



Synthesis and Antibacterial Activity of Pyrazolo[1,5-*a*]quinazoline-3-carbonitriles

AREESHA NAZEER¹, NAJMA PERVEEN², MISBAHUL AIN KHAN^{1,2}, MUHAMMAD NAEEM KHAN^{3,*}, MUNAWAR ALI MUNAWAR¹ and WHEI-OH LIN⁴

¹Institute of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

²Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

³Applied Chemistry Research Center, PCSIR Laboratories Complex, Lahore, Pakistan

⁴Departamento de Quimica, Universidade de Grande Rio, Rua Jose Herdy, Duque de Caxias, RJ, Brazil

*Corresponding author: E-mail: changwani_1@yahoo.com

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A method for the synthesis of 4,5,6,7, 8,9- hexahydro-5-arylpyrazolo[1,5-*a*]quinazoline-3-carbonitriles was developed. 5-Aminopyrazole-4-carbonitrile when reacted with aromatic aldehydes gives the schiff's base which on cyclization with cyclohexanone in the presence of glacial acetic acid produces a series of title compounds. The synthesized compounds were characterized through elemental analysis and spectroscopic techniques (FTIR, ¹H NMR and MS). The antibacterial activity of these compounds was also tested against six different bacterial strains by agar plate disc method.

Key Words: Pyrazoloquinazolines, Pyrazoles, Schiff's bases, Cyclizations.

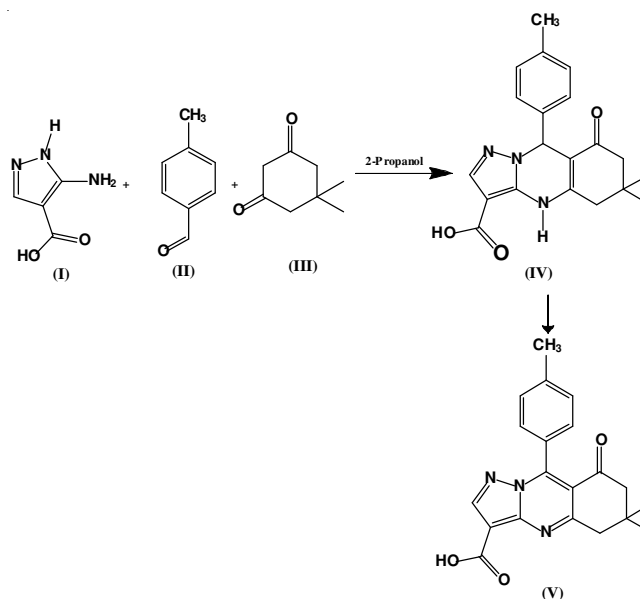
INTRODUCTION

Quinazoline is an important integral part of various physiologically and pharmaceutically important ring systems *e.g.* pyrazolo[5,1-*b*]quinazoline, pyrido[1,2-*a*]quinazoline and chromeno[3',4':4,5]pyrido[1,2-*a*]quinazoline¹. Their derivatives show a variety of biological activities, including antihypertensive^{2,3} antimicrobial^{4,5}, antihyperlipidemic^{6,7}, antiinflammatory^{8,9} and anticonvulsant¹⁰⁻¹⁴. Quinazoline derivatives also possess diverse pharmacological activities^{15,16}.

A large number of quinazoline derivatives were recently synthesized in order to design more effective biologically active molecules. A three component reaction of 3-amino-pyrazole-4-carboxylic acid (**I**), tolualdehyde (**II**) and dimedone (**III**) on refluxing in 2-propanol gives 6,6-dimethyl-8-oxo-9-*p*-tolyl-4,5,6,7,8,9-hexahydropyrazolo[5,1-*b*]quinazoline-3-carboxylic acid (**IV**), which on aromatization gives a tetrahydro product (**V**). A large number of medically important hexahydro- and tetrahydropyrazolo[5,1-*b*]-quinazolines bearing different substituents at C-3 (COOH, COOEt, CN) were synthesized in this way (**Scheme-I**)¹⁷.

Structure activity relationship of a new set of related 5,6-dihydropyrazolo[4,3-*h*]quinazolines (Fig. 1.), having different substituents (COOEt, Cl, Br, CH₃ and COOH) at position-1 was studied. These compounds were tested for their binding activity at the Gly/NMDA, AMPA and KA receptors. These studies showed that a C-1 anionic carboxylate residue on the

pyrazolo[1,5-*c*]quinazoline-2-carboxylate core enhances the receptor binding activity. Among the pyrazoloquinazoline series of compounds, 1,2-dicarboxylic acid derivative showed the highest receptor binding activity¹⁸.



