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A Facile Synthesis of 2-(2-hydroxyaryl)quinazolin-4(3H)-ones and Their Anticancer Activity Evaluation against MCF-7 Cells

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ABSTRACT

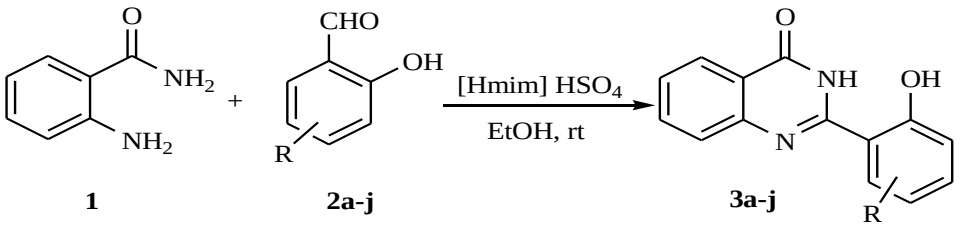
A convenient method was established for the preparation of 2-(2-hydroxyaryl)quinazolin-4(3H)-one derivatives *via* reaction of *o*-aminobenzamide and substituted salicylaldehyde using [Hmim]HSO_4 (10 mol %) as a catalyst. All prepared derivatives were tested for inhibition of cancer cell. The primary assays reveals that some of the prepared derivatives present considerably good inhibition activities against human breast cancer cell (MCF7) compared with the control (Adriamysin) which might be extended as lead scaffold for potential anticancer agents.

Keywords: Anticancer activity, Salicylaldehyde, *o*-aminobenzamide, [Hmim]HSO_4, 2-(2-hydroxyaryl)quinazolin-4(3H)-ones

INTRODUCTION

Heterocyclic synthesis is a noteworthy component of the organic chemistry due to the wide range of biological active hetero molecules. Among nitrogen heterocycles, quinazoline nucleus concern most important position with two nitrogen atoms in their structure and is generally observed in a broad range of synthetic pharmaceutical substances, natural products and additional functional materials [1]. Quinazoline derivatives have a various biological activities such as antioxidant [2], anticonvulsant [3], anticancer [4], antitubercular [5], antiviral [6], anti-HIV [7], anti-inflammatory [8], antimicrobial [9] and analgesic [10]. Moreover several studies have been conducted to explore the pharmacokinetics and toxicity of new quinazoline-based derivatives in different animal model to confirm the protected nature of their synthesized derivatives [11]. Quinazolines nucleus come out as building blocks for variety of alkaloids, noticed across the plant and animal kingdoms as well as numerous microorganisms such as *Bacillus cereus* [12], *Bouchardatia neurococca* [13], *Dichroa febrifuga* [14] and *Peganum nigellastrum* [15]. Due to broad scope of biological activities afforded by the quinazoline nucleus, drugs like gefitinib (Iressa) and erlotinib (Tarceva) enhanced awareness in the expansion of new synthetic approaches for the quinazoline derivatives.

Various synthetic approaches have been developed for the synthesis of quinazoline derivatives [16] and quinazolinone derivatives [17,18]. However, most of the methods require higher temperatures for the reaction. Due to awareness in the expansion for the heterocyclic synthesis catalyzed by ionic liquid, here we explain a convenient synthesis of 2-(2-hydroxyphenyl)quinazolin-4(3H)-one derivatives starting from *o*-aminobenzamide and substituted salicylaldehyde using [Hmim]HSO_4 as a catalyst at ambient temperature condition (Scheme 1).



Scheme 1: Heterocyclic synthesis

MATERIALS AND METHODS

The column chromatography was performed over silica gel (80-120 mesh). Melting points of all synthesized derivatives were carried out in open capillary tube and are uncorrected.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon-13-Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded on Bruker-300 MHz spectrometer in solvent Deuterated Chloroform ($CDCl_3$) with Tetramethylsilane (TMS) as an internal standard. Mass spectra were carried out on Polaris Q Thermo scientific GC-MS spectrometer.

