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Research Article

CONVENTIONAL AND MICROWAVE ASSISTED SYNTHESIS OF NOVEL QUINOLINE DERIVATIVES AND THEIR ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL

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ABSTRACT

Objective: To synthesize novel quinoline derivatives by conventional and microwave methods and to characterize them by using IR, ^{1}H NMR and mass spectra and to screen the molecules for antioxidant and antimicrobial activities.

Methods: Novel series of 5-(quinoline-4-yl)-1,3,4-thiadiazol-2-amine derivatives (AR-6 to AR-10) have been synthesized by cyclisation of aniline, substituted aldehyde and pyruvic acid which yields substituted quinoline carboxylic acid (AR-1 to AR-5) followed by reaction with phosphorous oxy trichloride and thiosemicarbazide.

Results: The synthesized compounds were characterized by IR, ^{1}H NMR and mass spectra and they were screened for their antimicrobial and antioxidant activities. Antimicrobial study of these compounds against Gram+ve and Gram-ve organisms such as Bacillus subtillus, Escherichia coli, Staphylococcus aureus and antifungal activity against Aspergillus. showed good activity Compounds AR-1, AR-3 exhibited broad spectrum antimicrobial activity at a concentration of 5µg/ml when compared to standard ciprofloxacin (1µg/ml). Compounds AR-10 (22.38µg/ml), AR-3 (24.97 µg/ml), AR-8(38.12µg/ml) showed comparable antioxidant activity compared to standard ascorbic acid(12.18µg/ml).

Conclusions: AR-1, AR-3 exhibited broad spectrum antimicrobial activity and AR-10,AR-3,AR-8 showed comparable antioxidant activity. The mechanism by which this molecules exhibit antioxidant activity is still under study.

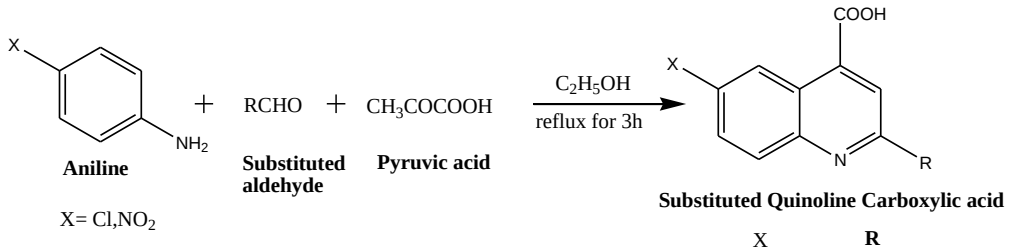
Keywords: Antioxidant, Antimicrobial, Conventional, Microwave, Quinoline.

INTRODUCTION

Quinoline is a heterocyclic aromatic organic compound characterized by benzene fused to pyridine at two adjacent carbon atoms..Heterocyclic compounds containing quinoline nucleus possess wide variety of pharmacological and biological activities. Quinoline nucleus have been found to possess anti-malarial[1], anti-cancer[2], antibacterial[3] activities. The quinoline was introduced for the treatment of urinary tract infections in 1963, the drugs containing quinolone nucleus includes oxolinic acid, norfloxacin, ciprofloxacin etc.[3] Since then this nucleus of quinoline has been explored widely and its derivatives have been found to possess various activities ranging from anti- HIV,[4,5] antimalarial,[6,7,8] anticancer,[9] anticonvulsant,[10,11] antituberculor,[12,13] anti-infective,[14] melanin concentrating hormone antagonists[15] etc. This nucleus still holds a broad potential. In the view of continuation of our research we report Conventional and microwave assisted synthesis of novel quinoline derivatives and their antimicrobial and antioxidant potential.

