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ONE-POT SYNTHESIS OF 2-(CYCLOHEXYLAMINO)-6,7-DIHYDRO-3-(2-HYDROXYPHENYL)-6,6-DIMETHYL-1*H*-INDOL-4(5H)-ONES AS POTENTIAL ANTICANCER AGENTS

Suresh C. Jadhavar, Hanmant M. Kasaraliker, Bhimrao C. Khade and Sudhakar R. Bhusare*

Department of Chemistry, Dnyanopasak College, Parbhani-431 401, MS, India.

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***Correspondence for Author**
Dr. Sudhakar R. Bhusare
Department of Chemistry,
Dnyanopasak College,
Parbhani-431 401, MS,
India.

ABSTRACT

An efficient method was described for the synthesis of 2-(cyclohexylamino)-6,7-dihydro-3-(2-hydroxyphenyl)-6,6-dimethyl-1H-indol-4(5H)-ones catalyzed by [Hmim]HSO_4. The one-pot synthesis was achieved *via* reaction of cyclohexyl isocyanide, substituted salicylaldehyde, dimedone and ammonium acetate in ethanol at room temperature condition. All synthesized derivatives were evaluated for inhibition of cancer cell. The obtained derivatives were evaluated for their in vitro antitumor activity against MCF-7 cell lines compared to the reference drug (Adrimycin). Compounds were found to be the most active against cell lines exhibiting IC50, TGI and GI50 values ranging MCF-7 cell lines.

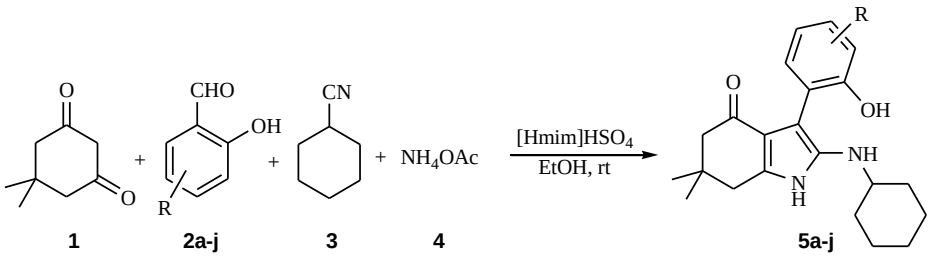
KEYWORDS: Anticancer activity ● Salicylaldehyde ● Cyclohexyl isocyanide ● Dimedone ● Indole-4(H)-ones.

INTRODUCTION

Multi-component reactions (MCRs) are effective and valuable synthetic method to produce complex molecules from simple precursors by a one-pot procedure. Since they are performed without the need to isolate any intermediate during their processes, this reduces the time and saves both energy and raw materials. MCRs play a key role in modern drug discovery processes.[1] Therefore, the development of MCRs has attracted remarkable attention from research scientists working in the field of medicinal chemistry, materials science and drug discovery.[2-4] The synthesis of indole derivatives are of much significance as they are extensively distributed in nature and reveal a wide range of biological activities.[5-7] Indoles

with aminoalkyl or aryl substituents at the 3-position are considered as venerable pharmacophores in drug discovery[8] and are found in several natural products[9] such as 5-HT1B/1D with receptor agonist activities used in the treatment of migraine, aromatase inhibitor for breast cancer[10] and HIV-1 integrase inhibitors[11-12] Gramine, Ergine, and Sumatriptant. Substituted indoles have a range of biological activity, for example antibacterial, antioxidative, and insecticidal[13], and some of the indole derivatives have been employed as antibiotics in pharmaceuticals.[14]

Indole derivatives have been explored for the anticancer activities. Several indole derivatives have shown tyrosine kinase inhibition in low micromolar range.[15] 3-Substituted 2,2'-dithiobis-(1H-indoles) have been reported to show inhibition against protein tyrosine kinases (PTKs), such as EGFR and non receptor v-Src tyrosine kinases.[16] Indole-3-carbinols have been reported earlier to reveal the anticancer activities against a number of human cancers through acting on different cellular signaling pathways.[17] 1-Aroylindoles and 3-aroylindoles have shown potent cytotoxicity against different human cancer cell lines.[18] The huge potential of indole nucleus as drug candidates prompted among the synthetic chemists to investigate the different methodology appropriate for the synthesis of indole derivatives. Herein, we report a an effcient methodology for the synthesis of indole derivatives by the reaction of cyclohexyl isocyanide, substituted salicylaldehyde, dimedone and ammonium acetate using ionic liquid [Hmim]HSO_4 at room temperature condition (**Scheme 1**).



