Heterocyclic Letters
Vol. 6| No.4 |717-723|Aug-Oct| 2016
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

A CONVENIENT ONE-POT SYNTHESIS OF HEXAHYDROQUINOLINES AND THEIR EVALUATION OF ANTICANCER ACTIVITY AGAINST MCF-7 CELLS

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ABSTRACT: A convenient protocol was described for the synthesis of hexahydroquinoline derivatives by reaction of a dimedone, substituted salicylaldehyde, malononitrile and ammonium acetate using [Msim]Cl (10 mol %) as a catalyst. All the synthesized derivatives were evaluated for inhibition of cancer cell. The initial assays reveals that some of the newly synthesized compounds displayed significantly good inhibition activities against human breast cancer cell (MCF7), cell lines compared with the control (Adriamysin), which might be developed as novel lead scaffold for potential anticancer agents.

Keywords: Anticancer activity, Salicylaldehyde, Malononitrile, [Msim]Cl, Hexahydroquinolines.

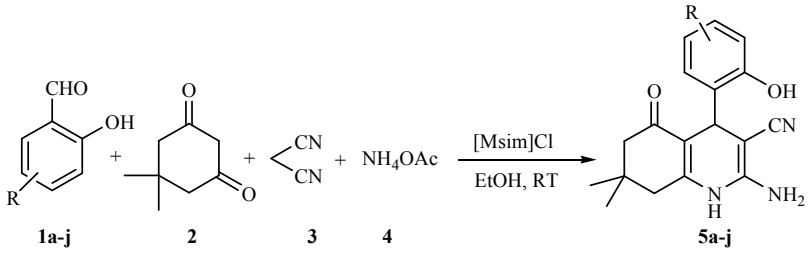
Introduction

Multicomponent reactions (MCRs) are a desirable synthetic approach, for the reason that complex products are produced in a single step and variety can be easily achieved by the changing the reaction components.[i-iii] Moreover, several molecular scaffolds in both biologically active and natural products can be produced with combinatorial approaches.[iv-v] Quinoline derivatives execute as a fundamental unit in numerous natural products and drugs attributing to their various applications in the pharmaceutical industries maintain a noteworthy place among the heterocyclic molecules.[vi] Quinolines having 1,4-DHP nucleus have been reported as important molecule due to their therapeutic and pharmacological properties such as antitumor, bronchodilator, vasodilator, geroprotective, anti-inflammatory, antimalarial, antiasthematic and antibacterial activities.[vii-viii] Especially, now days 1, 4-DHP nucleus containing drugs nimodipine, lacidipine posses improved calcium channel antagonist activity [ix] and the cardiovascular agents such as nifedipine, nicardipine and amlodipine are effective against treatment of hypertension.[x]

A literature survey shows that the synthesis of quinoline derivatives can be accomplished by using various catalysts such as CAN,[xi] organocatalysts,[xii] $Sc(OTf)_3$,[xiii] ionic liquids,[xiv] L-proline,[xv] and $Yb(OTf)_3$.[xvi] However, most of the reported methodology still suffer from several problem such as the long reaction time, unsatisfactory yields, drastic reaction

condition as well as costly catalysts and tedious work up procedures. For this reason there is need to develop an effective methodology for the synthesis of hexahydroquinoline derivatives.

As a interest in the development for the synthesis of heterocycles using catalytic amount of ionic liquid, herein we describe an efficient synthesis of hexahydroquinoline derivatives *via* four component condensation of dimedone, substituted salicyaldehydes, malononitrile and ammonium acetate using [Msim]Cl as a catalyst in acetonitrile solvent (**Scheme 1**). All the synthesized derivatives were evaluated for inhibition of Brest cancer cell.



Scheme 1

Results and Discussion

Initially in our investigation, we studied the effect of solvents on the synthesis of hexahydroquinolines under the ambient temperature condition using model reaction of dimedone, 5-chloro salicylaldehyde. malononitrile and ammonium acetate to give corresponding desired product 5d in presence of 10 mol% ionic liquid [Msim]Cl. The results are listed in Table 1 (Entries 1-7). In this optimization study, admirable was found to be an excellent solvent over other solvents such as methanol, water, acetonitrile, tolune, dichloromethane and DMF in terms of reaction time and product yields (Table 1, Entry 2). In the solvent methanol, product 5d was obtained in good yield 72% within 4 hours (Table 1, Entry 1). In the solvent acetonitrile and water, the reaction afforded 68 and 38 % product yield (Tabel 1, Entries 3 and 4 respectively). In the solvent dichloromethane and tolune, the corresponding product 5d was obtained in lower yield with increased reaction time (Table 1, Entries 5 and 6, respectively). In the solvent DMF, the reaction afforded 42 % product yield within the reaction time 7 hours (Table 1, Entry 7). Afterwards, we screened the effect of catalytic loading on the model reaction in solvent ethanol. Initially at the catalytic concentration of 5 mol % of [Msim]Cl, reaction was completed with extended reaction time and afforded 76% product yield (Table 1, Entry 8). Further increase in the catalytic loading up to 15 mol % did not show significant improvement in the yield even with extended reaction time (Table 1, Entry 9).

