND METHODS

Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-fourier transform infra red **(**FTIR)-8400 Spectrophotometer using KBr disc. 1H NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a SHIMADZU-GCMS-QC-2010**.**

Experimental procedure

Synthesis of 3, 5 dibromo Anthranilic Acid (1)

20gm of anthranilic acid dissolved in 25 c.c. bromine in glacial acetic acid(9 cc bromine in 25 cc glacial acetic acid) was added drop by drop from separating funnel till the reddish colour of the liquid persist. Then content was converted to a thick mass. So it will form dibromo anthranilic acid. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under vacuum when the reaction was completed. The solid was crystallized from methanol to give pure product (**2**). Their melting points, yields and molecular formula are given in Table-1.

Yield: 79%; mp 202-204 ºC; IR (cm-1): 3312 (N-H stretching of primary amine), 3066 (C-H stretching of aromatic ring), 1726, 1675 (C=O stretching of carboxylic acid), 1604 (N-H deformation of NH2 group), 1560 and 1432 (C=C stretching of aromatic ring), 1004 (C-H in plane bending for aromatic ring); 695(C-Br); 1H NMR ($CDCl_3$) δ ppm: 7.03-7.58 (m, 2H, ArH), 5.38 (s, 1H, NH), 9.94 (S, 1H, COOH); MS: *m/z* 293,275,213, 189, 174 , 134; Anal.Calcd. For $C_7H_5Br_2NO_2$: C, 28.51; H, 1.71; N, 4.75. Found: C, 28.80; H, 1.64; N, 4.77%.

Synthesis of 6, 8-dibromo-2-methyl-4*H*-3, 1-benzoxazin-4-one (2)

A mixture of 3, 5 dibromo Anthranilic Acid (1,0.01 mol) and acetic anhydride (10.2 ml(0.1 mol) was refluxed on gentle flame for 1 hr .The excess of acetic anhydride was distilled off under reduce pressure and the residue was dissolve in petroleum ether and kept a side for 1 hr. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under vacuum when the reaction was completed. The solid was crystallized from ethanol to give pure product (**2**). Their melting points, yields and molecular formula are given in Table-1.

Yield: 71%; mp 179-181 ºC; IR (cm-1): 3076,3000 (C-H stretching of aromatic ring), 1689 (C=O stretching of ***benzoxazin-4-one*** ring), 1639 (C=N stretching of pyridine ring), 1495 (C=C stretching of aromatic ring), 1127 (C0C stretching of ***benzoxazin-4-one*** ring),933 (C-H in plane bending for aromatic ring); 684(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.87 (s, 3H, CH_3), 7.39-7.89 (m, Ar-H); MS: *m/z* 317,289,276,237,213,158,105; Anal.Calcd. for $C_9H_5Br_2NO_2$: C, 33.89; H, 1.58; N, 4.39. Found: C, 33.91; H, 1.54; N, 4.42%.

Synthesis of 3-amino-6,8-dibromo-2-methylquinazolin-4(3*H*)-one (3)

A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of 6,8-dibromo-2-methyl-4*H*-3,1-benzoxazin-4-one (**2,** 0.01 mmol) in ethyl alcohol (30 mL),followed by the hydrazine hydrate (0.03 mmol) was added and the mixture

was reflux for 2 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table-1.

Yield: 69%; mp 138-140 ºC; IR (cm-1):3361(N-H stretching of primary amine), 3063(C-H stretching of aromatic ring),2914(CH_3 Str.),1682 (C=O stretching of ring), 1599(C=N stretching of pyridine ring), 1451 (C=C stretching of aromatic ring), 880(C-H in plane bending for aromatic ring); 688(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.84 (s, 3H, CH_3), 4.73 (s,2H,NH_2), 7.40-8.07(m,Ar-H); MS: *m/z* 331,314,303,290,251,227,211,172,103; Anal.Calcd. for C9H7Br2N3O: C, 32.46; H, 2.12; N, 12.62. Found: C, 32.49; H, 2.09; N, 12.58%.

Synthesis of *N*-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4*H*)-yl) acetamide (4)

A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of 3-amino-6, 8-dibromo-2-methylquinazolin-4(3*H*)-one (**3,** 0.01 mmol) in Glacial acetic acid (30 mL), followed by the acetic anhydride (0.03 mmol) was added and the mixture was reflux for 1 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table-1.