Scheme-I

A three component condensation reaction of aminopyrazoles, diones and arylaldehydes resulted in the formation

of a series of pyrazoloquinazolinones as a result of cascade cyclization¹⁹ while a new method for the synthesis of tetrahydropyrazolo[1,5-*b*]quinazoline involved the cyclocondensation of substituted 5-aminopyrazoles with benzylideneacetone gives dihydropyrazolo[1,5-*a*]pyrimidines which on heating under alkaline catalysis conditions readily undergo cyclocondensation with chalcones in butanol has been reported to give aryl-substituted tetrahydropyrazolo[1,5-*b*]quinazolines²⁰.

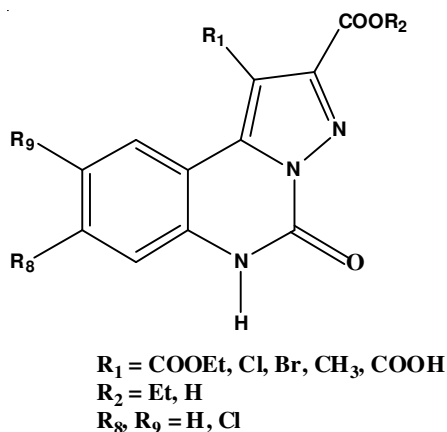


Fig. 1. C-1 substituted pyrazolo[1,5-*c*]quinazoline-2-carboxylate

A simple and facile synthesis of 9-oxo-1,9-dihydro-pyrazolo[5,1-*b*]quinazoline-3-carbonitrile involving refluxing a mixture of methyl 2-[2-cyano-3-(dimethylamino)acrylamido]benzoate and hydrazine hydrate in *n*-butanol for 12 h has been reported¹. Thiazolo[2,3-*b*]quinazolines (synthesized in one pot reaction by treating octahydroquinazoline with chloroacetic acid and aromatic aldehydes) on refluxing with hydrazine in ethanol gives pyrazolo[3',4':4,5]thiazolo[2,3-*b*]quinazolines²¹. A tricyclic ring system -4,5-dihydropyrazolo[4,3-*h*]quinazoline (Fig. 2) is reported to be one of the most promising enzyme inhibitor (MPS1)²². In addition some reviews have mentioned many important derivatives of quinazoline with important medicinal and biological properties^{23,24}.

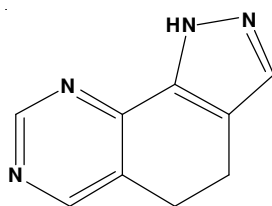


Fig. 2. Structure of 4,5-Dihydro-pyrazolo[4,3-*h*]quinazoline

In view of the foregoing, we would like to report our results of synthesis of some novel derivatives of the title ring system via Schiff's bases from 5-aminopyrazole-4-carbonitrile²⁵ and their reaction with cyclohexanone (**Scheme-II**). The synthesized compounds were screened for their antibacterial properties.

EXPERIMENTAL

All the chemicals used in this synthesis were of analytical grade and were mostly used without further purification. The starting material 5-aminopyrazole-4-carbonitrile was obtained

commercially. Melting points were determined on a Gallenkamp melting point apparatus. Infrared spectrometer Shimadzu-408 was used for measuring the spectra in KBr disks. Proton magnetic resonance spectra were taken on a Bruker 300 and 500 MHz spectrometer. Elemental analyses were determined on a Perkin-Elmer model 240. Mass spectra were recorded on a Finnigan MAT 112.

Synthesis of Schiff's bases (1a-g): These were prepared according to the reported method²⁵:

5-(Benzylideneamino)pyrazole-4-carbonitrile (1a): m.p. 98 °C; yield: 65 %.

5-(*o*-Nitrobenzylideneamino)pyrazole-4-carbonitrile (1b): m.p. 115 °C; yield: 62 %.

