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Synthesis and Evaluation of Anticancer Activity of Some New 3-Aminoalkylated Indole Derivatives

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ABSTRACT

An effective and economical protocol was developed for the synthesis of 3-substituted indoles by one-pot three-component coupling reaction of a substituted salicylaldehyde, N-methylaniline and indole using [Hmim] HSO_4 as a catalyst. All the synthesized derivatives were evaluated for inhibition of cancer cell. The initial assays indicated 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, One-pot synthesis, Salicylaldehyde, Indoles, [Hmim] HSO_4, 3-Aminoalkylated indoles.

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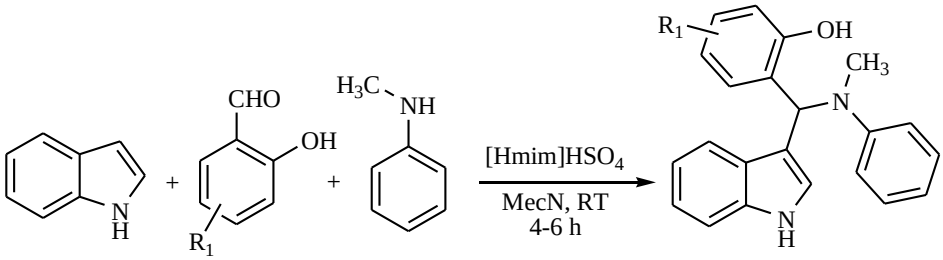
INTRODUCTION

The indole is an important structural design which presented in a variety of natural products and having pharmaceutical interest in a several therapeutic areas [1-2]. 3-Substituted indoles are of great significance as they are widely scattered in nature and having a wide range of biological activities [3]. 3-Substituted indoles with aminoalkyl/aryl substituents at 3-position are considered as impressive pharmacophores [4] and are found in a variety of natural products [5] such as 5-HT1B/1D with receptor agonist activities used in the treatment of migraine, aromatase inhibitor for breast cancer [6] and HIV-1 integrase inhibitors Gramine [7]. They have a variety of biological activities such as antibacterial, anticonvulsant and antihypertensive activity [8]. Some of indoles moiety possess in bioactive metabolites of terrestrial and marine organisms [9-10]. Because of significant chemical and biological properties of indole molecules, development in the efficient protocols for the synthesis of 3-substituted indoles constitutes a growing area in organic synthesis. Nowadays multi-component reactions (MCRs) have received a great importance of organic chemists as they offer remarkable advantages over conventional linear-type synthesis such as in terms of easy work-up procedures and purification, short reaction time, low cost and high atom economy [11-12]. Nowadays, a one-pot method was developed for the synthesis of 3-aminoalkylated indoles by the reaction of aldehyde, amine and indole [13-17]. However, some of the reported methods require prolonged reaction time, high temperature and is generally accompanied by formation of bis-indolyl compound. Herein we describe an efficient method for the synthesis of 3-aminoalkylated indoles using ionic liquid [Hmim]HSO_4 as catalyst under ambient temperature condition (**Scheme 1**). All the synthesized derivatives were evaluated for inhibition of cancer cell.



1 **2** **3** **4a-j**

Scheme 1

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.

Experimental

General procedure for the one-pot synthesis of 3-aminoalkylated indoles: A mixture of salicylaldehyde (1 mmol), N-methylaniline (2 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 indole (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, pet.ether-EtOAc) to obtain analytically pure product.

Anticancer Activity

The anti-cancer activity for these compounds was done in the Anti-cancer drug screening facility (ACDF), Tata memorial centre, A 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.