Anticancer activity

The anticancer activity of synthesized derivatives was carried out in the Anticancer Drug Screening Facility, Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC). The *in vitro* anticancer activity of all synthesized molecules tested using Adriamysin (ADR) as a standard drug and sulforhodamine B assay protocol as precisely illustrated by Skehan et al. The human breast cancer cell (MCF-7) in 96-well plate deal with distinct concentrations of corresponding molecules (10, 20, 40 and 80 µg/ml). Afterward the treatment, the cells were fixed in trichloroacetic acid and stained in presence of sulforhodamine B (0.4% w/v) prepared in 1% acetic acid for 30 min. Four washes with 1% acetic acid were given to remove unbound dye. 10 mM unbuffered tris base was utilized to extract protein bound dye and subjected for microtiter plate reader. The absorbance of dye was calculated at wavelength 565 nm. The absorbance is correlated with the net protein synthesis rate. The 50% inhibition of cell growth (GI_{50}), 50% lethal concentration (LC_{50}) and 100% Total Growth Inhibition (TGI) was measured. The GI_{50} value <10 µg/ml is considered to reveal activity in case of pure compound. This testing was carried out in triplicate. The average values were plotted against % control growth versus drug concentrations.

EXPERIMENTAL SECTION

General procedure for the synthesis 2-(2-hydroxyphenyl)quinazolin-4(3H)-one derivatives

In round bottom flask, a mixture of substituted salicylaldehyde (1 mmol), o-aminobenzamide (1 mmol), $[Hmim]HSO_4$ (10 mol %) and solvent ethanol (10 ml) was stirred at ambient temperature for suitable time. After the completion of reaction indicated by Thin Layer Chromatography (TLC), the reaction mixture was diluted with water (15 ml) and extracted with diethyl ether (3 × 4-5 ml). The organic phase was dried over magnesium sulphate and evaporated under reduced pressure. The corresponding crude product was purified by column chromatography to get pure corresponding product.

2-(5-bromo-2-hydroxyphenyl)quinazolin-4(3H)-one (3d)

^{1}H-NMR(300 MHz, $CDCl_3$): δ=10.58 (s, 1H), 7.48-7.72 (m, 4H), 6.97-7.09 (m, 3H), 5.39 (s, 1H); ^{13}C-NMR (300 MHz, $CDCl_3$): δ=112.4, 116.0, 120.4, 124.1, 126.8, 130.2, 130.8, 132.5, 134.1, 136.8, 150.2, 154.5, 159.0, 163.8; GC-MS, m/z: 317 (M^+); Elemental Analysis: Anal. Calcd for $C_{14}H_9BrN_2O_2$: C, 53.02; H, 2.86; N, 8.83; Found C, 53.05; H, 2.89; N, 8.88.

2-(2-hydroxy-3,5-diiodophenyl)quinazolin-4(3H)-one (3e)

^{1}H-NMR (300 MHz, $CDCl_3$): δ=10.72 (s, 1H), 7.62-7.90 (m, 4H), 7.02-7.12 (m, 2H), 5.50 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ=93.5, 98.0, 108.2, 114.7, 119.5, 124.0, 127.8, 130.0, 133.1, 142.0, 148.9, 154.2, 160.4, 168.0; GC-MS, m/z: 490 (M^+); Elemental Analysis: Anal. Calcd for $C_{14}H_8I_2N_2O_2$: C, 34.31; H, 1.65; N, 5.72; Found C, 34.35; H, 1.67; N, 5.75.

RESULTS AND DISCUSSION

Initially we examined the some catalyst with (10 mol %) on the model reaction of 5-bromo salicylaldehyde and o-amino benzamide in ethanol solvent at ambient temperature (Table 1, Entries 1-6). The model reaction was performed at the room temperature with different catalyst such as $ZnCl_2$, $Sc(OTf)_3$, $SnCl_2$, *L*-proline, [Msim]Cl and $[Hmim]HSO_4$ catalyst. With $ZnCl_2$ and $Sc(OTf)_3$ catalyst product obtained was good but unsatisfactory when compared to ionic liquid $[Hmim]HSO_4$ (Table 1, Entries 1 and 2 respectively). The catalyst $SnCl_2$ and *L*-proline offered 55 and 68% corresponding product yield (Table 1, Entries 3 and 4 respectively). We observed that desired product 3d was obtained with excellent yield in presence of catalyst $[Hmim]HSO_4$. The reaction afforded 91% excellent yield in short reaction time 3.5 h (Table 1, Entry 6). The other ionic liquid [Msim]Cl shows less effectiveness as compared to $[Hmim]HSO_4$ (Table 1, Entry 5). Subsequently, we studied the influence of solvents on model reaction with 10 mol % $[Hmim]HSO_4$ catalyst (Table 1, Entry 7-11). We observed that solvent ethanol was worthy solvent as compared to other solvents such as dichloromethane, methanol, acetonitrile, tolune and water considering both reaction time and product yield. The corresponding outcome given in Table 1 (Entry 7-11).