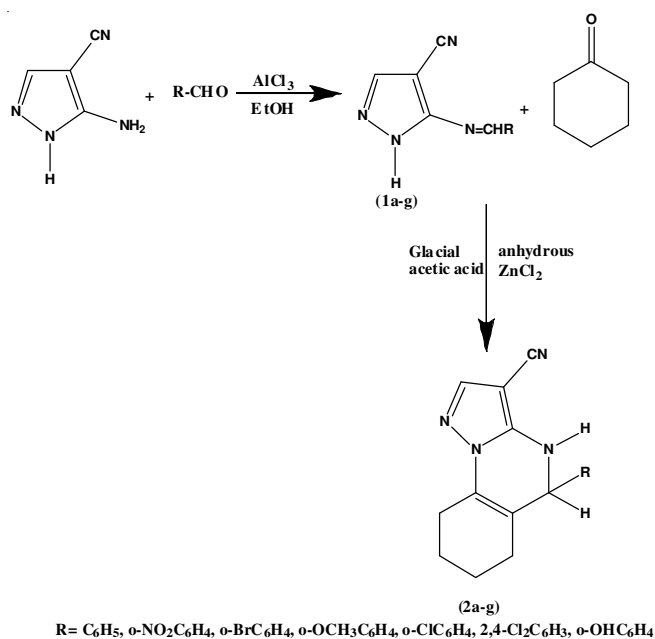
5-(*o*-Methoxybenzylideneamino)pyrazole-4-carbonitrile (1c): m.p. 130 °C; yield: 62%.

5-(*o*-Bromobenzylideneamino)pyrazole-4-carbonitrile (1d): m.p. 205 °C; yield: 68%.

5-(*o*-Chlorobenzylideneamino)pyrazole-4-carbonitrile (1e): m.p. 140 °C; yield: 72 %.

5-(2,4-Dichlorobenzylideneamino)pyrazole-4-carbonitrile (1f): m.p. 154 °C; yield: 75 %.

5-(*o*-Hydroxybenzylideneamino)pyrazole-4-carbonitrile (1g): m.p. 115 °C; yield: 73 %.



Scheme-II

Synthesis of 5-aryl-4,5,6,7,8,9-hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitriles (2a-g)

4,5,6,7,8,9-Hexahydro-5-phenylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2a): A mixture of schiff's base (1a), (1.96 g, 0.01 mol), cyclohexanone (0.98 g, 0.01 mol) and anhydrous zinc chloride (1.0 g) was refluxed in glacial acetic acid for 6 h. On cooling the reaction mixture, with constant stirring, was poured over crushed ice. The solid product which separated out was filtered, washed with excess of water, dried and recrystallized from ethanol to give the desired product. m.p. 160 °C; yield; 70 %; IR (KBr, ν_{max} , cm^{-1}): 3000, 2200 (CN), 1720, 1190, 1040.

TABLE-1
 PHYSICAL DATA FOR COMPOUNDS (2a-g)

Compound	m.p. (°C)	Yield (%)	R _f	IR (KBr) cm ⁻¹
2a	160	70	0.34	3000, 2200 (CN), 1720, 1190, 1040
2b	140	72	0.32	3050, 2200 (CN), 1722, 1520 (NO ₂), 1340, 1140, 1020
2c	212	62	0.36	3000, 2840 (OCH ₃), 2200 (CN), 1590, 1310, 1030
2d	206	65	0.31	3000, 2200 (CN), 1720, 1585, 1310, 1060, 1040
2e	230	63	0.34	3000, 2200 (CN), 1722, 1590, 1310, 1060, 1020
2f	245	68	0.33	3000, 2200 (CN), 1722, 1580, 1310, 1250, 1010
2g	215	65	0.32	3010, 2200 (CN), 1722, 1690 (OH), 1315, 1250, 1020

 TABLE-2
 ELEMENTAL ANALYSIS

Compound	m.f. (m.w.)	Calculated (%)				Found (%)			
		C	H	N	Halogen	C	H	N	Halogen
2a	C ₁₇ H ₁₆ N ₄ (276)	73.91	5.80	20.29		73.88	5.83	20.33	
2b	C ₁₇ H ₁₅ N ₅ O ₂ (321)	63.55	4.67	21.81		63.51	4.64	21.85	
2c	C ₁₈ H ₁₈ N ₄ O (306)	70.59	5.88	18.30		70.62	5.84	18.32	
2d	C ₁₇ H ₁₅ N ₄ Br (355)	57.46	4.23	15.77	22.54 Br	57.43	4.26	15.75	22.54 Br
2e	C ₁₇ H ₁₅ N ₄ Cl (310.5)	65.70	4.83	18.04	11.43 Cl	65.74	4.86	18.01	11.43 Cl
2f	C ₁₇ H ₁₄ N ₄ Cl ₂ (344)	59.13	4.06	16.23	20.58 Cl	59.10	4.02	16.27	20.56 Cl
2g	C ₁₇ H ₁₆ N ₄ O (292)	69.86	5.48	19.18		69.90	5.45	19.21	