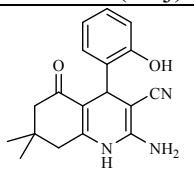
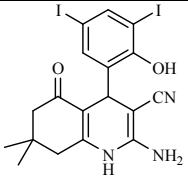
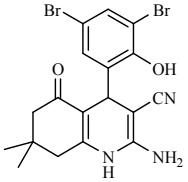
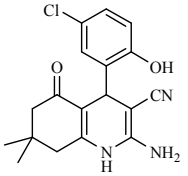
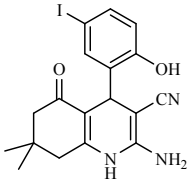
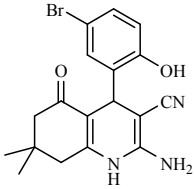
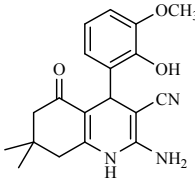
Table 1: The screening of solvents for the synthesis of hexahydroquinoline derivatives[a]

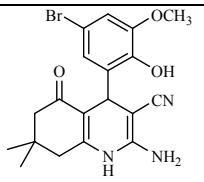
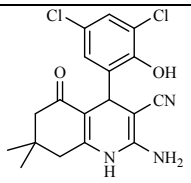
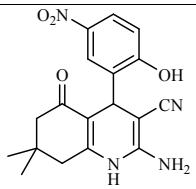
Sr. No.	Catalyst	Solvent	Catalyst (mol %)	Time (h)	Yield %
1	[Msim]Cl	MeOH	10	4	72
2	[Msim]Cl	**EtOH**	**10**	**2.5**	**91**
3	[Msim]Cl	CH_3CN	10	5	68
4	[Msim]Cl	H_2O	10	9.0	38
5	[Msim]Cl	DCM	10	8	35
6	[Msim]Cl	Tolune	10	8.5	28
7	[Msim]Cl	DMF	10	7	42
8	[Msim]Cl	EtOH	5	4	76
9	[Msim]Cl	EtOH	15	2.5	84

[a]Conditions: 5-chloro salicylaldehyde (1 mmol), dimedone (1 mmol), malononitrile (1mmol), ammonium acetate (1mmol), solvent (10 mL) at room temperature condition. Reaction was monitored by thin layer chromatography.

Optimistic by these noteworthy results, we screened a variety of substituted salicylaldehydes for the synthesis of corresponding hexahydroquinoline derivatives. We observed all products are obtained with excellent yields (Table **2).**

Table 2: [Msim]Cl catalyzed an efficient synthesis of hexahydroquinoline derivatives[a]

Sr. No.	Products (3a-j)	Time (h)	Mp. (^{O}C)	Yield[b] (%)
1	 5a	3.50	226-228	88
2	 5b	3.40	278-280	86
3	 5c	3.30	252-254	89
4	 5d	2.50	234-236	91
5	 5e	3.00	210-212	87
6	 5f	3.20	221-223	90
7	 5g	2.20	198-200	89

8	 5h	2.50	258-260	88
9	 5i	3.10	268-270	89
10	 5j	3.50	282-284	85

[a]Conditions: Substituted salicylaldehyde (1 mmol), dimedone (1 mmol), malononitrile (1mmol), ammonium acetate (1.2mmol), [Msim]Cl (10 mol%), EtOH (10 ml) at room temperature condition. Reaction was monitored by thin layer chromatography. [b]Isolated Yield

Based on the precedence of known anticancer activity of known quinoline derivatives we were interested to test anticancer properties in vitro. We evaluated our compounds for their anti-proliferative properties in vitro against cancer cell lines for human breast cancer cell line MCF7. The test compounds were examined at various concentrations in a MTT (3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and the LC 50, TGI and GI 50 values obtained for each compounds are summarized in Table 3. ADR (Adriamysin or Doxorubicin known drug) compounds showed cytotoxicity against LC50, TGI and GI 50 was used as a reference compound. While most of these compounds showed MCF7 activity shown by LC 50, TGI and GI50 values.

Table 4: In vitro cytotoxic activity of the synthesized compounds against human breast cancer cell line (MCF7).