Yield: 74%; mp 126-128 ºC; IR (cm-1):3366(N-H stretching of primary amine), 3082(C-H stretching of aromatic ring), 2870(CH_3 Str.), 1703(C=O stretching of ***Acetyl group***), 1673(C=O stretching of ring), 1609(C=N stretching of pyridine ring), 1462 (C=C stretching of aromatic ring), 900(C-H in plane bending for aromatic ring); 689(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.83 (s, 3H, CH_3), 2.50 (s, 3H, CH_3),8.94(s,1H,NH),7.40-8.17(m,Ar-H); MS: *m/z* 373,345,331,314,293,278,269,190,103; Anal.Calcd. for C118H9Br2N3O2: C35.23; H, 2.42; N, 11.20. Found: C 35.19; H, 2.44; N, 11.24%.

Synthesis of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide (5a)

A mixture of N-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4*H*)- yl) acetamide (4,0.01 mol) was dissolved in ethanol. Then sodium hydroxide(5 ml,30%) solution and salisaldehyde (0.01 mol) were added to the resulting solution with continuous stirring for 10 hr then solution so obtained refluxed on gentle flame for 6 hr . The reaction was monitored by TLC. After complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water .The solid was crystallized from ethanol to give pure product (**5a**).5b were prepared similarly. Their melting points, yields and molecular formula are given in Table-1.

Yield: 70%; mp 152-154 ºC; IR (cm-1):3471(O-H stretching of Hydroxy group) ,3356(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2915(CH_3 Str.), 1730(C=O stretching of keto group), 1673 (C=O stretching of ring), 1607(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 876(C-H in plane bending for aromatic ring); 687(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.84 (s, 3H, CH_3), 10.83 (s, 1H, OH),9.01(s,1H,NH),6.210-6.216(d,1H,CH),7.19-7.25(d,1H,CH),7.41-8.10(m,Ar-H);MS: *m/z* 477,459,449,397,331,316,*314*,163,161,103; Anal.Calcd. for C18H13Br2N3O3: C, 45.12; H, 2.73; N, 8.77. Found: C, 45.16; H, 2.69; N, 8.81%.

Synthesis of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide (5b)

A mixture of N-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4*H*)- yl) acetamide (4,0.01 mol) was dissolved in ethanol. Then sodium hydroxide(5 ml,30%) solution and benzaldehyde(0.01 mol) were added to the resulting solution with continuous stiring for 10 hr then solution so obtained refluxed on gentle flame for 6 hr. The reaction was monitored by TLC. After complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water .The solid was crystallized from ethanol to give pure product (**5a**). Their melting points, yields and molecular formula are given in Table-1.

Yield: 73%; mp 151-153 ºC; IR (cm-1):3377(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2932(CH_3 Str.), 1727(C=O stretching of ***keto group***),1670 (C=O stretching of ring), 1616(C=N stretching of pyridine ring), 1485,1460 (C=C stretching of aromatic ring), 924(C-H in plane bending for aromatic ring); 659(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.80 (s, 3H, CH_3), 9.73(s,1H,NH),6.210-6.216(d,1H,CH),7.19-7.27(d,1H,CH),7.40-8.57(m,Ar-H); MS:*m/z* 461,433,381,331,314,235,147,103; Anal.Calcd. for C18H13Br2N3O2: C, 46.68; H, 2.83; N, 9.07. Found: C, 46.66; H, 2.81; N, 9.10%.

Synthesis of 6, 8-dibromo-3-{[5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one (6a)

A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide **(5a,** 0.01 mol) in Glacial acetic acid (30 mL), followed by the Hydrazine hydrate (0.01 mol) was added and the mixture was reflux for4 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table-1.

Yield: 69%; mp 234-236 ºC; IR (cm-1):3472(O-H stretching of Hydroxy group) ,3354(N-H stretching of primary amine), 3074(C-H stretching of aromatic ring), 2901(CH_3 Str.), 1733(C=O stretching of keto group), 1671 (C=O stretching of ring), 1608(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 875(C-H in plane bending for aromatic ring); 688(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.84 (s, 3H, CH_3), 10.95 (s, 1H, OH),9.19(s,1H,NH),4.50(s,1H,NH)6.21-6.91(t,1H,CH),7.19-7.29(d,1H,CH),7.40-8.27(m,Ar-H);MS:*m/z*491,473,463,411,397,331,178,160,135,120,103; Anal.Calcd. for C18H15Br2N5O2: C, 43.84; H, 3.07; N, 14.20. Found: C, 43.88; H, 3.10; N, 14.22%.