Scheme

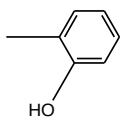
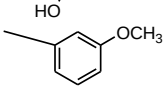
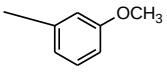
Step-1



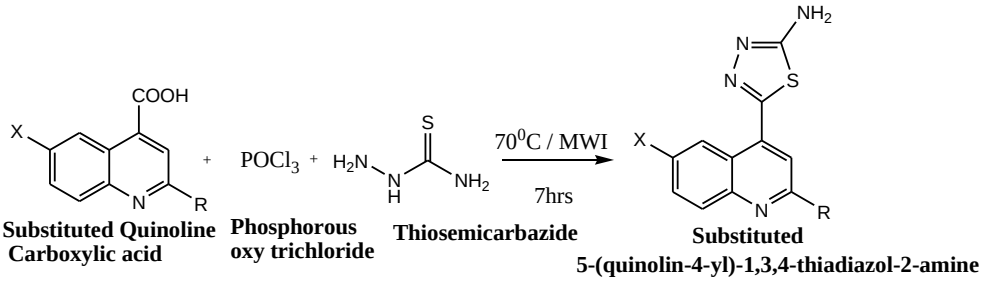
Aniline **Substituted aldehyde** **Pyruvic acid**

X= Cl,NO_2

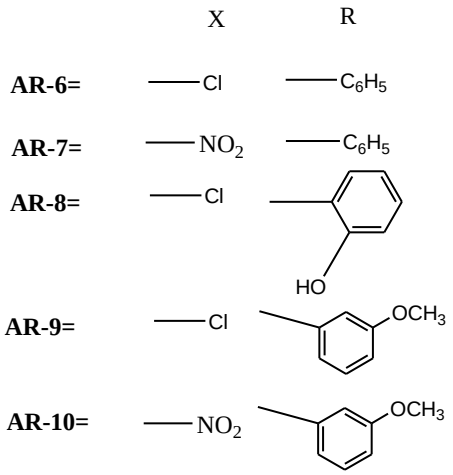
Substituted Quinoline Carboxylic acid

	X	R
AR-1)=	Cl	C_6H_5
(AR-2)=	NO_2	C_6H_5
(AR-3)=	Cl	
(AR-4)=	Cl	
(AR-5)=	NO_2	

Step- 2



Substituted Quinoline Carboxylic acid | **Phosphorous oxy trichloride** | **Thiosemicarbazide** | **Substituted 5-(quinolin-4-yl)-1,3,4-thiadiazol-2-amine**



MATERIALS AND METHODS

Synthesis of Substituted/Unsubstituted 6-chloro-2-phenylquinoline-4-carboxylic acid[16]

Conventional Method

In a 500 ml round bottom flask, equipped with a reflux condenser, 12.5 g (12 ml, 0.118 mol) of purified substituted/unsubstituted benzaldehyde, 11 g (8.66 ml, 0.125 mol) of freshly distilled pyruvic acid and 100 ml of absolute ethanol was placed. The mixture was heated to the boiling point on a water bath and a solution of 15.8 g (0.124 mol) of pure *p*-chloroaniline in 100 ml of absolute ethanol was added slowly to it, with frequent shaking. The addition occupied about one hour. The mixture was then refluxed on a water bath for 3 h and then allowed to stand overnight. The crude quinoline-4-carboxylic acid was filtered off at the pump and crystals were washed with a little ether. The crude products were recrystallised from methanol to give a white colored compound.

Microwave irradiation method

A mixture of substituted/unsubstituted benzaldehyde (0.125 mol), pyruvic acid in DMF and p-chloroaniline in 100ml of absolute ethanol was irradiated at 300 watts for different time (table-1) for different reaction mixtures. The completion of the reaction was monitored continuously by TLC after every minute. The products obtained were dried and recrystallised from methanol.

Synthesis of 5-(quinolin-4-yl)-1,3,4-thiadiazol-2-amine

Conventional method

Substituted/unsubstituted quinoline carboxylic acid (0.3M), Phosphorous oxychloride (3.5ml) and thiosemicarbazide (0.25M) were taken in two necked round bottom flask and slowly heated to 70˚c and maintained at that temperature for 7hours. After cooling poured into ice water and made basic with concentrated ammonia. The product obtained was filtered, washed with water and recrystallised from ethanol.