 TABLE-3
¹H-NMR DATA

Compound	¹ H-NMR data δ (ppm)
2b	7.15 (s, 1H, Pz-2H), 8.80-7.95 (m, 4H, Ar-H), 1.20-3.20 (m, 8H, 4×CH ₂), 10.50 (s, 1H, NH), 5.50 (s, 1H, Qz-H)
2c	7.10 (s, 1H, Pz-2H), 9.25-7.90 (m, 4H, Ar-H), 1.50-3.00 (m, 8H, 4×CH ₂), 10.00 (s, 1H, NH), 3.51 (s, 3H, OCH ₃), 5.55 (s, 1H, Qz-H)
2d	7.05 (s, 1H, Pz-2H), 9.05-8.15 (m, 4H, Ar-H), 1.32-4.30 (m, 8H, 4×CH ₂), 9.90 (s, 1H, NH), 5.55 (s, 1H, Qz-H)
2e	6.90 (s, 1H, Pz-2H), 9.35-7.90 (m, 4H, Ar-H), 1.50-4.15 (m, 8H, 4×CH ₂), 10.05 (s, 1H, NH), 5.50 (s, 1H, Qz-H)
2f	6.87 (s, 1H, Pz-2H), 9.32-7.93 (m, 4H, Ar-H), 1.30-4.28 (m, 8H, 4×CH ₂), 10.10 (s, 1H, NH), 5.35 (s, 1H, Qz-H)

Compounds 2b-2g were prepared from their respective Schiff's bases 1b-1g by this general method: 4,5,6,7,8,9-Hexahydro-5-*o*-nitrophenylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2b): m.p. 140 °C; yield; 72 %; IR (KBr, ν_{max}, cm⁻¹): 3050, 2200 (CN), 1722, 1520 (NO₂), 1340 (NO₂), 1140, 1020.

4,5,6,7,8,9-Hexahydro-5-*o*-methoxyphenylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2c): m.p. 212 °C; yield; 62 %; IR (KBr): 3000, 2840 (OCH₃), 2200 (CN), 1590, 1310, 1030 cm⁻¹.

5-*o*-Bromophenyl-4,5,6,7,8,9-hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2d): m.p. 206 °C; yield; 65 %; IR (KBr, ν_{max}, cm⁻¹): 3000, 2200 (CN), 1720, 1585, 1310, 1060, 1040.

5-*o*-Chlorophenyl-4,5,6,7,8,9-hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2e): m.p. 230 °C; yield; 63%; IR (KBr, ν_{max}, cm⁻¹): 3000, 2200 (CN), 1722, 1590, 1310, 1060, 1020.

5-(2,4-Dichlorophenyl)-4,5,6,7,8,9-hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2f): m.p. 245 °C; yield; 68 %; IR (KBr, ν_{max}, cm⁻¹): 3000, 2200 (CN), 1722, 1580, 1310, 1250, 1010.

4,5,6,7,8,9-Hexahydro-5-*o*-hydroxyphenylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2g): m.p. 215 °C; yield; 65 %; IR (KBr, ν_{max}, cm⁻¹): 3010, 2200 (CN), 1722, 1690 (OH), 1315, 1250, 1020.

RESULTS AND DISCUSSION

5-Aminopyrazole-4-carbonitrile was used as a starting material for the preparation of 4,5,6,7,8,9-hexahydro-5-

arylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2a-g). The cyano group may, if desired, could help in derivitization of the cyclized products. The presence of cyano group, during cyclization, blocks the C-4 position of the pyrazole thus encouraging the cyclization occurring at NH of the pyrazole ring.

A number of Schiff's bases were earlier obtained by condensing aromatic aldehydes with 5-aminopyrazole-4-carbonitrile in the presence of various catalysts in absolute ethanol²⁵. These schiff's bases on reaction with cyclohexanone in the presence of glacial acetic acid produces the corresponding hexahydropyrazolo[1,5-*a*]quinazolines (Scheme-II).

Although quinazolines can generally be prepared from anthranilic acid and formamide by fusion²⁶, in the present work reaction of a Schiff's base of an aminopyrazole carrying a free ring NH, with cyclohexanone leads to the desired hexahydro product. This, if desired, may be eventually aromatized.