RESULTS AND DISCUSSION

Initially we studied the effect of solvent on the synthesis of 3-aminoalkylated indoles under the ambient temperature condition using model reaction of indole, salicylaldehyde and N-

methylaniline to give corresponding product 4a in presence of 10 mol% ionic liquid [Hmim]HSO_4. The results are summarized in Table 1 (entries 1-5). Among different solvents used, [Hmim]HSO_4 gave superior yield of corresponding product 4a in solvent acetonitrile (Table 1, entry 3). Among the other solvent, ethanol and methanol gave good yield of 4a in 72 & 62 % respectively of 4a (Table 1, entries 1 and 2). Solvents tetrahydorfuran and dichloromethane afford 58 and 46 % yield respectively with enhanced reaction time (Table 1, entry 4 and 5). Further we also examined the effect of other catalyst on the model reaction in solvent acetonitrile. The model reaction was performed at this optimum condition with other catalyst $FeCl_3$, *L*-proline and $ZnCl_2$ catalyst. With $FeCl_3$ and *L*-proline catalyst, product obtained was good but unsatisfactory when compared to [Hmim]HSO_4 (Table 1, entries 6 and 7). $ZnCl_2$ afforded 56 % product yield with extended reaction time (Table1, entry 8). To establish the significance of [Hmim]HSO_4 as catalyst, a reaction was carried out in the absence of catalyst finding that only 21 % yield of desired product was obtained after 16 hours (Table 1, entry 11).

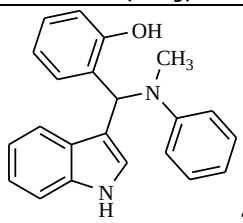
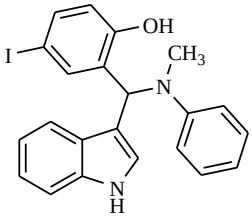
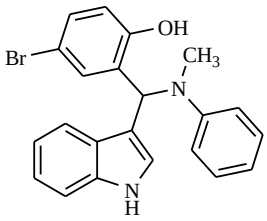
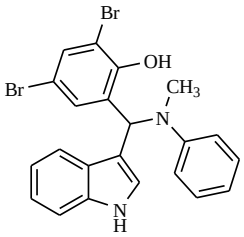
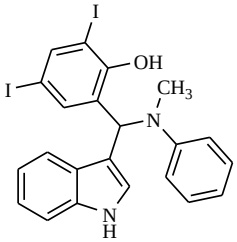
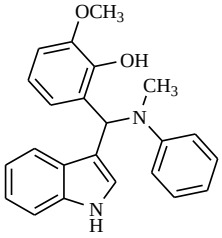
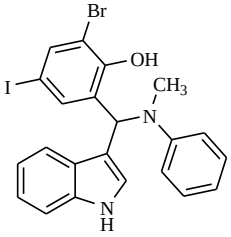
Table 1: Optimization conditions for the synthesis of 3-amino alkylated indole[a]

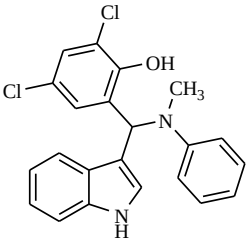
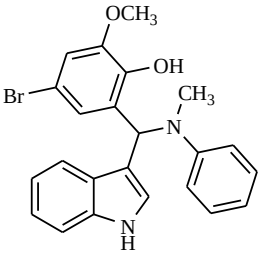
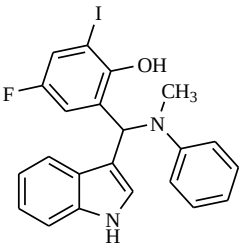
Entry	Solvent	Catalyst (Mol %)	Time (h)	Yield[b] (%)
1	EtOH	[Hmim]HSO_4 (10)	8	72
2	MeOH	[Hmim]HSO_4 (10)	10	62
3	CH_3CN	[Hmim]HSO_4 (10)	6	92
4	THF	[Hmim]HSO_4 (10)	12	58
5	DCM	[Hmim]HSO_4 (10)	14	46
6	CH_3CN	$FeCl_3$(10)	12	64
7	CH_3CN	*L*-Proline (10)	10	60
8	CH_3CN	$ZnCl_2$ (10)	14	56
9	CH_3CN	[Hmim]HSO_4 (5)	8	65
10	CH_3CN	[Hmim]HSO_4 (15)	6	90
11	CH_3CN	none	16	21

[a]Conditions: Salicylaldehyde (1 mmol), N-methyl aniline (2 mmol), indole (1 mmol), catalyst (mol %), Solvent (10 mL) at room temperature. Reaction was monitored by thin layer chromatography.
[b]Isolated yield

At the catalytic loading 5 mol % and 15 mol %, reaction afforded 65 % and 90 % yield respectively (Table 1, entry 9 and 10 respectively). The excellent result for the reaction was offered at the catalytic loading 10 mol% of the catalyst [Hmim]HSO_4 in solvent acetonitrile. The reaction was completed within 6 hours and offered the desired product 4**a** in excellent yield 92% (Table 1, entry 3). Optimistic by these remarkable results, we screened a variety of substituted salicylaldehydes for the synthesis of corresponding 3-substituted indole derivatives. We observed all products are obtained with excellent yields (Table 2).