Microwave irradiation method

Substituted/unsubstituted quinoline carboxylic acid (0.3M), Phosphorous oxychloride (3.5ml) and thiosemicarbazide (0.25M) were taken in two necked round bottom flask and was irradiated at 300 watts for different time (table-1) maintained at that temperature for 7hours. . The completion of the reaction was monitored continuously by TLC after every minute. After cooling poured into ice water and made basic with concentrated ammonia. The product obtained was filtered, washed with water and recrystallised from ethanol.

Table 1: Comparison of yields and reaction time in case of Microwave and conventional methods of synthesis of Quinoline derivatives

Compd Code	% Yield		Reaction Time		M.P (˚c)	Retention Time (R_t)	Mol.Wt.
	Conventional	Microwave	Conventional	Microwave			
AR-1	32	60	3hrs	2minutes	150	0.321	283.71
AR-2	45	78	3hrs	3minutes	128	0.458	294.26
AR-3	54	87	3hrs	2minutes	176	0.569	299.71
AR-4	48	78	3hrs	4minutes	189	0.792	313.74
AR-5	52	86	3hrs	7minutes	114	0.864	324.29
AR-6	49	88	7hrs	10 minutes	168	0.215	338.81
AR-7	58	90	7hrs	9minutes	154	0.389	349.37
AR-8	40	76	7hrs	25minutes	189	0.457	354.81
AR-9	67	79	7hrs	18minutes	135	0.587	368.84
AR-10	64	92	7hrs	20minutes	112	0.643	379.39

Spectral data

Antioxidant activity

Antioxidant activity was evaluated using **Nitro blue tetrazolium chloride method**

Nitro blue tetrazolium chloride method[17]

The reaction mixture contained 2.5ml phosphate buffer, 0.1ml NBT, 0.2ml Potassium cyanide (KCN), 0.5ml riboflavin and different concentrations of aspirin and aspirin derivatives (AR-01, AR-02,AR-03,AR-04,AR-05,AR-06,AR-07,AR-08,AR-09,AR-10) in a final volume of 3ml.The tubes were illuminated with an incandescent lamp for 15 minutes. Optical density was measured at 532nm before and after illumination. The percentage of inhibition of super oxide generation was evaluated by comparing the absorbance value of control and test.

Percentage inhibition $= \frac{C-T}{C} \times 100$ Where,

C= Absorbance of control

T= Absorbance of test

Anti microbial activity

Preparation of stock solution

Stock solutions of the synthesized compounds used were prepared in DMSO in the concentration of 0.5, 1, 5, 10, 50 and 100 µg/ml. The stock solution of standard drug (ciprofloxacin) drug was prepared using distilled water in the concentration of 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 20 and 40 µg/ml.

Cultures used

Standard cultures of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* species.

Strain No.

Bacillus subtilis (ATCC 6633)

Staphylococcus aureus (ATCC 6538)

Escherichia coli (ATCC 10536)

Pseudomonas aeruginosa (ATCC 10145)

Composition of nutrient broth media used for bacteria

S. No.	Ingredients	Weight (g)
1.	Beef extract	1.50
2.	Peptic digest of animal tissue	5.00
3.	Yeast extract	1.50
4.	Sodium chloride	5.00
Final pH at 25 ° C 7.4 ± 0.2		

Composition of nutrient agar media or agar plates

S. No.	Ingredients	Weight (g)
1.	Beef extract	1.50
2.	Peptic digest of animal tissue	5.00
3.	Yeast extract	1.50
4.	Sodium chloride	5.00
5.	Agar	15.00
Final pH at 25 ° C 7.4 ± 0.2		

Procedure

Disc diffusion method

The petri dishes were washed thoroughly and sterilized in hot air oven at 170˚C for one hour. Around 120 ml of sterile nutrient agar medium for bacteria was poured into sterile petri dishes and allowed to solidify. The petri dishes were incubated at 37˚C for 24 h to check for sterility.