4,5,6,7,8,9-Hexahydro-5-phenylpyrazolo[1,5-*a*]quinazoline-3-carbonitrile (2a) was also isolated from a reaction of 5-aminopyrazole-4-carbonitrile, benzaldehyde and cyclohexanone with anhydrous ZnCl₂ catalyst in glacial acetic acid. When benzaldehyde was replaced by other aromatic aldehydes containing substituents such as *o*-nitro, *o*-bromo, *o*-methoxy, *o*-chloro, 2,4-dichloro and *o*-hydroxy, various corresponding pyrazolo[1,5-*a*]quinazolines (2b-g) were isolated.

The preliminary investigation of compounds was carried out by melting points, TLC and IR data (Table-1). Subsequent characterization of the synthesized compounds was based on satisfactory elemental analyses (Table-2), ¹H NMR (Table-3) and mass spectroscopy.

The infrared spectra of all the Schiff bases (**1a-g**) showed a strong absorption at 1680 cm^{-1} , correspond to $\text{N}=\text{CH}$ confirmed the synthesis of the Schiff's base. The presence of CN and NH of pyrazole was indicated by characteristic absorptions at 2200 and $3350\text{-}3300\text{ cm}^{-1}$ respectively. The infrared spectrum of **1b** showed strong absorptions of NO_2 at 1520 and 1340 cm^{-1} . The absorptions at 2840 and 1700 cm^{-1} correspond to the presence of OCH_3 and OH in **1c** and **1g** respectively.

IR spectra of pyrazolo[1,5-*a*]quinazoline-3-carbonitrile (**2a-g**) showed a sharp absorption peak at 2200 cm^{-1} due to cyano group and the absence of absorption at $1690\text{-}1670\text{ cm}^{-1}$ due to $\text{N}=\text{CH}$ (Schiff's base) indicated the formation of the cyclized products.

The $^1\text{H NMR}$ data (Table-3) of compounds (**2b-g**) showed a singlet at $\delta 7.15$ ppm corresponding to H-2 of pyrazole, the signals of aromatic protons centred at $\delta 7.90\text{-}9.35$ as multiplets. Multiplet signals at $\delta 1.20\text{-}3.20$ ppm appeared due to the presence of 8H ($4 \times \text{CH}_2$). The quinazoline ring proton at C-5 appeared as a singlet between $\delta 5.35\text{-}5.55$ ppm.

$^1\text{H NMR}$ data of compound **2b** showed a singlet at $\delta 7.15$ (1H, s, pyazole H-2), multiplet at $\delta 8.80\text{-}7.95$ (4H, m, Ar-H), singlet at $\delta 10.50$ (1H, s, NH). Compound **2c** showed a singlet at $\delta 3.51$ (3H, s, OCH_3), singlet at $\delta 7.10$ (1H, s, pyazole H-2), multiplet at $\delta 9.25\text{-}7.90$ (4H, m, Ar-H). $^1\text{H NMR}$ spectra of **2g** showed a singlet at $\delta 5.45$ (1H, s, OH), 7.15 (1H, s, pyazole H-4) and a multiplet centred at $\delta 7.34$ was assigned to aromatic protons.