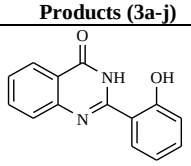
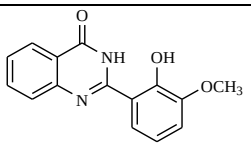
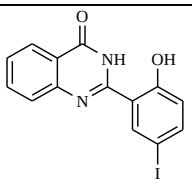
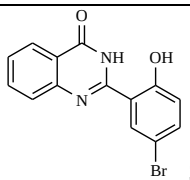
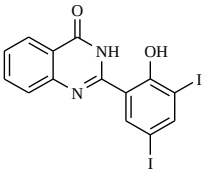
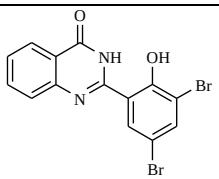
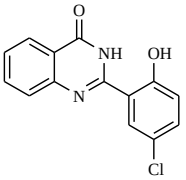
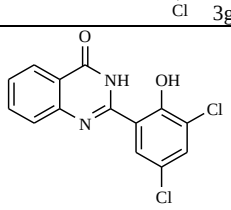
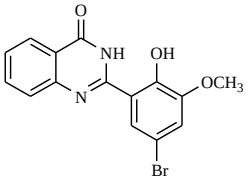
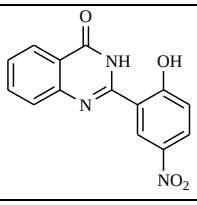
Table 1: The screening for the synthesis of 2-(5-bromo-2-hydroxyphenyl)quinazolin-4(3H)-one

S. No.	Catalyst	Solvent	Catalyst mol %	Reaction time (h)	Yield[a] (%)
1	$ZnCl_2$	EtOH	10	5.3	59
2	$Sc(OTf)_3$	EtOH	10	5.0	62
3	$SnCl_2$	EtOH	10	6.0	55
4	*L*-Proline	EtOH	10	5.5	68
5	[Msim]Cl	EtOH	10	5.0	72
6	**$[Hmim]HSO_4$**	**EtOH**	**10**	**3.5**	**91**
7	$[Hmim]HSO_4$	MeOH	10	5.0	75
8	$[Hmim]HSO_4$	DCM	10	8.0	31
9	$[Hmim]HSO_4$	Tolune	10	8.5	24
10	$[Hmim]HSO_4$	MeCN	10	5.0	76
11	$[Hmim]HSO_4$	H_2O	10	9.0	38

[a]Isolated yield

Optimistic by these results, we employed a several substituted salicylaldehyde and in each case we observed good to excellent yields (Table 2, Entries 1-10).

Table 2: An efficient synthesis 2-(2-hydroxyphenyl)quinazolin-4(3H)-one derivatives

S. No.	Products (3a-j)	Reaction time (h)	Melting point (°C)	Yield[a] (%)
1	 3a	3	210-211	90
2	 3b	3.15	197-199	93
3	 3c	3.25	229-231	87
4	 3d	3.30	223-225	91
5	 3e	3.35	245-248	85
6	 3f	3.05	238-240	88
7	 3g	3.45	216-218	90
8	 3h	3.20	232-234	92
9	 3i	3.30	205-207	88
10	 3j	3.50	256-258	86

[a]Isolated yield

Biological evaluation

Considering the precedence of known anticancer activity of known 2-(2-hydroxy phenyl) quinazolin-4(3H)-ones, we were concerned to check the *in vitro* anti-cancer. We tested corresponding derivatives for their antiproliferative properties *in vitro* against cancer cell lines for human Breast cancer cell (MCF-7). The synthesized molecules were scrutinized at several concentrations in a 3-(4,5-Dimethyl Thiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) assay. The LC_{50}, GI_{50} and TGI values obtained for each molecule are given in Table 3. Adriamycin drug showed cytotoxicity against LC_{50}, GI_{50} and TGI was used as a standard compound. Though most of these synthesized derivatives reveal the MCF-7 activity as shown by LC_{50}, GI_{50} and TGI values.

Table 3: *In vitro* cytotoxicity of the 2-(2-hydroxyphenyl)quinazolin-4(3H)-ones against human breast cancer cell line (MCF7)