Synthesis of 6, 8-dibromo-3-{[5-(phenyl)-4, 5-dihydro-1H-pyrazol-3-yl] amino} quinazolin-4(3H)-one (6b)

A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(phenyl) prop-2-enamide **(5a,** 0.01 mol) in Glacial acetic acid (30 mL),followed by the Hydrazine hydrate (0.01 mol) was added and the mixture was reflux for4 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table-1.

Yield: 78%; mp 230-231 ºC; IR (cm-1):3322(N-H stretching of primary amine), 3079(C-H stretching of aromatic ring), 2932(CH_3 Str.), 1727(C=O stretching of ***keto group***),1672 (C=O stretching of ring), 1616(C=N stretching of pyridine ring), 1485,1460 (C=C stretching of aromatic ring), 924(C-H in plane bending for aromatic ring); 650(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.84 (s, 3H, CH_3), 9.00(s,1H,NH),6.20-6.22(d,1H,CH),7.16-7.25(d,1H,CH),7.42-8.07(m,Ar-H); MS: *m/z* 475,447,395,371,331,316,161,159,144,119,104; Anal.Calcd. for C18H15Br2N5O: C, 45.31; H, 3.17; N, 14.68. Found: C, 45.37; H, 3.20; N, 14.71%.

Synthesis of 6,8-dibromo-3-({5-(2-hydroxyphenyl)-1-[(4-nitro Phenylamino)methyl]-4,5-dihydro-1*H*-pyrazol-3-yl}amino)quinazolin-4(3*H*)-one($7a_1$)

To a mixture of 6,8-dibromo-3-{[5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one **(6a)** (0.005 mol) ,

37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-nitro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table-1.

Yield: 81%; mp 170-172 ºC; IR (cm-1): 3476(O-H stretching of Hydroxy group) ,3350(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2918(CH_3 Str.), 1732(C=O stretching of keto group), 1670 (C=O stretching ring), 1609(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 879(C-H in plane bending for aromatic ring); 685(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.84 (s, 3H, CH_3), 9.72(s,1H,NH),4.47(s,1H,NH)6.57-6.91(t,1H,CH),7.16-7.26(d,1H,CH),7.40-8.11(m,Ar-H); [13]C NMR (400 MHz $CDCl_3$): 19.10, 32.40, 33.29, 36.24, 37.45, 40.32, 41.43, 43.05, 46.54, 48.15, 50.28, 51.44, 70.12, 101.12-101.15, 104.76, 105.25, 129.23, 144.12, 154.55 MS:*m/z* 641,623,613,561,520,327,285,150,121,104; Anal. Calcd. for $C_{25}H_{21}Br_2N_7O_4$: C, 46.48; H, 3.29; N, 15.24. Found: C, 46.46; H, 3.32; N, 15.29%.

Synthesis of 6,8-dibromo-3-({5-(2-hydroxyphenyl)-1-[(4-chloro-Phenylamino)methyl]-4,5-dihydro-1*H*-pyrazol-3-yl}amino)quinazolin-4(3*H*)-one($7a_2$)

To a mixture of 6,8-dibromo-3-{[5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one **(6a)** (0.005 mol) , 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-chloro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table-1.

Yield: 79%; mp 168-169 ºC; IR (cm-1):3475(O-H stretching of Hydroxy group) ,3354(N-H stretching of primary amine), 3078(C-H stretching of aromatic ring), 2815(CH_3 Str.), 1734(C=O stretching of keto group), 1672 (C=O stretching ring), 1617(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 877(C-H in plane bending for aromatic ring); 686(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.88 (s, 3H, CH_3), 9.84(s,1H,NH),5.11(s,1H,NH),3.12-3.16(d,1H,CH),3.80-3.91(t,1H,CH),7.40-8.12(m,Ar-H); [13]C NMR (400 MHz $CDCl_3$) 19.40, 32.40, 33.99, 36.24, 37,78, 39.86, 40.41,41.43,42.50,43.05,46.51,47.55,48.43,50.28,51.84,72.12,101.11 ,101,26,103.20,105.25,123.25,129.23,144.12,156.99 MS:*m/z* 630, 612, 604, 550, 520, 491, 316, 299, 274, 139,127,104; Anal.Calcd. for $C_{25}H_{21}Br_2ClN_6O_2$: C, 47.46; H, 3.35; N, 13.28. Found: C, 47.51; H, 3.38; N, 13.31%.

