International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.6, pp 782-788, 2015

Synthesis and Antibacterial Screening of 3-(4,5-Dihydro-5-Aryl-1*H*-Pyrazol-3-YL)-4-Hydroxyquinolin-2(1*H*)-Ones

S. Sarveswari* and V. Vijayakumar

Centre for Organic and Medicinal Chemistry, VIT University, Vellore-632014, India

Abstract: A series of 3-(4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-ones were synthesized by an efficient microwave assisted synthesis and the product were screened for their antibacterial activity towards *Pseudomonas aeruginosa (*ATCC-27853), *Proteus mirabilis* ATCC-19181, *Staphylococcus aureus* (ATCC-700699), *Klebsiella pneumonia (*ATCC-10273), *Salmonella typhi (*ATCC-700931), *Entrococci* Clinical sample. Compound 3b and 3d found to show good activity against *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia.*
Key words: Synthesis, Pyrazoiline derivatives, Antibacterial screening.

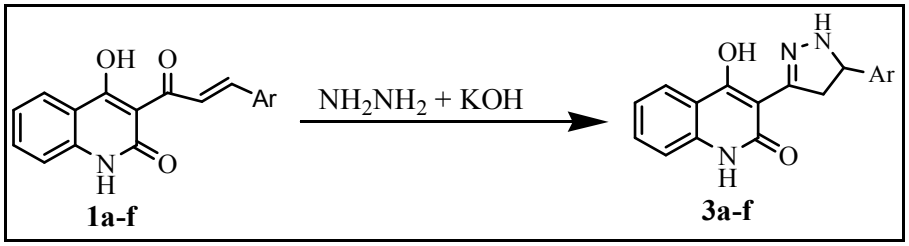
Introduction

Pyrazole belongs to the family of azoles. The dihydro pyrazoles are called pyrazolines. The interest of scientists in this class of compounds has been stimulated by their biological, pharmacological and industrial importance. Pyrazoline derivatives have been found to possess a broad spectrum of biological activities such as tranquillizing, muscle relaxant, psycho analeptic, anticonvulsant, antihypertensive, antidepressant[1], antibacterial[2], antifungal, antiviral, antiparasitic, antitubercular, insecticidal, antiinflammatory, antidiabetic, anaesthetic and analgesic properties[3]. Among various pyrazoline derivatives, 2-pyrazolines showed an interesting *in vitro* antimycobacterial activity. 1-unsubstituted-3,5-diaryl-2-pyrazolines were reported to exhibit inhibition of human acyl CoA cholesterol acyl transferase as well as low-density lipoprotein oxidation,[4] whereas 1,3,5-triaryl-2-pyrazolines were reported to possess antidepressant properties in addition to monoamine oxidase inhibitory activities[5-8] Recent progressive interest was directed towards nano-crystal investigation of 1,3,5-triaryl-2-pyrazoline derivatives[9]. Our initial attempt of conversion of chalcones (**1a-f**) into pyrazolines using domestic microwave oven was failed. It may be due to the loss of hydrazine from the reaction mixture upon irradiation, since the reaction was carried out in an open vessel. But the target was achieved at that time by conventional heating[10] in continuation of our earlier interest on the synthesis[10,11] of pyrazolines, herein we report a microwave assisted synthesis of 3-(4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-ones using scientific microwave oven and the spectral characterization of synthesized compounds. The comparative yield and time required for the reaction are given in experimental section.

Experimental

Materials and methods

All the reagents used for the synthesis were purchased from Aldrich and used without further purification. Progress of the reactions were monitored by thin layer chromatography (TLC) using a mixture of 8:2 n-hexane and ethyl acetate as an eluent. The NMR spectra were recorded on Bruker 400 MHz instrument. Microwave assisted reaction were carried out in CATAR (Scientific Microwave oven). The melting points were determined by using a Metler Toledo melting point apparatus by the open capillary tube method and uncorrected.