Human breast cancer cell line (MCF-7)																
% 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
3a	46	27.9	17.4	13.5	44.9	31.7	23.1	20	44.6	28	16.7	15.8	45.2	29.2	19.1	16.4
3b	58.3	39.2	28.3	21.4	58.6	42.3	34.7	32.3	56.4	39.4	28.3	27.4	57.6	55.7	30.4	27
3c	56.4	37.3	26.3	19.3	56.3	40.3	33.6	30.1	54.5	37.3	26.4	25.3	55.7	38.3	28.7	24.9
3d	50.1	31..2	20.2	15.7	48.1	34.3	27.4	24.1	48.2	31.4	20.3	19.3	48.8	32.3	28.6	19.7
3e	40	25	15.2	10.2	36.6	30.3	21.3	17.4	38.8	27.8	14.3	10.9	38.4	27.7	16.9	12.8
3f	54.3	35.4	24.3	17.8	54.4	38.1	31.3	28.4	52.4	35.3	24.1	23.3	53.7	36.2	26.4	23.1
3g	48.2	29.1	18.7	14.8	46.3	32.9	25.4	22.3	46.7	29.7	18.3	17.1	47	30.5	20.8	18
3h	43.1	26.2	16.3	11.7	40.4	31.1	22.2	18.7	41.3	28.3	15.7	12.8	41.6	32.8	18	14.4
3i	52.2	33.2	22.4	16.3	52.1	36.3	29.4	26.3	50.6	33.3	22.4	21.3	51.6	34.2	24.3	21.3
3j	60.3	41.4	30.3	23.1	61.1	44.1	36.2	34.4	58.4	41.3	30.2	29.4	59.9	42.2	32.2	28.9
ADR	5.7	4.1	-0.8	-30	1.4	5	-2.2	-32	1.2	6.2	2.5	-36	2.8	5.1	-0.2	-32.7

Drug concentration mg/ml calculated from graph			
MCF-7	**LC_{50}**	**TGI**	**GI_{50}**
3a	>80	>80	20.9
3b	>80	>80	34.4
3c	>80	>80	31.7
3d	>80	>80	25.1
3e	70.7	67.9	16.3
3f	>80	>80	29.4
3g	>80	>80	22.9
3h	75.3	73.62	18.3
3i	>80	>80	26.4
3j	>80	>80	36.8
ADR	>80	43.7	<10
GI_{50}	Growth inhibition of 5% considered from [(Ti-Tz)/(C-Tz)] × 100=50, drug concentration ensuing in 50% reduction in net protein increase		
TGI	Total growth inhibition calculated from Ti=Tz		
LC_{50}	Lethal concentrations is calculated from [(Ti-Tz)/Tz] × 100=-50.		

The notable results were achieved using molecules 3e, 3h (Table 3). All tested compounds were found to be active against breast cancer cells and reveals worthy activities against breast cancer cells. To recognize the process of action, some synthesized derivatives were tested their inhibitory potential against sirtuins. Inhibition of sirtuins permits the reexpression of silenced tumor suppressor genes leading to diminished growth of cancer cells. The activity of tested derivatives was established by Sirt1 fluorescence activity assay using suramin a known inhibitor of Sirt1 as a reference compound. At the concentration of 10 mg/ml, the compounds 3e, 3h gives 38 and 41 inhibition respectively. While for the concentration at 80 mg/ml, the compounds 3e, 3h gives 12 and 14 inhibition respectively. Compared to ADR 2.8 and -32.4 inhibition shows that the anti-cancer properties of these compounds are probably owing to their sirtuin inhibiting properties. The molecule 3b shows notable inhibition activities against human breast cancer cell (MCF-7) in Figure 1.

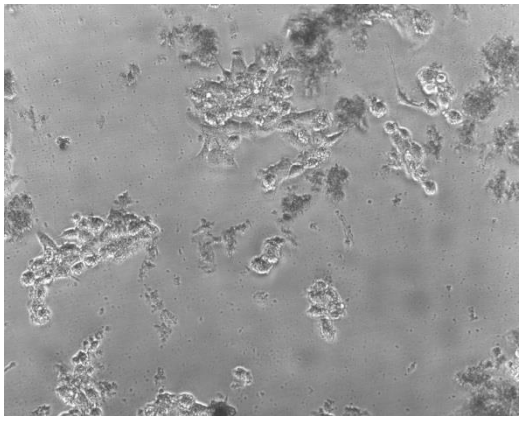


Figure 1: MCF-7 of 3b

CONCLUSION

In conclusion, we established an efficient synthesis 2-(2-hydroxyphenyl)quinazolin-4(3H)-ones starting from substituted salicylaldehyde and *o*-benzamide catalyzed by ionic liquid [Hmim]HSO_4. The satisfying features of this methodology are simple work-up, environmentally kind catalyst, short reaction time and superior yield of 2-(2-hydroxyphenyl)quinazolin-4(3H)-ones. The starting assays showed that some synthesized derivatives presented extensively notable inhibition activities against human breast cancer cell lines (MCF-7) compared with the standard ADR, which might be resulted as novel lead scaffold for potential anti-cancer agents.

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