Synthesis of 6, 8-dibromo-3-({5-(phenyl)-1-[(4-nitro Phenylamino) methyl]-4,5-dihydro-1*H*-pyrazol-3-yl}amino)quinazolin-4(3*H*)-one ($7b_1$)

To a mixture of 6,8-dibromo-3-{[5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one **(6a)** (0.005 mol), 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-nitro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table-1.

Yield: 76%; mp 164-166 ºC; IR (cm-1):3323(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2811(CH_3 Str.), 1724(C=O stretching of ***keto group***),1672 (C=O stretching), 1611(C=N stretching of pyridine ring), 1471(C=C stretching of aromatic ring), 917(C-H in plane bending for aromatic ring); 687(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.89 (s, 3H, CH_3), 9.81(s,1H,NH),5.10(s,1H,NH)3.86-3.96(t,1H,CH),3.11-3.16(d,1H,CH),7.41-8.12(m,Ar-H);[13]CNMR (400MHz, $CDCl_3$) : 18.18, 32.40, 33.99, 36.04,37.05, 39.66,40.41,41.05, 42.12, 46.51, 47.51, 48.72, 49.51, 72.03, 100.01-100.07, 103.70, 104.76, 105.75, 123.95, 129.23, 144.02, 145.02. MS: *m/z* 625, 608, 597, 545, 331, 309, 294, 150, 138, 121, 104; Anal.Calcd. For $C_{25}H_{21}Br_2N_7O_3$: C, 47.87; H, 3.37; N, 15.63. Found: C, 47.84; H, 3.41; N, 15.83%.

Synthesis of 6,8-dibromo-3-({5-(phenyl)-1-[(4-chloro-Phenylamino)methyl]-4,5-dihydro-1*H*-pyrazol-3-yl}amino)quinazolin-4(3*H*)-one ($7b_2$)

To a mixture of 6,8-dibromo-3-{[5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one ($6b_1$) (0.005 mol) , 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-chloro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table-1.

Yield: 78%; mp 162-163 ºC; IR (cm-1): 3364(N-H stretching of primary amine), 3028(C-H stretching of aromatic ring), 2928(CH_3 Str.), 1665(C=O stretching of ***keto group***), 1586(C=N stretching of pyridine ring), 1466,1451 (C=C stretching of aromatic ring), 909(C-H in plane bending for aromatic ring); 751(C-Cl stretching of aromatic ring), 654(C-Br stretching of aromatic ring); 1H NMR ($CDCl_3$) δ ppm: 1.91 (s, 3H, CH_3), 9.89(s,1H,NH),5.09(s,1H,NH)3.81-3.90(t,1H,CH),3.10-3.14(d,1H,CH),7.43-8.19(m,Ar-H); [13]C NMR (400 MHz, $CDCl_3$): 18.44, 32.40, 33.99, 36.04, 37.78, 39.66, 40.41, 41.03, 42.12, 46.05, 47.55, 48.43, 49.04, 49.47, 74.44, 100.00-100.17, 101.11, 103.07, 104.76, 105.25, 129.13, 144.12. MS: *m/z* 614,586,534,283,139,127,104; Anal. Calcd. for $C_{25}H_{21}Br_2ClN_6O$: C, 48.69; H, 3.43; N, 13.63. Found:C, 48.37; H, 3.49; N, 13.59%.

Antibacterial activity

In the present investigation the antibacterial activity of all the synthesized compounds was carried out by cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculum. The test compounds were introduced into the discs and the diameter of the zone of inhibition was measured.

All the test compounds were evaluated for their antibacterial activity against. *Staphylococcus aureus, Bacillus subtilis, (gram-positive), Escherichia coli,, Pseudomonas aeruginosa (gram negative)* following agar diffusion method of assay[21].

Solution of the test compounds were prepared by dissolving 10mg each in dimethyl formamide (10ml-analytical grade) A reference standard from gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of Ampicilin respectively in sterile distilled water, separately.

The nutrient agar medium was stabilized by autoclaving at 121^0 (15lb/sq.inch). The Petri-plates, tubes and flask plugged with cotton were sterilized in hot-air oven at 160^0, for an hour. In each stabilized Petri-plate (10cm diameter), about 30ml of molten nutrient agar medium inoculated with the respective strain of bacteria (6ml of inoculum to 300ml, of nutrient agar medium) was transferred, aseptically. The plates were left at room temperature to allow the solidification. In each plate, three discs of 6mm diameter were made with a sterile borer. Accurately 0.1ml (1000μg/ml conc.) of the test solution was added to the cups, aseptically and labelled accordingly. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into nutrient agar medium. After incubation of the plates at 37^0C for 24 hours, the diameter of zone of inhibition surrounding each of the discs was measured with the help of an antibiotic zone reader". All the experiments were carried out in triplicate simultaneously; controls were maintained employing 0.1ml of dimethyl formamide to observe the solvent effects.