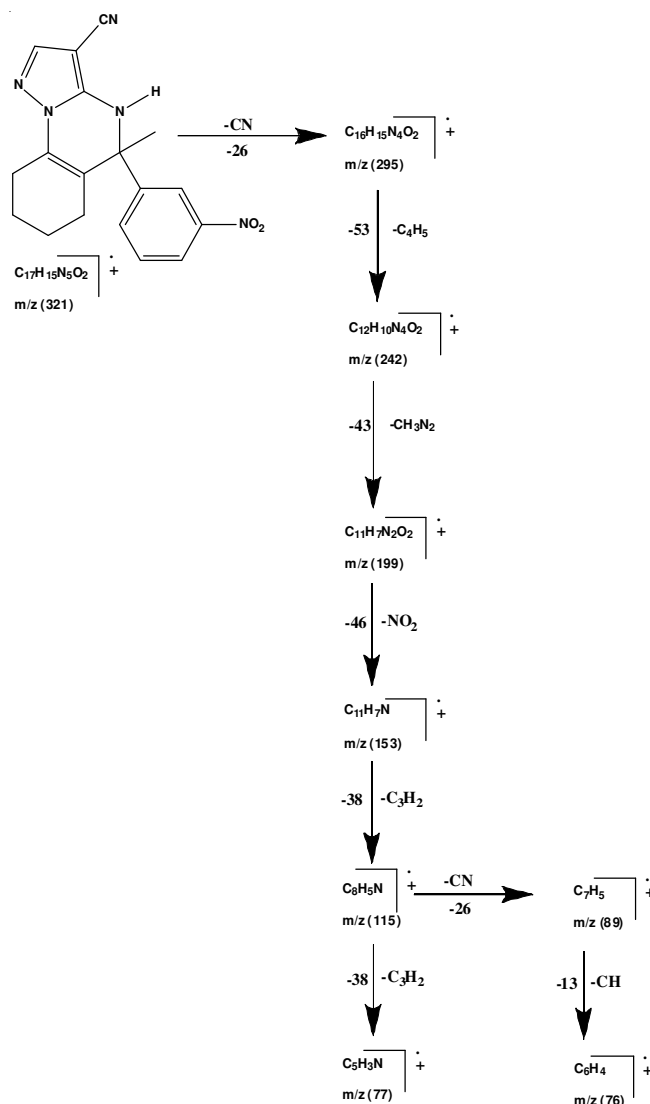
Satisfactory elemental analyses (Table-2) and mass spectral data correspond to a particular molecular ion peak of all the cyclized products confirmed the formation of 5-aryl 4,5, 6,7,8,9-hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitriles (**2a-g**). The mass spectra of **2b** showed molecular ion peak at m/z 321.3 as expected, agreed with calculated ($\text{C}_{17}\text{H}_{15}\text{N}_5\text{O}_2 = 321$). Compound **2g** showed molecular ion peak at m/z 292.1 agreed with calculated ($\text{C}_{17}\text{H}_{16}\text{N}_4 = 292$). The results of the IR and mass spectra strongly suggest that in the cyclization step no oxidation is indicated and the structures are **2a-2g** as represented in the Scheme-II. Also the appearance of a one proton signal around $\delta 5.00$ ppm for a quinazoline proton strongly supports this assertion.

The low resolution electron impact mass spectrum of **2b** showed molecular ion peak at (m/z 321). A peak has been observed at (m/z 242) which is obtained by the removal of CN (m/z 26) and C_4H_5 fragment (m/z 53). The base peak is obtained by removal of CH_3N_2 (m/z 43) at m/z 199. Which further fragments by successively losing NO_2 (m/z 46), (C_3H_2 m/z 38), CN (m/z 260) and CH (m/z 13) till a fragment which corresponds to C_6H_4 (m/z 76).

A tentative partial mass spectral fragmentation pattern for the compound **2b** is given in the Scheme-III.

Antibacterial activity: Antibacterial activity of hexahydropyrazolo[1,5-*a*]quinazoline-3-carbonitriles (**2a-g**) was tested by Agar plate disc diffusion method²⁷ against six different bacterial strains; *Staphylococcus aureus* (gram +ve), *Staphylococcus cocci* (gram +ve), *Streptococcus viridens* (gram +ve), *Pseudomonas* (gram -ve), *Escherichia coli* (gram -ve) and *Klebsiella* (gram -ve). The filter paper disc (6.00 mm in diameter) was soaked with the solution of 50 mg of compound in

1 mL of chloroform was placed in the centre of plate. The plates were incubated with the growing cultures of bacterial strains.



Scheme-III: Mass fragmentation pattern of **2b**

The six different types of bacterial cultures were inoculated and were further incubated at 37°C for 24-48 h. Vibramycin and cefizox were used as standard or positive control while chloroform and ceclor were used as negative controls.

The test organism *S. aureus*, *S. cocci* and *E. coli* showed an inhibition zone of (30 mm) for vibramycin while *Pseudomonas* and *S. viridens* showed resistance. *Klebsiella* showed an inhibition zone of (10 mm) for vibramycin and (20 mm) for cefizox, while in case of cefizox no antibacterial activity was observed in *S. aureus* and *E. coli* but *S. cocci*, *S. viridens* and *Klebsiella* showed an inhibition zone of 15 mm, 20 mm and 22 mm respectively.

In case of antibacterial activity of synthesized compounds (**2a-g**) results were not very significant against these bacterial strains.

Conclusion

The method employed for the synthesis is an efficient one for the construction of pyrazolo[1,5-*a*]quinazoline system. In

this case free NH of pyrazole ring was situated nearest to the amino group, cyclization preferentially occurring on this nitrogen. Since in the case of 5-aminopyrazole-4-carbonitrile, a cyano group has blocked the four position, the cyclization exclusively takes place on the ring nitrogen giving a bridge-head nitrogen ring system. A Schiff's base being the reactive intermediate, its pre-formation gave satisfactory results. Zinc chloride and acetic acid both act as acid catalyst for cyclization reaction. The present synthesis unambiguously provides the 5-aryl products.

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