The medium was seeded with the organism by spread plate method using sterile cotton swabs and then placed the disc of Whatmann filter paper, pre-saturated with different sterile dilutions of **AR(1,2,3,4,5,6,7,8,9,10)** the synthesized compounds and a standard solution of ciprofloxacin on the agar media.[18] The petri plates were incubated for 24 h at 37˚C and then the zones of inhibition were measured.

RESULTS AND DISCUSSION

Chemistry

IR,^{1}HNMR(300MHz,$CDCl_3$),Mass analysis of synthesized compounds

AR-1: IR (KBr): 3325 (-OH str.), 3061(Ar-C-H str.), 1663(-C=O str), 1594 (C=N str).

^{1}HNMR (300MHZ, $CDCl_3$): 6.049 (s, 1H,Ar-H),6.982-6.999(d, 2H, Ar-H), 7.235-7.241 -(m,4H,Ar-H),7.301-7.503(d, 2H, Ar-H), 10.1 (s, 1H, -COOH)).MS: m/z 282.95[M^+].

AR-2: IR (KBr): 3334 (-OH str.), 3065 (Ar-C-H str.), 1657(-C=O str), 1598 (C=N str),1555(-N=O str), 1330(-N=O str).^{1}HNMR (300MHZ, $CDCl_3$): 7.212 (s, 1H, Ar-H), 7.409(s, 1H, Ar-H), 7.450(d, 2H, Ar-H), 7.417-8.144 (m,4H,Ar-H), 9.332 (s, 1H, -COOH)).MS: m/z 293.68[M^+].

AR-3: IR (KBr): 3331 (-OH str.), 3058 (Ar-C-H str.), 1689(-C=O str), 1574 (C=N str),.^{1}HNMR (300MHZ, $CDCl_3$):2.166(s,1H,-OH), 6.929 (s, 1H, Ar-H), 6.969-7.224(m, 4H, Ar-H), 8.596(s, 1H, Ar-H), 12.20 (s, 1H, -COOH)).MS: m/z 298.68[M^+].

AR-4: IR (KBr): 3315 (-OH str.), 3063 (Ar-C-H str.), 1658(-C=O str), 1598 (C=N str),1248(C-O str. (-OCH_3).^{1}HNMR (300MHZ, $CDCl_3$):3.83(s,3H,-OCH_3), 6.83 (d, 2H, Ar-H), 7.2(m, 4H, Ar-H), 7.35(d,2H,Ar-H),7.47(d, 1H, Ar-H), 9.232 (s, 1H, -COOH)).MS: m/z 312.96[M^+].

AR-5: IR (KBr): 3324 (-OH str.), 3099 (Ar-C-H str.), 1657(-C=O str), 1598 (C=N str),1257(C-O str. (-OCH_3),1332(-N=O symmetric str.).^{1}HNMR (300MHZ, $CDCl_3$):3.78(s,3H,-OCH_3), 7.151 (s,1H, Ar-H), 7.309-7.331(d,2H, Ar-H), 7.443-7.466(d,2H,Ar-H),8.12-8.14(d, 1H, Ar-H), 9.52 (s, 1H, -COOH)).MS: m/z 324.15[M^+].

AR-6: IR (KBr): 3106 (-N-H str.), 3050(Ar-C-H str.), 1595 (C=N str),.^{1}HNMR (300MHZ, $CDCl_3$):4.12(s,2H,-NH_2), 7.63 (d, 1H, Ar-H), 7.29-7.99(m, 6H, Ar-H), 7.66(s,1H,Ar-H),MS: m/z 337.96[M^+-1].

AR-7: IR (KBr): 3617 (-N-H str.), 3062 (Ar-C-H str.), 2446(-C-S-C str), 1332 (N=O str),. ^{1}HNMR (300MHZ, $CDCl_3$): 6.60-6.65 (d, 2H,NH_2),7.18-7.44(m,8H,Ar-H),8.10(s, 1H, Ar-H),.MS: m/z 348.67[M^+].