Scheme 1 Synthesis of 3-(4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-ones (3a-f).

S. No	Ar	S. No	Aryl
1a	Phenyl	**1d**	4-methoxyphenyl
1b	4-chlorophenyl	**1e**	3,4-dimethoxyphneyl
1c	2,4-dichlorophenyl	**1f**	3-nitrophenyl

General procedure for the synthesis of 3-(4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-ones (3a-f).

A mixture of chalcones (**1a-f)** (0.1g 3mmol), hydrazine hydrate (4ml, 30mmol) and KOH (1mg) in ethanol was irradiated for about for 15 min. at power level 320 W. Lower power levels and lesser duration led to incompletion of reaction, higher power level and longer reaction duration resulted charring of reaction mixture. The reaction mixture was poured onto ice and acidified with dil. HCl. The resultant solid was filtered, dried, and purified by column chromatography using 1:1 chloroform and methanol. The product formation was confirmed by the spectral data. The reaction was repeated with **1b-f** to check the reproducibility. All the newly synthesized compounds 3-(4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-ones **(3a-f)** have been characterized by IR, ^{1}H NMR and mass spectral data. The compound **3f** has been taken as representative and its IR, ^{1}H NMR chemical assignments are discussed. Compounds **1a-f** were synthesized using the procedure available in literature[10].

Spectral Data

3-(4,5-dihydro-5-phenyl-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3a): Yield: 78% (by conventional method), 62% (by Microwave method); Melting Point: 240-41°C; IR (KBr) cm^{-1}: 3422 (NH), 1670 (C=O), 1607 (C=N); Compound is insoluble in normal NMR solvents. ES-MS: *m/z* 306.1[M+1]$^+$.

3-(4,5-dihydro-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3b): Yield: 52% (by conventional method), 54% (by Microwave method); Melting Point: 230-32° C; IR (KBr) cm^{-1}: 3423 (NH), 1656 (C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.05-3.09 (m, 2H), 3.19-3.25 (m, 1H), 8.01 (d, 1H, *J* = 7.8 Hz), 7.17-7.28 (m, 3H), 7.48 (t, 1H, *J* = 7.8 Hz), 7.40 (d, 1H, *J* = 8.0 Hz), 7.32 (d, 1H, 8.2); 11.24 (bs, NH), 13.75 (s, OH).

3-(4,5-dihydro-5-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3c): Yield: 65% (by conventional method), 56% (by Microwave method); Melting Point: 280-81 °C; IR (KBr) cm^{-1}: 3434 (NH), 1659 (C=O), 1607 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.12 (dd, 2H, *J* = 19.2 Hz, 2.0 Hz), 3.7 (m, 2H) 5.62 (dd, *J* = 4.6 Hz, 12.0 Hz) , 7.90-8.00 (m, 1H), 7.18-7.67 (m, 6H) 11.24 (bs, NH), 13.74 (s, OH); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 158.90, 150.05, 143.5, 137.86, 133.89, 131.81, 131.29, 129.55, 129.13, 128.47, 127.95, 127.22, 122.02, 121.71, 115.96, 31.29, 26.90; ES-MS: *m/z* 378.1[M+4]$^+$.

3-(4,5-dihydro-5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3d): Yield: 76% (by conventional method), 61% (by Microwave method); Melting Point: 254-55 °C; IR (KBr) cm^{-1}: 3358 (NH), 1624 (C=O), 1590 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.64 (dd, 1H, *J* = 19.5 Hz, 4.2 Hz), 4.11 (dd, 1H, *J* = 19.4 Hz, 11.7 Hz), 5.62 (dd, 1H, *J* = 4.2 Hz, 11.7 Hz), 8.11 (d, 1H, *J* = 7.7 Hz), 7.27-7.34 (m, 3H), 7.61 (t, 1H, *J* = 7.2), 7.50 (s, 1H), 7.17 (d, 1H, *J* = 8.4 Hz), 6.80 (d, 2H, *J* = 8.5 Hz), 3.30 s (OCH_3), 11.24 (bs, -NH), 13.70 (s, -OH).

