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One-Pot Synthesis of some new 2-Amino-4-phenylquinoline-3-carbonitrile derivatives and Anticancer Evaluation against MCF-7 Cells

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Abstract: An effective methodology was established using ammonium metavanadate as catalyst to obtained 2-amino-4-phenylquinoline-3-carbonitrile derivatives under reflux stirring. The synthesis of 2-amino-4- phenylquinoline-3-carbonitrile derivatives has been achieved via one pot reaction of substituted benzaldehydes, malononitrile and various anilines using ammonium metavanadate as catalyst in solvent ethanol. All the synthesized derivatives were evaluated for *in-vitro* anti-proliferative activity of cancer cell line. The initial assays reveal that some of the newly synthesized compounds displayed significantly good inhibition activities against human breast cancer cell (MCF-7), as compare to standard drug Adriamysin which might be developed as novel lead scaffold for potential anticancer agents.

Keywords: Anticancer activity, Anilines, Ammonium Metavanadate, Malononitrile, Benzaldehydes, 2-Amino-4- phenylquinoline-3-carbonitrile.

Introduction:

Quinoline derivatives are the common in nature and many of the derivatives exhibit various biological activities, such as anti-malarial, antitumor, anthelmintic, antibacterial, antiasthmatic and antiplatelet [1-6].

Due to these useful pharmacophoric properties they were extensively studied [7-14].

In this regard, the development of new multi component protocols for the synthesis of new quinoline incorporating heterocycles have attracted considerable interest in the recent years [15-18].

Therefore herein, we have developed an ammonium metavanadate catalyzed synthesis of 2-amino-4-phenylquinoline-3-carbonitrile derivatives by the one-pot reaction of substituted benzaldehydes, malononitrile and anilines in solvent ethanol under reflux stirring.

Experimental:

All solvents were employed as

commercial anhydrous grade without further purification. The column chromatography was carried out over silica gel (100-120 mesh). Melting points were determined in open capillary tube and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-300 MHz spectrometer in $CDCl_3$ solvent.

General procedure for the synthesis 2-amino-4-phenylquinoline-3-carbonitrile derivatives:

In oven dried 50 ml round-bottom flask a mixture of substituted benzaldehyde (1.0 mmol), ammonium metavanadate (10 mol %) stirred at reflux temperature for few minutes in 20 ml ethanol then malononitrile (1.2 mmol) were added and again stirred at reflux temperature for few minutes till yellow colour develops and finally aniline (1.1 mmol) were added to it. This mixture was then stirred at reflux temperature for appropriate time (**Table 2**).

2-Amino-6-chloro-4-(4-fluorophenyl)-quinoline-3-carbonitrile (4d): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 2H, NH_2), 7.24-7.29 (m, 4H), 7.93-7.99 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1, 120.9, 122.8, 128.3, 129.1, 129.9, 130.0, 132.1, 135.0, 136.2, 137.3, 149.2, 158.0, 168.2.

2-Amino-6-bromo-4-(4-chlorophenyl)-quinoline-3-carbonitrile (4e): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (s, 1H, NH), 1.55 (s, 1H NH,), 6.10-6.22 (d, 2H), 6.40-6.47 (m, 1H), 6.70-6.79 (m, 1H), 7.45-7.55 (m, 2H), 7.74-7.80 (d, 2H), 7.85-7.95 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.4, 120.2, 122.5, 129.4, 130.2, 131.9, 141.3, 149.3, 158.3, 166.3.

2-Amino-6-methoxy-4-(4-nitrophenyl)-quinoline-3-carbonitrile (4i): ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 2H, NH_2), 3.84 (s, 3 H), 6.74-6.88 (q, 1H), 7.21-7.26 (t, 2H) 7.74 (s, 1H), 7.94-7.98 (q, 2H) 8.34 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7, 83.6, 101.4, 105.3, 114.5, 115.3, 118.5, 127.1, 134.1, 135.4, 136.6, 145.3, 148.8, 158.5, 165.6.

Results and discussion:

Model reactions were carried between 4-nitro benzaldehydes, malononitrile, ammonium metavanadate and 4-methoxy aniline under reflux stirring. The screening to get appropriate conditions for the designed protocol based on the reaction of 4-NO_2-benzaldehyde (1i), malononitrile (2) and 4-methoxy aniline (3i) as a model substrate, we used different catalysts, solvents and temperature; the results are reported in **Table 1**.

It was found that, when the reaction was carried out without any catalyst, no product was observed even after 24 h (Table 1, entry 1). To obtain desired product (**4i**), we tested the reaction using some different Lewis acids as catalyst (Table 1, entries 2-5). As shown, the use of these catalysts, led to the formation of product after 10 h (Table 1, entries 2-5), which indicates a crucial role of the catalyst. Thus, ammonium metavanadate suitable to catalyze reaction smoothly as the best catalyst was tested for this reaction. When catalytic loading was 10 mol % for the catalyst ammonium metavanadate the product obtained with 94 % yield only in 3.5 h (Table 1, entry 6). To check the performance of the catalyst without solvent, the reaction was carried out in the presence of ammonium metavanadate at reflux stirring the product obtained 60 % in 4 h (Table 1,

entry 7).