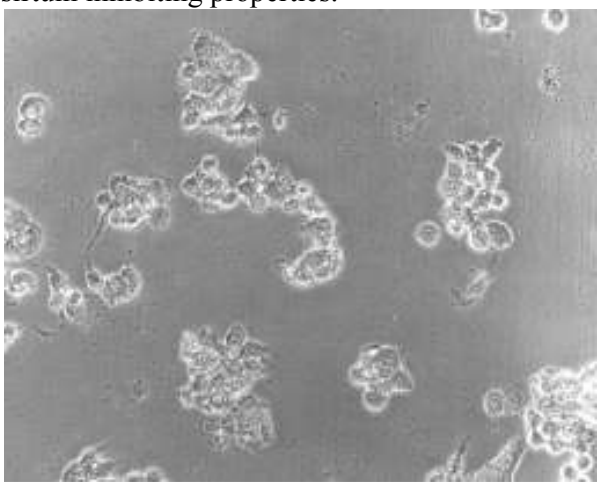
Human Breast Cancer cell line MCF7																
%control growth																
Drug concentration (mg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
5a	69.3	33.5	11	7.5	66.8	39.4	14.5	9.6	56	41.3	14.6	9.4	64.0	38.1	13.3	8.8
5b	81.4	41.1	13.3	12.5	79.8	48.1	17.8	13.5	70.1	53.2	16.5	12.6	77.1	35.2	9.9	8.7
5c	79.1	39.4	14.4	11.1	77.3	46.3	16.8	12.3	68	52.4	16.1	11.7	74.8	46.0	15.7	11.7
5d	76.3	37.8	11.2	8.9	73.1	41.2	15.5	10.2	61.3	44.4	17.6	12.1	70.2	41.1	14.7	10.4
5e	78.2	38.6	12.2	10.5	76.8	45.1	16.3	12.7	68	50	15.7	11.6	74.3	44.5	14.7	11.6
5f	76.2	37.1	11.4	9.2	74.3	43.1	15.5	11.2	63.1	47.2	16.3	12.4	71.2	42.4	14.4	10.9
5g	65.4	32.1	8.9	5.9	62.3	36.5	12.8	7.7	53.6	38.1	12.3	7.2	60.4	35.5	11.3	6.9
5h	73.7	35.7	10.9	8.5	68.4	41.8	15.3	10.6	62.4	44.6	16.2	11.3	68.1	31.2	14.1	10.1
5i	72.2	34.1	9.8	6.4	66.4	37.4	13.2	9.5	57	41.4	14.3	9.8	65.2	37.6	12.4	8.5
5j	84.1	44.1	16.2	15.4	82.1	50.4	19.5	15.2	73.4	54.6	17.4	16.2	79.8	49.7	17.7	15.6
ADR	5.7	4.1	-0.8	-30	1.4	5.0	-2.2	-32	1.2	6.2	2.5	-36.	2.8	5.1	-0.2	-32.7

	Drug concentrations mg/ml calculated from graph		
MCF7	**LC 50**	**TGI**	**GI50**
5a	>80	64.2	30.3
5b	>80	63.4	29.9
5c	>80	>80	40.2
5d	>80	75.8	35.8

5e	>80	64.2	39.9
5f	>80	79.4	37.5
5g	>80	50.8	23.7
5h	>80	64.2	34.7
5i	>80	61.8	29.2
5j	>80	>80	53.7
ADR	>80	43.7	<10

GI50	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

The good results however were obtained using compounds 5g, 5i (Table 3). Interestingly, all the compounds were found to be active against Breast cancer cells and showed good activities against breast cancer cells. In order to understand the mechanism of action some of the compounds were tested for their inhibitory potential against sirtuins. Being considered as important targets for cancer therapeutics sirtuins (class III NAD-dependent deacetylases) are shown to unregulated in various types of cancer. Inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. The activity of test compounds was determined using Sirt1 fluorescence activity assay using suramin, a known inhibitor of Sirt1 as a reference compound. At the concentration of 10 mg/ml compounds 5g, 5i showed 60.4, 68.4 where as for concentration 80 mg/ml for 5g and 5i showed 6.9 and 8.5 inhibition, respectively, in compared to Adriamysin 2.8 and -32.4 inhibition indicating that the anticancer properties of these molecules are possibly due to their sirtuin inhibiting properties.



MCF7 of 5a

Materials and method

All solvents were used as commercial anhydrous grade without further purification. The column chromatography was carried out over silica gel (80–120 mesh). Melting points were determined in open capillary tube and are uncorrected. ^{1}H spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$ solvent and TMS as an internal standard. ^{13}C NMR spectra were recorded on a Bruker-300 MHz spectrometer in $CDCl_3$ solvent. Mass spectra were taken on Polaris-Q Thermoscintific GC-MS.