Table 2: One pot synthesis of 3-aminoalkylated indole derivatives[a]

Entry	Product (4a-j)	Time (h)	Mp (^{o}C)	Yield[b] (%)
1	 **4a**	6	135-138	92
2	 **4b**	5	145-147	90
3	 **4c**	5	139-141	94
4	 **4d**	4	168-170	90
5	 **4e**	5	182-184	88
6	 **4f**	6	143-145	86
7	 **4g**	4	200-202	89

8	 **4h**	6	148-150	90
9	 **4i**	5	158-160	87
10	 **4j**	4	152-154	88

[a]Conditions: Substituted salicylaldehyde (1 mmol), N-methyl aniline (2 mmol), indole (1 mmol), [Hmim]HSO_4 (10 mol %), Acetonitrile (10 mL) at room temperature. Reaction was monitored by thin layer chromatography. [b]Isolated yield.

N-((2-hydroxyphenyl)(1H-indol-3-yl)methyl)-N-methylbenzenamine (4a): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (br s, 1H), 7.38 (d, 2H, *J* = 4.8 Hz), 7.10-7.19 (m, 4H), 6.91-6.98 (m, 6H), 6.68(s, 1H), 6.56 (d, 2H, *J* = 7.2 Hz), 5.80 (s, 1H), 2.86 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 36.2, 58.2, 106.8, 108.2, 111.2, 113.7, 116.0, 118.2, 120.0, 122.3, 124.8, 126.5, 129.0, 129.8, 130.4, 134.2, 138.2, 142.0, 152.0, 158.0; GC-MS, *m*/*z*: 328 (M+); Anal. Calcd for $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53; Found: C, 80.48; H, 6.18; N, 8.56.

N-((5-bromo-2-hydroxyphenyl)(1H-indol-3-yl)methyl)-N-methylbenzenamine (4c): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.43 (d, 2H, *J* = 7.4 Hz), 7.23 (d, 2H, *J* = 4.6 Hz), 7.09-7.16 (m, 5H), 6.84 (d, 2H, *J* = 4.6 Hz), 6.62(s, 1H), 6.52 (d, 2H, *J* = 4.2 Hz), 6.34 (d, 2H, *J* = 7.8 Hz), 5.67 (s, 1H), 2.98 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 38.0, 59.0, 107.2, 109.3, 112.4, 114.6, 116.5, 118.8, 120.7, 122.8, 125.4, 126.9, 129.7, 129.9, 130.2, 135.2, 139.5, 144.2, 152.8, 159.0; GC-MS, *m*/*z*: 407 (M+); Anal. Calcd for $C_{22}H_{19}BrN_2O$: C, 64.87; H, 4.70; N, 6.88; Found: C, 64.90; H, 4.73; N, 6.86.

Table3: Anticancer activity of 3-aminoalkylated indoles against human breast cancer cells