Subsequently, the same reaction was carried out to optimize the effect of solvent under different protic and aprotic solvents and it is observed that the reaction proceeds faster in protic solvent such as ethanol with maximum yield in less reaction time when compared with non protic solvents with lower yield (Table 1, entries 8-12).

We have also investigated the catalytic loading of the catalyst for the reaction and it was observed that increasing amount of the catalyst does not affect the yield of the reaction (Table 1, entries 15-16). Thus 10 mol % of ammonium metavanadate is sufficient in ethanol solvent to give final product in good to excellent yield. Finally, to optimize reaction temperature we carried out stirring of the reaction at temperature 35 ^{o}C gives yield of 76% (Table 1, entry 17).

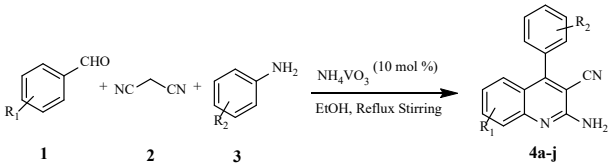
Table 1. Optimization of catalyst, solvent, and temperature for the construction of products.

Entry	Solvent	Catalyst	Catalyst (mol %)	Time (h)	Yield[a] (%)
1	Ethanol	-	-	24	-
2	Ethanol	L-Proline	10	8	27
3	Ethanol	$LaCl_3$	10	6	22
4	Ethanol	$FeCl_3$	10	6	25
5	Ethanol	$AlCl_3$	10	8	21
6	**Ethanol**	**NH_4VO_3**	**10**	**3.5**	**94**
7	-	NH_4VO_3	10[b]	4	60
8	Acetonitrile	NH_4VO_3	10	8	21
9	THF	NH_4VO_3	10	8	19
10	1,4-Dioxane	NH_4VO_3	10	8	23
11	DCM	NH_4VO_3	10	8	20
12	Toluene	NH_4VO_3	10	8	17
13	Methanol	NH_4VO_3	10	8	34
14	Water	NH_4VO_3	10	6	36
15	Ethanol	NH_4VO_3	15	4	87
16	Ethanol	NH_4VO_3	20	4	87
17	Ethanol	NH_4VO_3	10[c]	4	76

[a]Isolated yield; [b]Reaction temperature 80 ^{o}C; [c]Reaction temperature 35 ^{o}C

Under these optimized reaction conditions (reflux stirring in ethanol and 10 mol % catalytic loading of ammonium metavanadate), a series of 2-amino-4-phenylquinoline-3-carbonitrile derivatives were prepared and obtained results are summarized in Table 2. Encouraged by these noteworthy results, we used a variety of substituted benzaldehyde and various anilines (Scheme 2) for the synthesis of corresponding quinoline-3-carbonitrile derivatives. We observed that all products were obtained with good to excellent yields (Table 2, Entries 1-10).

As shown in Table 2, both electron deficient and electron rich substituted benzaldehydes and various anilines were applicable to the reaction, affording the products in excellent yields. Benzaldehydes with halogens at ortho, meta, para positions and electron withdrawing group such as -NO_2 at para position and electron donating -OH group at para position were used. In addition to that, the halogen, -OCH_3 and -NO_2 groups at para position containing anilines were also been tested. The anilines with halogen groups at para position reacted rapidly and gave the excellent yield in minimum time as compared to anilines having 4-NO_2 group.



Scheme 2

Table 2. The construction of 2-amino-4-phenylquinoline-3-carbonitriles

Entry	Aldehyde (R_1)	Amine (R_2)	Products (4a-j)	Time (h)	Yield[a] (%)
1	4-Cl	4-Cl	4a	5.00	85
2	3-Cl	4-Cl	4b	4.50	87
3	2-Cl	4-Cl	4c	5.50	83
4	4-F	4-Cl	4d	4.00	92
5	4-Cl	4-Br	4e	4.00	93
6	3-Cl	4-Br	4f	4.50	84
7	2-Cl	4-Br	4h	4.50	86
8	4-F	4-Br	4g	4.00	91
9	4- NO_2	4-OCH_3	4i	3.50	94
10	4-OH^2	4- NO_2	4j	6.00	82

[a]Isolated yield

The reaction between 4-Cl, 3-Cl, 2-Cl and 4-F benzaldehyde and 4-Cl aniline with malononitrile afforded up to 85, 87, 83, 92 % yield of corresponding products (compound 4a, 4b, 4c and 4d) respectively. The reaction between 4-Cl, 3-Cl, 2-Cl and 4-F-benzaldehyde and 4-Br aniline with malononitrile afforded up to 93, 84, 86, 91 % yield of corresponding products (compound 4e, 4f, 4g and 4h) respectively. Using 4-Br and 4-Cl aniline with 4-F benzaldehyde, the 2-amino-4-phenylquinoline-3-carbonitrile products were obtained with the excellent isolated yields. Highest yield and minimum reaction time were obtained for the reaction between 4-nitrobenzaldehyde and 4-methoxyaniline to get product yield of 94 % in 3.5 hours.