3-(4,5-dihydro-5-(3,4-dimethoxyphenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3e): Yield: 59% (by conventional method), 62% (by Microwave method); Melting Point: 330-32 °C; IR (KBr) cm^{-1}: 3426 (NH), 1647 (C=O), 1602 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.80-3.95 (m, 2H), 4.72 (dt, 1H, *J* = 4.3 Hz,

10.4 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.24 (d, 1H, *J* = 8.0 Hz), 7.16 (t, 1H, *J* = 7.4 Hz), 7.51 (t, 1H, *J* = 7.5 Hz), 7.58 (bs, 1H), 7.03 (s, 2H), 6.92 (m, 2H)**,** 3.76 s (-OCH_3), 3.77 s (-OCH_3), 11.30 (bs, -NH), 14.30 (bs, -OH).

3-(4,5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3f): Yield: 61% (by conventional method), 48% (by Microwave method); Melting Point: 292-293 °C; IR (KBr) cm^{-1}: 3425 (NH), 1659 (C=O), 1607 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.04 (t, 2H, *J* = 12.4 Hz), 4.99 (t, 1H, *J* = 12.4 Hz), 8.27 (s, 1H), 8.14 (d, 1H, *J* = 7.6 Hz), 7.91-7.88 (m, 3H), 7.67 (t, 1H, *J* = 7.9 Hz), 7.51 (t, 1H, *J* = 7.3 Hz); 7.24 (d, 1H, *J* = 8.1 Hz), 7.16 (t, 1H, *J* = 7.1 Hz), 11.36 (bs, NH), 14.14 (bs, OH). ES-MS: *m/z* 351$[M+1]^+$.

Anti Bacterial Screening of 4-Hydroxy-3-(5-Phenyl-4,5-Dihydro-1*H*-Pyrazol-3-YL)-Quinolin-2(*1H*)-Ones (3a-f):

All the synthesized 4-hydroxy-3-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-quinolin-2(*1H*)-ones **(3a-f)** were screened for their antibacterial activity against *Pseudomonas aeruginosa (*ATCC-27853), *Proteus mirabilis* ATCC-19181, *Staphylococcus aureus* (ATCC-700699), *Klebsiella pneumonia (*ATCC-10273)*, Salmonella typhi (*ATCC-700931), *Entrococci* Clinical sample. Minimum inhibitory concentration (MIC) values were found using broth dilution method (Table-1), optical density values measured for various concentrations, the concentration at which the optical density becomes zero correspond to the MIC of that particular compound.

Table-1 MIC values of 4-hydroxy-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-quinolin-2(*1H*)-ones (3a-f)

S.No	MIC values µg and Name of the organism					
	P. aeruginosa	**S. typhi**	**K .pneumonia**	**P. mirabilis**	**S. aureus**	**Enterococci**
3a	400	300	400	500	500	200
3b	200	200	300	200	600	300
3c	300	300	200	200	400	600
3d	200	300	300	400	600	300
3e	300	200	200	300	400	300
3f	300	300	400	500	300	600

Antibacterial Screening (Well Diffusion Method)

Table 2 Zone of inhibition values of 4-hydroxy-3-(5-aryl-4,5-dihydro-1*H*- pyrazol-3-yl)-quinolin-2(*1H*)-ones (3a-f)

S. No	*Name of the organisms*											
	Pseudomonas aeruginosa ATCC-27853				***Salmonella typhi ATCC-70099***				**Klebsiella pneumonia ATCC-10273**			
	100 µg/ mL	**200 µg/ mL**	**300 µg/ mL**	**Std***	**100 µg/ mL**	**200 µg/ mL**	**300 µg/ mL**	**Std***	**100 µg/ mL**	**200 µg/ mL**	**300 µg/ mL**	***Std****
3a	5	6	7	30	6	8	10	34	-	6	8	*36*
3b	5	7	10	30	7	8	10	34	9	13	13	*36*
3c	6	9	11	30	5	6	7	34	11	13	14	*36*
3d	8	10	13	30	5	7	9	34	7	8	10	*36*
3e	7	9	12	30	9	11	14	34	7	8	8	*36*
3f	*7*	*8*	*12*	*30*	*7*	*9*	*14*	*34*	*7*	*9*	*10*	*36*