Human Breast Cancer cell line MCF7																
% control growth																
Drug concentration (µg/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
4a	87.8	80.4	60.3	19.5	79.4	81.5	50.4	44.2	83.7	84.2	62.2	53.4	83.6	82.03	57.63	39.03
4b	96.8	88.2	70.3	25.3	88.3	90.4	58.3	52.4	93.7	92.5	70.3	63.1	92.93	90.36	66.3	46.93
4c	91.3	83.4	64.2	20.6	89.3	91.4	51.3	53.2	93.4	92.6	70.9	63.4	91.33	89.13	62.13	45.63
4d	94.7	86.1	67.1	22.5	86.1	87.7	55.3	50.6	91.4	90.3	68.7	61.3	90.7	88.00	63.7	44.8
4e	92.5	84.3	65.4	21.1	83.3	85.7	53.2	48.3	89.7	88.6	65.4	58.6	88.5	86.2	61.3	42.6
4f	86.2	78.4	58.3	17.5	77.4	79.5	48.4	42.2	81.7	82.2	60.2	51.4	81.76	80.03	55.63	37.03
4g	90.2	82.6	63.3	20.4	81.3	83.2	51.8	46.7	86.7	85.9	63.3	56.7	86.	83.9	59.4	41.26
4h	88.3	80.2	60.7	18.4	79.3	81.4	49.7	44.8	83.9	84.8	62.1	54.6	83.83	82.13	57.5	39.26
4i	90.4	82.8	48.4	19.4	84.4	85.8	50.4	44.4	88.6	84.4	64.3	55.8	87.8	86.73	54.3	39.86
4j	98.3	90.8	69.9	27.6	90.3	92.3	60.3	54.6	95.4	94.3	72.4	65.7	94.6	92.46	67.53	49.3
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

Table 2: Drug concentrations µg/ml calculated from graph

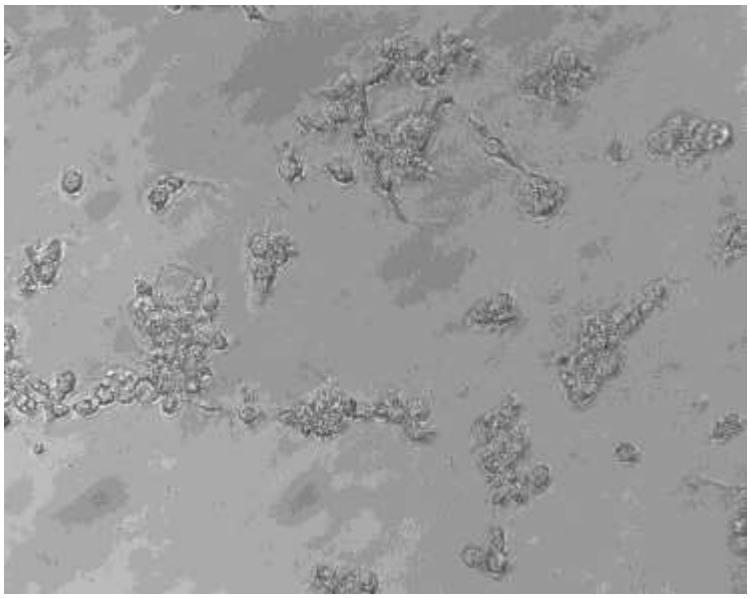
MCF7	LC 50	TGI	GI50
4a	>80	>80	62
4b	>80	>80	73
4c	>80	>80	71
4d	>80	>80	70
4e	>80	>80	66
4f	>80	>80	58
4g	>80	>80	65
4h	>80	>80	62
4i	>80	>80	62
4j	>80	>80	77
ADR	>80	43.7	<10

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.

Cells were plated in 96-multiwell plate (10 cells/ well) for 24 hrs before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different, concentrations of the compounds under test (10, 20, 40, 80 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37 °C and in atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained with Adriamysin (ADR). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each breast cancer cell line after the specified compound was added. IC50, TGI and GI50 values of the tested compounds were calculated by Graph Pad Prism 5 software. All synthesised derivatives show good to moderate activity (MCF7) against human breast cancer cells in Table 3. From above data by comparison with anticancer drug (Adrimycin) above values are good, which are near to known drug Adrimycin. This study provides insights for further optimizing of substituted indoles for the discovery of the anticancer activity.



MCF7 of 4d

CONCLUSION

In conclusion, we have developed a facile and convenient synthesis of 3-aminoalkylated indoles by one-pot three-component reaction of a substituted salicylaldehyde, N-methylaniline and indole in presence of ionic liquid [Hmim] HSO_4 as catalyst. This modified method offers improved performance over the many conventional methods. The delightful features of this protocol are easy work up, use of environmentally benign catalyst, short reaction time and excellent yields of 3-aminoalkylated indoles. All the synthesized derivatives were evaluated for their anticancer activities. The initial assays indicated 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.

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