Anticancer activity:

In vitro MTT assay for anticancer activity

The all ten synthesized 2-amino-4-phenylquinoline-3-carbonitrile derivatives were tested for *in vitro* anticancer activity against MCF7 (human breast cancer) cells using MTT reduction assay, which was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. In detail the 1mg/ml stock solution of all the compounds were prepared individually using DMSO. The working solution was prepared by serial dilution with complete medium to get the test concentration ranging from 0.001, 0.01, 0.1, 1.0 and 10uM. The 96 well plates seeded with MCF-7 breast cancer cells and treated with different concentrations of the test compounds incubated for 96 hours and temperature of 37 °C with 5% CO_2 concentration to maintain pH of the system.

After the incubation MTT reagent was added to the wells and further incubated for 4 hours; Supernatant from each well containing the dark blue formazan product formed by the cells was carefully removed was dissolved in 100 ul of DMSO under a safety cabinet and absorbance was read at 550nm by UV-Visible spectrophotometer to determine the cell viability. Percentage inhibitions were calculated using following formula and plotted against the concentrations used to calculate the IC_{50} values.

$$\%\ \text{cell inhibition} = \frac{(\text{OD control} - \text{OD treated})}{\text{OD control}} \times 100$$

MTT assay is based on the principle of enzymatic reduction of MTT dye [(3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyl tetrazolium bromide)]. Pale-yellow colored MTT is reduced by mitochondria of living cells to produce a dark blue formazan product. The amount of blue formazan production is directly proportional to the number of viable cells present and can be quantified by colorimetric methods.

Here to determine the anticancer activity of all synthesized compounds, MTT assay was performed and the results were compared with standard anticancer drug Adriamycin. From the assay it was found that the entire compound shows some

amount of anti-proliferative activity against MCF7 cells with IC_{50} value ranges from 1uM to 31uM Table 3. From the results it was evident that compounds 4d (4C) and 4e (5C) are most active compound against the MCF7 cell line as compared to other synthesized quinoline compounds. The compound 4d (4C) with IC_{50} values of 1 uM was found to be most potent as compared with later one with IC_{50} of 13 uM figure 1. The results show that the substitution of 4-F and 4-Cl with 4-Cl and 4-Br have positive impact on the growth inhibitory activity of 2-amino-4-aryl quinoline-3- carbonitrile derivatives which increases by 13 fold.

Table 3: In vitro MTT assay for anticancer activity of the synthesized 2-Amino-4-aryl quinoline-3-carbonitriles against cell line MCF7.

Entry	Product	Aldehyde (R_1)	Aniline (R_2)	Anticancer Activity (MFC7)					
				0.001	0.01	0.1	1	10	IC-50 (uM)
1	4a	4-Cl	4-Cl	0.12	1.1	10.4	17.6	22.1	27.07
2	4b	3-Cl	4-Cl	1.1	1.2	8.4	16.2	21.3	21.95
3	4c	2-Cl	4-Cl	1.5	2.4	7.8	11.4	18.3	25.87
4	4d	4-F	4-Cl	1.23	2.14	16.32	28.15	33.25	13.94
5	4e	4-Cl	4-Br	3.27	12.36	21.2	48.26	55.61	1.0
6	4f	3-Cl	4-Br	1.3	2.4	7.3	10.6	16.4	28.79
7	4g	4-F	4-Br	1.2	3.1	6.9	9.6	17.1	27.85
8	4h	2-Cl	4-Br	1.6	3.4	7.2	11.1	14.8	31.59
9	4i	4-NO_2	4-OCH_3	2.1	4.7	8.9	10.7	15.3	30.67
10	4j	4-OH	4-NO_2	1.7	3.6	7.9	9.8	16.2	29.25
ADR	Adriamycin			13.42	27.36	49.64	82.49	96.71	0.57

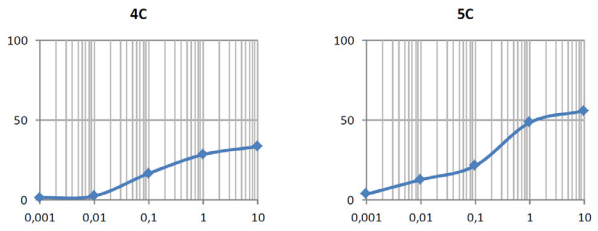


Figure 1: percent cell inhibition activity of compounds 4d (4C) and 4e (5C)

Conclusion:

In conclusion, we have developed an expedient approach for the synthesis of 2-amino-4-phenylquinoline-3-carbonitrile derivatives by the one-pot reaction of substituted benzaldehydes, malononitrile and anilines in solvent ethanol under reflux stirring condition using ammonium metavanadate as catalyst. The satisfying features of this method are simple work up, cost efficiency and excellent yields of the corresponding quinoline derivatives. Anticancer activity of synthesized derivatives (4a-j) reveals that newly synthesized compounds displayed significant good inhibition activities against human breast cancer cell (MCF-7) cell lines.

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