A suspension of *S. aureus* was added to the sterile *Muller Hinton* agar medium at 45 °C in aseptic environment. The mixture was transferred to sterile Petri dishes and allowed to solidify. Wells of 6mm in diameter were made using well-borer, Specified concentration of synthesized compounds and the standard were applied on the surface of agar plates. DMSO was used as control. All the plates were left for 1h-4h as a period of pre incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 37 ±1°C for 18 -24 h and were observed for antibacterial activity. The diameter of the zone of inhibition was measured for the plates in which the zone of inhibition was observed, the process was triplicated and average zone of inhibitions were calculated. The results of

antimicrobial screening of 4-hydroxy-3-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-quinolin-2(*1H*)-ones **(3a-f)** are given in Table-2 and Table-3.

Table 3 Zone of inhibition values of 4-hydroxy-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-quinolin-2(*1H*)-ones (3a-f)

S. No	*Name of the organisms*											
	Proteus mirabilis ATCC-19181				*Staphylococcus aureus* ATCC-700699				Entrococci *Clinical sample*			
	100 µg/ mL	200 µg/ mL	300 µg/ mL	Std*	100 µg/ mL	200 µg/ mL	300 µg/ mL	Std*	100 µg/ mL	200 µg/ mL	300 µg/ mL	*Std**
3a	-	-	8	30	-	-	5	24	6	9	10	*35*
3b	9	10	13	30	-	-	5	24	8	10	14	*35*
3c	5	7	11	30	5	7	9	24	-	-	6	*35*
3d	-	-	7	30	-	-	7	24	6	8	11	*35*
3e	7	8	12	30	-	-	8	24	5	6	7	*35*
3f	-	-	*8*	*30*	*7*	*8*	*12*	*24*	-	-	*7*	*35*

Results and Discussions

The compound **3f** has been taken as representative and its IR, ^{1}H NMR chemical assignments are discussed.

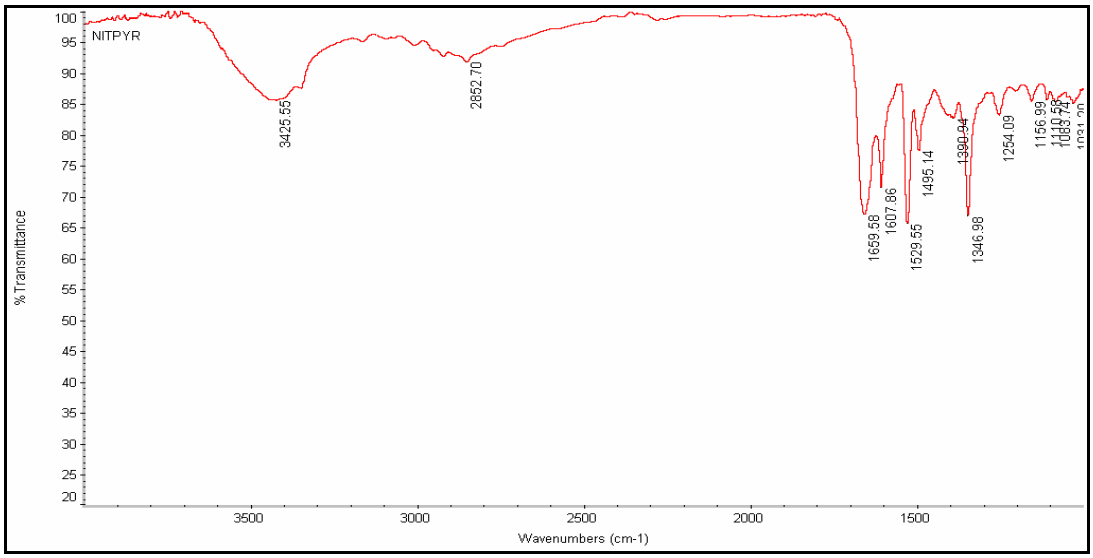


Fig. 1 IR spectrum of 3-(4, 5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxy quinolin-2(1*H*)-one (3f)