AR-8: IR (KBr): 3312(-OH str.), 3059 (Ar-C-H str.), 1658(-C=O str), 1598 (C=N str),1248(C-O str. (-OCH_3).^{1}HNMR (300MHZ, $CDCl_3$):5.12(s,1H,-OH), 6.76-7.82 (m,4H, Ar-H), 7.62-7.99(m, 3H, Ar-H), 7.66(s,1H,Ar-H),.MS: m/z 312.96[M^+].

AR-9: IR (KBr): 3100(-N-H str.), 3050 (Ar-C-H str.), 2210(-C-S-C str), 1601 (C=N str),1251(C-O str.).^{1}HNMR (300MHZ, $CDCl_3$):3.90(s,3H,-OCH_3), 6.63-6.95 (d, 2H, NH_2), 6.99-8.60(m, 8H, Ar-H),MS: m/z 368.14[M^+].

AR-10: IR (KBr): 3117(-N-H str.), 3077 (Ar-C-H str.), 2295(-C-S-C str), 1595 (C=N str), 1330(-N=O str.), 1254(-C-O str.). ^{1}HNMR (300MHZ, $CDCl_3$):3.91(s,3H,-OCH_3), 6.60-6.65 (d, 2H, NH_2), 6.99-8.26(m, 8H, Ar-H),.MS: m/z 379.15[M^+].

All the synthesized compounds were confirmed for their structure by IR, ^{1}H NMR, Mass spectras.

Antioxidant activity

The IC_{50} values of standard in comparison prepared compounds are as below Table 2:

Compounds AR-1, AR-3 exhibited broad spectrum antimicrobial activity at a concentration of 5µg/ml when compared to standard ciprofloxacin (1µg/ml). Compound AR-2 exhibited broad spectrum antimicrobial activity at a concentration of 25µg/ml. Compounds AR-7,AR-8 were active against *Bacillus subtilis, Staphylococcus aureus* at 50µg/ml,100µg/ml concentrations respectively. Compounds AR-4(50µg/ml), AR-5(100 µg/ml) exhibited antimicrobial activity at, concentrations respectively against *Bacillus subtilis, Pseudomonas aeruginosa.* Compounds AR-6,9 were found to be devoid of antimicrobial activity.

Table 2: IC_{50} values of Standard Ascorbic acid vs Prepared compounds

Compound name	IC_{50} (µg/ml)
Ascorbic acid	12.18
AR-1	131.34
AR-2	79.18
AR-3	24.97
AR-4	135.23
AR-5	54.67
AR-6	98.45
AR-7	115.35
AR-8	38.12
AR-9	108.18
AR-10	22.38

Compounds AR-10,AR-8,AR-5 exhibited significant antioxidant activity when compared to standard ascorbic acid while compounds AR-2,AR-6,AR-9,exihibited moderate antioxidant activity.

Table 3: The MIC values of synthesized compounds

Compound	MIC (µg/ml) against *Bacillus subtilis*	MIC (µg/ml) against *Staphylococcus aureus*	MIC(µg/ml) against *Escherichia Coli*	MIC(µg/ml) against *Pseudomonas aeruginosa*
Ciprofloxacin	1	1	1	1
AR-1	5	5	5	5
AR-2	25	25	25	25
AR-3	5	5	5	5
AR-4	50	-	-	50
AR-5	100	-	-	100
AR-6	-	-	-	-
AR-7	50	50	-	-
AR-8	100	100	-	-
AR-9	-	-	-	-
AR-10	100	100	100	-

CONCLUSIONS

Novel quinoline derivatives synthesized were found to possess antimicrobial and antioxidant potential. Compounds AR-1, AR-3(quinoline carboxylic acids were found to possess broad spectrum antimicrobial activity). Compounds AR-10 (22.38µg/ml), AR-3 (24.97 µg/ml), AR-8(38.12µg/ml) showed comparable antioxidant activity compared to standard ascorbic acid(12.18µg/ml).The mechanism by which the compounds exhibit antioxidant activity is yet to be studied.

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