Scheme 1

MATERIALS AND METHODS

Experimental

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. 1H spectra were recorded on a Bruker 300 MHz spectrometer in CDCl3 solvent and TMS as an internal standard. 13C NMR spectra

were recorded on a Bruker-300 MHz spectrometer in CDCl3 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 plates 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.

Preparation of 2-(cyclohexylamino)-6,7-dihydro-3-(2-hydroxyphenyl)-6,6-dimethyl-1H-indol-4(5H)-ones: A mixture of substituted salicylaldehyde (1 mmol), dimedone (1 mmol), ammonium acetate (1.5 mmol) and ionic liquid [Hmim]HSO_4 (10 mol %) in acetonitrile solvent (10mL) was stirred at room temperature for 30 min followed by addition of cyclohexyl isocyanide (1 mmol) and stirring is continued till the completion of the reaction as indicated by thin layer chromatography (Table 2). After the completion of reaction, mixture was diluted with water (15 mL) and extracted with diethylether (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-(cyclohexylamino)-6,7-dihydro-3-(2-hydroxy-3,5-diiodophenyl)-6,6-dimethyl-1H-indol-4(5H)-one (5c): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94(s, 3H, CH3), 1.03(s, 3H, CH3), 1.38-1.51(m, 6H, 3x CH2), 1.62-1.80(m, 4H, 2x CH2), 2.32-2.51 (m, 4H, 2x CH2), 2.72(m,

1H, CH), 5.35 (s, 1H, OH), 7.32-7.52(m, 2H, Ar-H), 8.35 (s, 1H, NH), 9.64(s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3): δ 24.2, 27.0, 29.4, 33.0, 36.1, 46.8, 50.4, 54.9, 92.4, 96.2, 115.2, 122.8, 125.4, 129.8, 138.0, 143.2, 147.4, 165.3, 194.5; GC-MS, m/z: 604 (M+).

3-(3,5-dichloro-2-hydroxyphenyl)-2-(cyclohexylamino)-6,7-dihydro-6,6-dimethyl-1H-indol-4(5H)-one (5h): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98(s, 3H, CH3), 1.09(s, 3H, CH3), 1.41-1.58 (m, 6H, 3 x CH2), 1.70-1.84(m, 4H, 2x CH2), 2.39-2.56 (m, 4H, 2 x CH2), 2.68(m, 1H, CH), 5.30 (s, 1H, OH), 7.20-7.35(m, 2H, Ar-H), 8.24 (s, 1H, NH), 9.42 (s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3): δ 23.9, 27.4, 29.0, 32.9, 35.7, 48.0, 51.8, 56.2, 115.2, 120.2, 123.8, 126.4, 128.2, 129.8, 133.1, 136.2, 143.1, 160.1, 192.9; GC-MS, m/z: 420 (M+).

RESULTS AND DISCUSSION

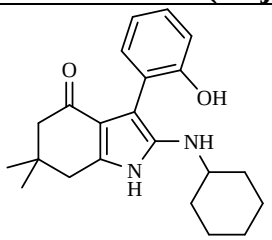
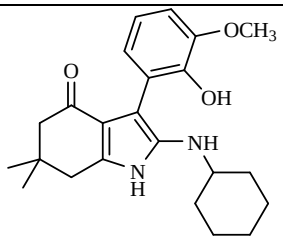
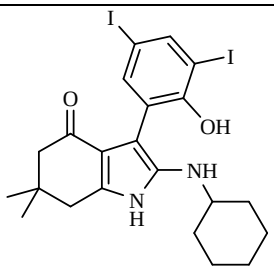
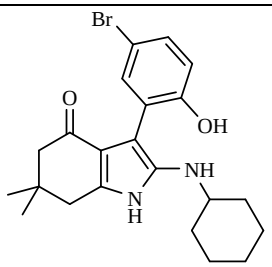
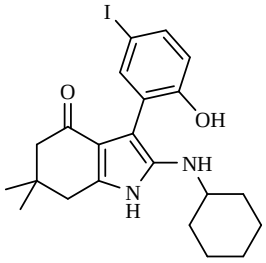
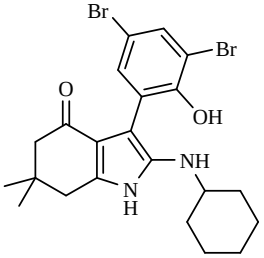
Initially we screened the effect of solvent on the synthesis of indole derivatives at room temperature condition with the model reaction of dimedone, 3-methoxysalicylaldehyde, cyclohexyl isocyanide and ammonium acetate to give corresponding product 5b using 10 mol% of ionic liquid $[Hmim]HSO_4$ as catalyst. The results are listed in Table 1 (Entries 1-7). Among different solvents utilized, $[Hmim]HSO_4$ offered excellent yield of corresponding product in solvent ethanol (Table 1, Entry 2). Among the other solvents, methanol and acetonitrile gave good yield of product in 69 & 66 % respectively (Table 1, Entries 1 and 3 respectively). The reaction in solvents dichloromethane and toluene afforded 27 and 38 % product yield respectively with extended reaction time (Table 1, Entry 4 and 5). Moreover we also performed the reaction in solvents DMF and water. The model reaction afforded lower yield of corresponding product (Table 1, Entry 6 and 7 respectively).