IR spectrum of the compound **3f** displays (Fig. 1) the following stretching frequencies 3425 cm^{-1} (-NH); 1659 cm^{-1}(carbonyl group). ^{1}H NMR spectrum (Fig.2.40 and 2.41) of **3f** displays the following peaks. A singlet at δ 8.27 ppm may be due to H-7 since it is *ortho* to strongly electron withdrawing nitro group and pyrazoline ring so more deshielded so appears at downfield compare to other protons of this molecule and a doublet at δ 8.14 ppm with *J* value of 7.6 Hz is due to H-9. H-9 is also *ortho* to strongly electron withdrawing nitro group so it appears slightly up field compared to the H-7 since it is under the influence of only one electron withdrawing group. A triplet at δ 7.51 ppm with coupling constant of 7.51 Hz integrating for one proton may be due to H-7′ proton of quinoline ring. The signal due to H-5′ appear as multiplet in a range of δ 7.91-7.88 which integrates for three protons and hence assigned to H-5′, H-7, NH of pyrazoline ring. A triplet at δ 7.67 ppm [*J* = 7.9 Hz] integrating for one proton is due to H-10. A triplet at δ 7.16 ppm [*J* = 7.1 Hz] integrating for one proton may be due to H-6′. Two triplets in the aliphatic region of spectrum at δ 4.99 ppm [*J*=12.4 Hz] integrating for one proton and δ 4.04 ppm with [*J* = 12.4 Hz] integrating for two protons are due to protons of pyrazoline ring.

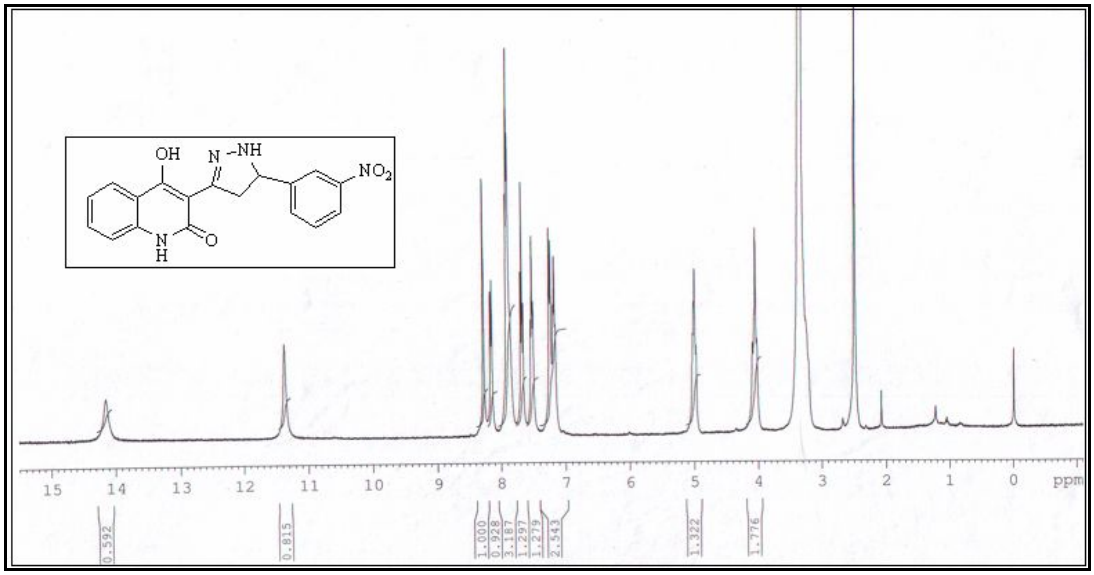


Fig. 2 ^{1}H NMR spectrum of 3-(4, 5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (121f)