Anticancer activity

The anti-cancer activity for these compounds was done in the Anti-cancer drug screening facility (ACDF), Tata memorial centre, advanced centre for treatment, research and education in cancer (ACTREC). The in-vitro anti-cancer activity for the corresponding compounds and ADR (Adriamysin or doxorubicin) taken as a known drug, tested using SRB (sulforhodamine B) assay protocol as exactly described by Skehan P. et al. Briefly, SRB is a dye binds to the protein. The human breast cancer cell line MCF7 cultured in 96 well plate treated with different concentrations of given compounds (10, 20, 40 and 80 µg/ml). After treatment the cells were fixed in trichloroacetic acid and stained using sulforhodamine B (0.4% wt/vol) prepared in 1% acetic acid for 30 minutes. Four washes with 1% acetic acid were given to remove unbound dye. 10 mM unbuffered tris base was used to extract protein bound dye and subjected for microtiter plate reader. The absorbance of dye was measured at wavelength 565 nm. The absorbance is correlated with the net protein synthesis rate. 50% inhibition of cell growth (GI50), 50% cell kill or lethal concentration (LC50) and 100% (total) growth inhibition (TGI) was calculated. The GI50 value $\leq$10 µg/ml is considered to demonstrate activity in case of pure compound. This experiment was done in triplicate and the average values were plotted against % control growth versus drug concentrations.

General procedure for the synthesis hexahydroquinoline derivatives: A mixture of dimedone (1 mmol), ammonium acetate (1.2 mmol), [Msim]Cl (10 mol %) and solvent acetonitrile (10 ml) was taken in a 50 ml round bottom flask and stirred at room temperature for 30 minute. Then malononitrile (1 mmol), and substituted salicyaldehyde (1 mmol) were added, and the reaction mixture was stirred at room temperature for appropriate time (Table 2). After the completion of reaction indicated by thin layer chromatography, mixture was diluted with water (15 mL) and extracted with ethylacetate (3 x 4-5mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, petether-EtOAc) to obtain analytically pure product.

2-amino-4-(3,5-dibromo-2-hydroxyphenyl)-1,4,5,6,7,8-hexahydro-7,7-dimethyl-5-oxoquinoline-3-carbonitrile (5c): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.20 (br s, 1H, NH), 7.13-7.27 (m, 2H, Ar-H), 5.98 (s, 2H, NH_2), 5.47 (s, 1H, OH), 4.39(s, 1H, CH), 2.46-2.78 (m, 4H, 2x CH_2), 1.02 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CDCl3): δ 27.6, 29.8, 33.4, 45.6, 54.2, 59.8, 110.2, 114.5, 118.4, 121.2, 125.8, 130.2, 133.7, 147.5, 151.2, 159.7, 195.2; GC-MS, m/z: 467 (M^+).

2-amino-4-(3,5-dichloro-2-hydroxyphenyl)-1,4,5,6,7,8-hexahydro-7,7-dimethyl-5-oxoquinoline-3-carbonitrile (5i): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.95 (br s, 1H, NH), 6.97-7.18 (m, 2H, Ar-H), 5.87 (s, 2H, NH_2), 5.40 (s, 1H, OH), 4.32(s, 1H, CH), 2.39-2.68 (m, 4H, 2x CH_2), 1.01 (s, 3H, CH_3), 0.92 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CDCl3): δ 27.4, 29.2, 33.0, 42.7, 49.8, 58.0, 110.6, 115.0, 122.1, 124.8, 128.2, 130.4, 134.2, 146.5, 150.4, 160.5, 194.1; GC-MS, m/z: 377 (M^+).

Conclusion

In conclusion, we have developed a facile synthesis of hexahydroquionoline derivatives by the reaction of a substituted salicylaldehyde, dimedone, malononitrile and ammonium acetate in presence of ionic liquid [Msim]Cl as catalyst. This modified methodology offers improved performance over the many conventional methods. The remarkable features of this protocol are easy work up, use of environmentally benign catalyst, short reaction time and excellent yields of corresponding derivatives. All the synthesized derivatives were evaluated for their anticancer activities. The initial assays reveals that some of the newly synthesized

compounds showed significantly good inhibition activities against human breast cancer cell (MCF7), cell lines compared with the control (Adriamysin), which might be developed as novel lead scaffold for potential anticancer agents.

Acknowledgements
We are thankful to Dr. P. L. More, Principal, Dr. W. N. Jadhav, Dnyanopasak College, Parbhani and Dr. Balasaheb Chavan, Principal, Yogeshwari Mahavidyalaya, Ambajogai for providing necessary facilities to the research work. We are also thankful to Tata Memorial Centre Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi Mumbai for providing anticancer activity.

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Received on October 24, 2016.