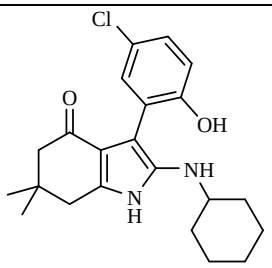
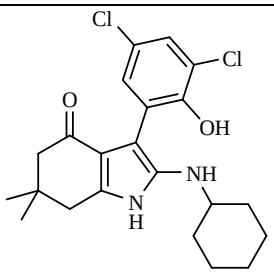
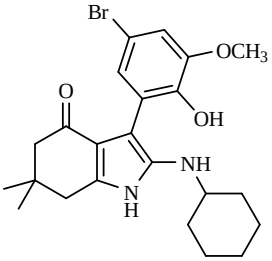
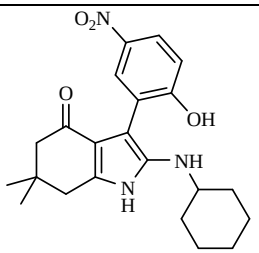
Table 1: The screening of solvents for the synthesis of indole derivatives[a]

Sr. No.	Solvent	Catalyst (mol %)	Time (h)	Yield %
1	MeOH	10	4.30	69
2	**EtOH**	**10**	**3.00**	**92**
3	CH_3CN	10	5.00	66
4	DCM	10	8.30	27
5	Tolune	10	8.00	38
6	DMF	10	7.50	44
7	H_2O	10	7.00	36

[a]Conditions: 3-methoxy salicylaldehyde (1 mmol), dimedone (1 mmol), cyclohexyl isocyanide (1mmol), ammonium acetate (1.5 mmol), solvent (10 mL), $[Hmim]HSO_4$ (10 mol %) at room temperature condition. Reaction was monitored by thin layer chromatography.

Table 2: One-pot four component synthesis of 2-(cyclohexylamino)-6,7-dihydro-3-aryl-1H-indole-4(5H)-ones using [Hmim]HSO_4 catalyst.[a]

Sr. No.	Products (5a-j)	Time (h)	Mp. (^{o} C)	Yield[b] (%)
1	 5a	3.50	210-214	90
2	 5b	3	202-206	92
3	 5c	3.30	265-267	85
4	 5d	3.20	223-227	86
5	 5e	3.30	245-248	83
6	 5f	3.00	238-242	84

7	 5g	3.20	216-220	88
8	 5h	3.00	232-237	85
9	 5i	3.30	207-209	89
10	 5j	3.50	250-252	82

[a]Conditions: Substituted salicylaldehyde (1 mmol), dimedone (1 mmol), cyclohexyl isocyanide (1mmol), ammonium acetate (1.5 mmol), EtOH (10 mL), $[Hmim]HSO_4$ (10 mol %) at room temperature condition. Reaction was monitored by thin layer chromatography. [b]Isolated Yield.

Therefore as a results, we continued our study in the solvent ethanol at room temperature condition using $[Hmim]HSO_4$ (10 mol%) catalyst. To study the scope of this methodology under the optimized reaction conditions, the different substituted salicylaldehydes were employed to give the corresponding indole derivatives. All the reactions proceeded efficiently with variety of salicylaldehyde having electron donating and electron withdrawing substitutents offering good to excellent yield of the corresponding products. The results are summarized in Table 2 (Table 2, Entries 1-10).