Protons of -NH and -OH groups of quinolone ring appears as a singlet at δ 11.36 ppm, δ 14.14 ppm and the extreme down field shift of these signals (than the normal), indicates that they are involved in hydrogen bonding. Presence of amide ring carbonyl stretching 1659 cm^{-1} indicates the retaining of 4-hydroxy group and keto group at 2′ so that it helps to eliminate the possibility of angularly cyclized product.. In the similar way, proton chemical values of other members in the series are assigned and given in the experimental section. The ^{13}C NMR spectrum of **3f** has been given in Fig. 3. The molecular ion peak at 351 in ESI mass spectrum (Fig. 4) confirm the formation of product (**3f**). All these discussion regarding the proton chemical shift assignment are summarized and given in (Fig. 5).

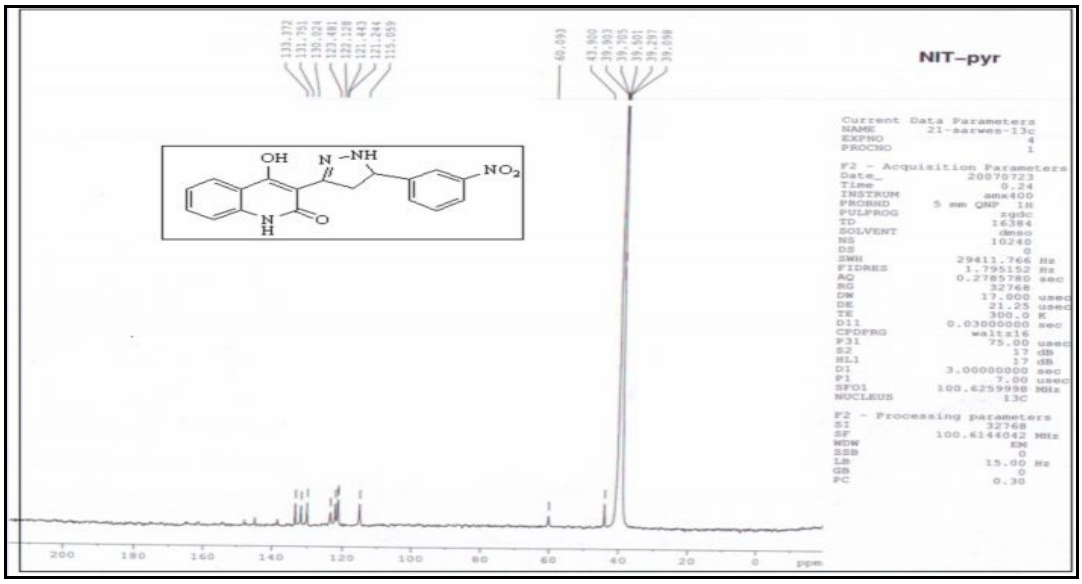


Fig. 3 ^{13}C NMR spectrum of 3-(4, 5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (121f)