General Procedures

SCHEME

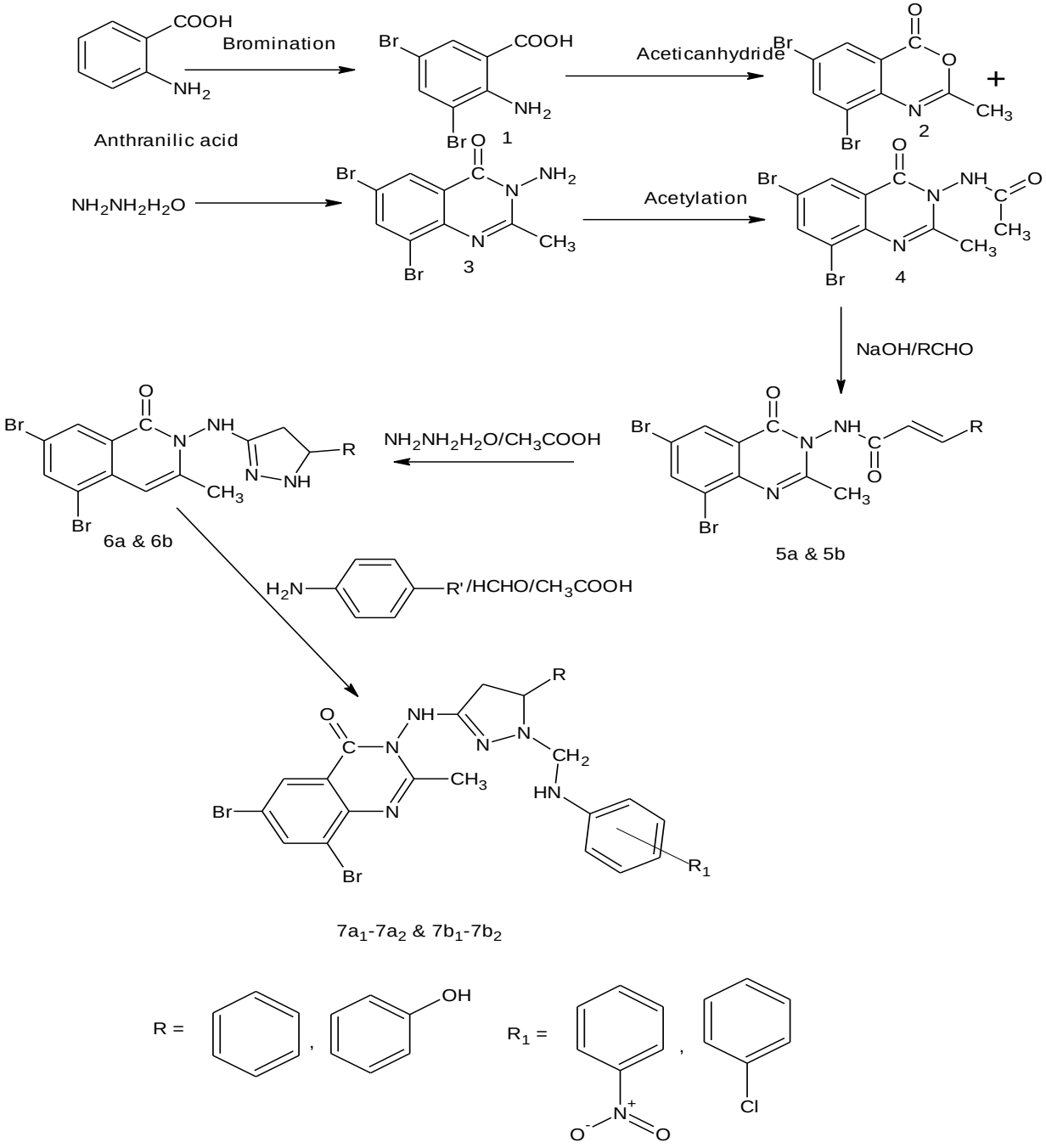

Table 1: Physical data of synthesized compounds

Comp. No.	Mol. Formula	Colour	MP (ºC)	Yield (%)
1	$C_7H_5B_rNO_2$	Reddish brown	202-204	73
2	$C_9H_5Br_2NO_2$	Orange	179-181	71
3	$C_9H_7Br_2N_3O$	Dirty Yellow	138-140	69
4	$C_9H_9Br_2N_3O_2$	Pale Yellow	126-128	74
5a	$C_{17}H_{11}Br_2N_3O_3$	Grey	152-154	70
5b	$C_{17}H_{11}Br_2N_3O_2$	Pale Yellow	151-153	73
6a	$C_{17}H_{13}Br_2N_5O_2$	Brick red	234-236	69
6b	$C_{17}H_{13}Br_2N_5O$	Yellowish brown	230-231	78
7a1	$C_{24}H_{19}Br_2N_7O_4$	Dirty Yellow	170-172	81
7a2	$C_{24}H_{19}Br_2N_6O_2Cl$	Brick red	168-169	79
7b1	$C_{24}H_{19}Br_2N_7O_3$	Reddish Yellow	164-166	76
7b2	$C_{24}H_{19}Br_2N_6OCl$	Brownish yellow	162-163	78

RESULT

All the newly synthesized compounds (**$7a_1$,$7a_2$,$7b_1$ & $7b_2$**)-series were screened for their antibacterial activity in-vitro at the doses of 100 µg in 0.1ml of DMF against *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Escherichia coli (E. Coli), Pseudomonas aeruginosa (P. aeruginosa)*.The results cited in Table 2 represents that many compounds registered mild to moderate to strong antibacterial activity against the tested microorganisms while some compounds does not showed any activity.

The Compounds **$7a_1$** and **$7b_1$** showed the marked zone of inhibition between **24 to 27mm** against S. aureus while standard drug Ampicillin registered **27mm**. Compounds **$7a_2$** & **$7b_2$** shows **14-16 mm** respectively towards S. aureus .Similarly, compound nos. **$7a_1$** and **$7b_1$** registered zone of inhibition between **21 to 23mm** against B. subtilis while the standard drug exhibited **24mm**. compounds **$7a_2$** & **$7b_2$** shows **12-17 mm** respectively towards B. subtilis .The most promising compounds among all the synthesized compounds showing a maximum zone of inhibition are **$7a_1$ (27mm)** and **$7b_1$ (24mm)** against *S. aureus* and for *B. subtilis*, the compounds are **$7a_1$ (23mm)** and **$7b_1$ (21mm).**