Table 3: In vitro cytotoxic activity of the synthesized quinoline derivatives 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	34.2	19	14	12.4	33	15	12.8	10.4	23.4	14.8	12.2	12.2	30.2	16.3	13	11.7
5b	32.4	17.8	12.4	10.6	31.2	12.8	10.6	8.6	21.2	10.4	8.8	10.4	28.3	13.7	10.6	9.9
5c	33.5	18.4	13.3	11.5	32.3	14.2	11.6	9.6	22.4	12.5	10.3	11.4	29.5	15.1	11.8	10.7
5d	31.3	16.4	11.7	9.7	31.9	15.4	10.9	9.9	19.9	13.3	11.5	10.7	29.8	12.7	11.9	10.3
5e	30.3	18	12.9	11.8	31.3	14.9	11.7	9.3	20.8	14.1	11.9	11.4	29.9	14.7	12.9	10.8
5f	32.2	17.5	12.5	10.6	31.3	13.4	10.8	9.3	20.4	11.7	9.8	10.4	28.5	13.8	10.7	10.1
5g	34.1	18.7	13.7	12.2	32.7	14.4	11.6	9.07	22.8	13.7	11.3	11.9	28.7	14.5	12.3	10.4
5h	31.2	15.4	10.7	10.4	29.7	12.4	9.8	8.7	19.6	18.8	8.7	9.2	27.7	12.6	13.9	9.8
5i	33.3	18.3	12.7	10.4	32.7	17.3	11.7	10.8	10.4	14.3	12.8	11.8	30.7	15.7	12.8	11.7
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	75.9	10.3
5b	>80	72.6	8.9
5c	>80	73.3	9.3
5d	>80	72.7	8.6
5e	>80	70.3	7.3
5f	>80	71.4	8.7
5g	>80	74.5	9.8
5h	>80	69.4	9.4
5i	>80	75.9	10.3
ADR	>80	43.7	<10

I50	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.

We evaluated our compounds for their anti-proliferative properties in vitro against cancer cell lines for human breast cancer cell line MCF7. The test of compounds were examined at various concentrations in a MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Table 3) and the LC50, TGI and GI50 values obtained for each compounds are summarized in Table 3. ADR (Adriamysin or Doxorubicin known drug) compounds

showed cytotoxicity against LC50, TGI and GI50 was used as a reference compound. While most of these compounds showed MCF7 activity shown by LC50, TGI and GI50 values. The good results however were obtained using compounds 5b, 5h (Table 3). Interestingly, all 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 upregulated 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 5b, 5h showed 28.3 and 27.7 inhibition where as for concentration 80 mg/ml for 5b, 5h showed 10.6 and 13.9 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. The compound 5b shows good inhibition activities against human breast cancer cell (MCF7) in **Figure 1**.

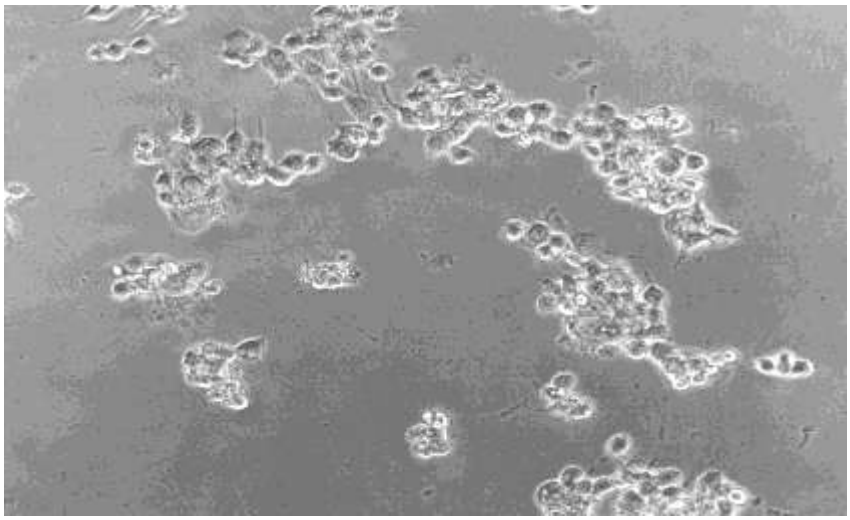


Figure1: MCF7 of 5b

CONCLUSION

In conclusion, we have demonstrated a convenient protocol for the one-pot four component synthesis of indole derivatives by the reaction of dimedone, substituted salicylaldehyde, cyclohexyl isocyanide and ammonium acetate in solvent ethanol using [Hmim]HSO_4 as catalyst. The delightful features of this protocol are use of environmentally benign catalyst, easy work up and excellent yields of corresponding products. All the synthesized derivatives

were evaluated for their anticancer activities. The initial assays reveals that some of the newly synthesized compounds shows 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.

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