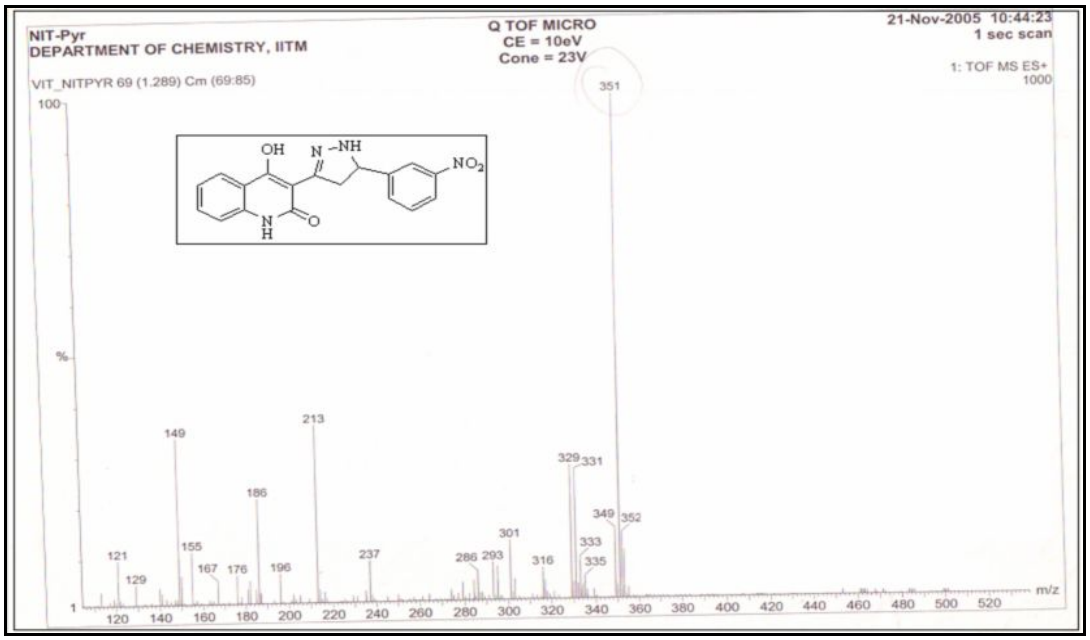


Fig. 4 ESI Mass spectrum of 3-(4,5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3f).

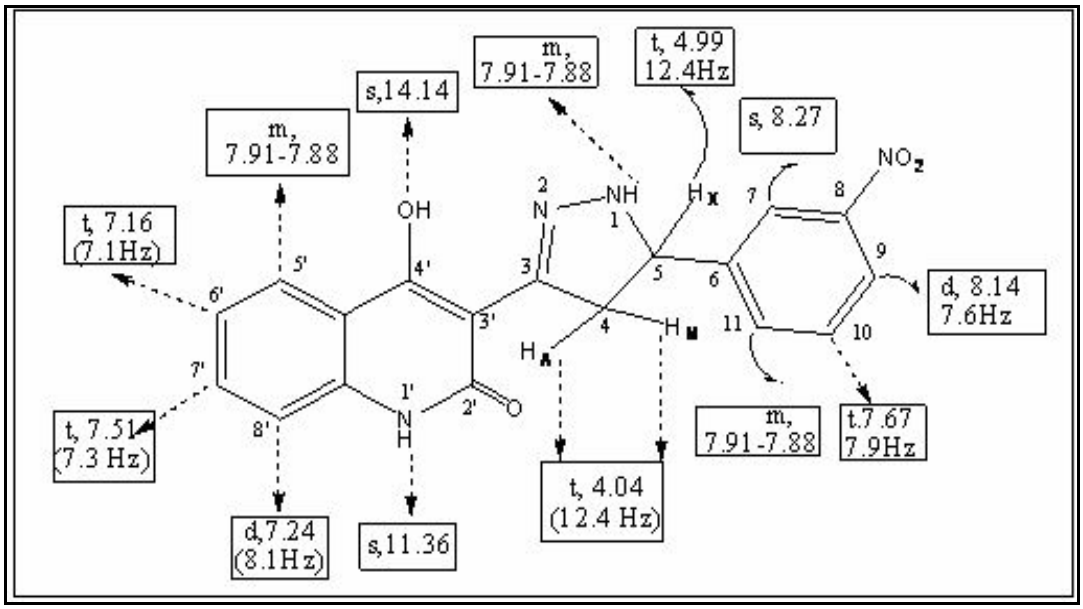


Fig. 5 Summary of proton chemical shift values of 3-(4, 5-dihydro-5-(3-nitrophenyl)-1*H*-pyrazol-3-yl)-4-hydroxyquinolin-2(1*H*)-one (3f)