In case of Gram-negative bacteria, compound nos. **$7a_1$** and **$7b_1$** possess zone of inhibition between **25 to 28**mm while the standard drug Ampicillin registered **22mm** against E. coli. Compounds **$7a_2$** & **$7b_2$** shows **18-19 mm** respectively towards E. coli. Similarly, compound nos. **$7a_1$** and **$7b_1$** showed zone of inhibition between **26 to 31mm**, at the same time the standard drug resulted zone of inhibition of **20mm** against P.aeruginosa. Compounds **$7a_2$** & **$7b_2$** shows **14-18 mm** respectively towards P.aeruginosa. The most promising compounds against gram-negative bacteria are **$7a_1$** and **$7b_1$** against E. coli and compounds **$7a_1$** and **$7b_1$** against P. aeruginosa.

In the anti-bacterial study, compounds **$7a_1$** and **$7b_1$** has significant anti-bacterial against both tested gram positive and gram-negative bacteria while other promising compounds mentioned earlier showed significant narrow spectrum activity. The different spectrum of activity by the test compounds against the tested microorganisms may be due to different substituents present in the substituted pyrazole nucleus. The experiment was performed in triplicate in order to minimize the errors and results are presented in Table 2.

In the study of antibacterial activity, the synthesized had shown comparatively better activity against tested bacteria. The difference spectrum of activity against bacteria may be due to chemical modification of test compounds which relate to the Structure Activity Relationship (SAR).

Table 2: Antibacterial activity data in Zone of Inhibition

S. No.	Compound	Zone of Inhibition			
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	7a1	24	21	25	26
2	7a2	14	12	18	14
3	7b1	27	23	28	31
4	7b2	16	17	19	18
5	Solvent	-	-	-	-
6	Ampicilin	27	24	22	20

CONCLUSION

In conclusion, four (7a1, 7a2, 7b1, 7b2) new biologically active pyrazol were synthesized for the first time in this study. The structures of novel compounds were determined by FT-IR, 1H NMR and 13C NMR spectroscopic techniques and analytical methods.

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