Conclusion

The pyrazolines **3a-f** were subjected to antibacterial activity screening, the results revealed that these pyrazolines are not very much active against test strains of this work. Some of the compounds like **3a, 3b, 3d** and **3b, 3c** show activity towards *Entrococci* and *Proteus mirabilis* respectively. It has been found that 4-methoxy and 4-chloro (**3b, 3d**) analogues show better activity than the other derivatives of this series against *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia.*

References

1. Li. J.T., Xiao-Hui. Z., Lin. Z.P, An improved synthesis of 1,3,5-triaryl-2-pyrazolines in acetic acid aqueous solution under ultrasound irradiation, Beilstein Journal of Organic Chemistry, 2007, 3, 13-17.

2. Taylor, E.C. Patel, H. H., Synthesis of pyrazolo 3,4-*d* pyrimidine analogues of the potent agent N-4-2-2-amino-4-(3*H)*-oxo-7*H*-pyrrolo-2,3.*d*pyrimidin-5-yl ethylbenzoyl-L-glutamic acid (LY231514), Tetrahedron, 1992, 48, 8089-8100.
3. Bonacorso H.G., Oliveira, M.R. Costa, M.B. da Silva, L.B. Zanatta, N. Martins, M.A.P. Flores, A.F.C. Regiospecific Synthesis of New Non-Condensed Heteropolycyclic Systems from β-Heteroaryl-β-methoxyvinyl Trihalomethyl Ketones, J. Braz. Chem. Soc., 2005, 16, 868-873.
4. Jeong, T.S., K.S. Kim, S.J. Ana, K.H. Choa, S. Leea and W.S. Lee (2004). Novel 3,5-diaryl pyrazolines as human acyl-CoA:cholesterol acyltransferase inhibitors, *Bioorg. Med. Chem. Lett*., Vol. 14, No.11, pp. 2715-2717.
5. Palaska, E., Aytemir, M., Tayfun, I. U. and Erol, D., Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines, Eur. J. Med. Chem., (2001). 36, 539-543.
6. Bilgin, A.A., Palaska, E. Sunal, R., Gumusel. B Some 1,3,5- triphenyl-2-pyrazolines with antidepressant activities. *Pharmazie,* 1994, 49, 67-69.
7. Soni, N., Pande, K., Kalsi,R, Gupta, T.K., Parmar S.S., Barthwal, J.P. Inhibition of rat brain monoamine oxidase and succinic dehydrogenase by anticonvulsant pyrazolines, Res. Commun. Chem. Pathol. Pharmacol, 1987, 56, 129-132.
8. Parmar, S.S., Pandey, B.R. Dwivedi. Harbinson, R.D. Anticonvulsant activity and monoamine oxidase inhibitory properties of 1,3,5-trisubstituted pyrazolines, J. Pharm. Sci., 1974, 63, 1152-1153
9. Barsoum, F.F., Hosni H.M, Girgis, A.S., Novel bis(1-acyl-2-pyrazolines) of potential anti-inflammatory and molluscicidal properties, 2006, Bioorg. Med. Chem, 14, 3929–3937
10. S. Sarveswari S, Raja T.K., A Rapid Microwave assisted synthesis of 3-acetyl-4-Hydroxyquinolin-2(1H)-one and its conversion to quinoline-3-yl isoxazolines and pyrazolines, Indian. J. Heterocyclic chem., 2006, 16, 171-174.
11. Sarveswari. S and Vijayakumar. V., An efficient microwave assisted eco-friendly synthesis of 6-chloro-3-(3-arylacryl oyl)-2-methyl-4-phenylquinolines and their conversion to 6-chloro-3-(1-phenyl-5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-2-methyl-4-phenylquinolines, J. Chin. Chem. Soc., 2012, 59, 66-71.

*****