Chemistry & Biology Interface, 2015, 5, 3, 226-232

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

[Hmim]HSO_4 Catalyzed an Expedient One-pot Synthesis of Quinazoline Derivatives as Potential Anticancer Agents

Suresh C. Jadhavar, Hanmant M. Kasaraliker, Shrikant S. Pendalwar and Sudhakar R. Bhusare*

Department of Chemistry, Dnyanopasak College, Parbhani-431 401, MS, India
E-mail: bhusare71@yahoo.com
Received 6 May 2015; Accepted 14 June 2015

Abstract: An expedient approach for the synthesis of quinazoline derivatives was developed by the one-pot three-component reaction of 2-chloro acetophenone, aqueous ammonia and substituted salicylaldehyde catalyzed by ionic liquid [Hmim]HSO_4. Further their biological evaluations as anticancer agent are studied against the Brest cancer cell lines. 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 $IC_{50,}$ TGI and GI50 values ranging MCF-7 cell lines.

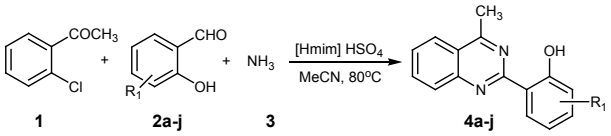
Keywords: Anticancer activity, 2-Chloro acetophenone, Salicylaldehyde, [Hmim]HSO_4, Quinazolines.

Introduction

The quinazoline moiety generally exists in a variety of naturally occurring alkaloids presenting a broad range of the biological activities [1]. Quinazolines retains an important place in synthetic organic chemistry for their significant appliances in clinical, chemical and biological area. Medicinally quinazoline has been applied in several areas such as an anti-oxidant[2], analgesic[3], anti-inflammatory [4], anti-cancer drugs[5-7], anti-bacterial[8], anti-convulsant[9], anti-mycobacterial [10-11] and anti-fungal[12] agents. It has also been found in the treatment of malaria[13-14]. Because of the availability of quinazoline nucleus in several naturally occurring alkaloids, it has a huge significance to chemists and biologists.

Multicomponent reactions (MCRs) are much essential for the construction of many heterocycles and this methodology has been applied efficiently in the synthesis of several bioactive substances and natural products [15-

16]. In continuation of our interest on MCRs [17], herein we have developed an efficient approach for the synthesis of quinazoline derivatives with excellent yields using ionic liquid [Hmim]HSO_4 as a catalyst. The synthesis of quinazolines was achieved from the reaction of 2-chloroacetophenone, substituted salicylaldehyde and aqueous ammonia in a solvent acetonitrile at 80 °C (**Scheme** 1).



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. ^1H 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 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.

General procedure for the synthesis of quinazoline derivatives: In 50ml round-bottom flask, a mixture of 2-chloro acetophenone (1 mmol), substituted salicyaldehyde (1mmol) and 25% aqueous ammonia (1 ml) were added in solvent acetonitrile (15 ml). Then catalytic amount of [Hmim]HSO_4 (10 mol %) was added to the reaction mixture. The reaction mixture was then heated in oil bath at 80 ^oC for appropriate time (Table 2). After the completion of reaction indicated by thin layer chromatography, the reaction mixture was cooled to room temperature and the reaction mixture was extracted with ethyl acetate (3 x 4-5 ml). Organic layer was washed thoroughly with saturated NaCl aqueous solution, 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.

2,4-diiodo-6-(4-methylquinazolin-2-yl) phenol (4b): ^1H NMR (300 MHz, $CDCl_3$): δ 7.68-7.91 (d, 2H, *J* = 7.8 Hz), 7.38-7.52 (t, 2H, *J* = 7.4 Hz), 7.02-7.13 (d, 2H, *J* = 7.8 Hz), 5.30 (s, 1H, OH), 2.54 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CDCl3): δ 25.2, 95.1, 98.2, 120.8, 124.7,

126.0, 128.2, 130.7, 134.0, 138.6, 146.2, 152.1, 158.1, 164.2, 166.4; GC-MS, m/z: 487 (M+).

2,4-dichloro-6-(4-methylquinazolin-2-yl) phenol (4g): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72-7.98 (d, 2H, *J* = 7.6 Hz), 7.42-7.59 (t, 2H, *J* = 7.2Hz), 7.08-7.21 (d, 2H, *J* = 7.6 Hz), 5.37 (s, 1H, OH), 2.54 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CDCl3): δ 26.4, 96.4, 99.0., 121.2, 124.9, 127.2, 129.8, 132.0, 135.1, 139.4, 144.8, 153.4, 159.7, 163.4, 167.5; GC-MS, m/z: 304 (M+).

2-(4-methylquinazolin-2-yl)-4-nitrophenol (4j): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62-7.80 (m, 3H), 7.35-7.51 (t, 2H, *J* = 7.0 Hz), 7.16-7.28 (d, 2H, *J* = 7.8 Hz), 5.42 (s, 1H, OH), 2.71 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CDCl3): δ 26.4, 96.4, 99.0., 121.2, 124.9, 127.2, 129.8, 132.0, 135.1, 139.4, 144.8, 153.4, 159.7, 163.4, 167.5; GC-MS, m/z: 281 (M+).

Results and discussion

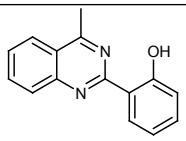
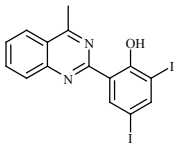
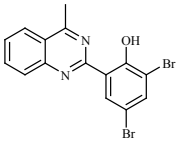
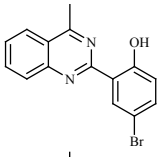
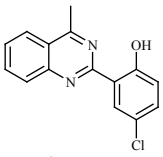
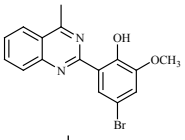
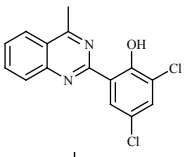
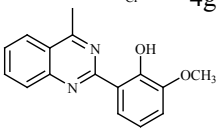
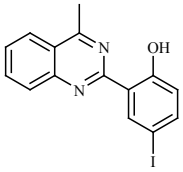
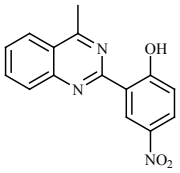
Herein we describe the synthesis of quinazoline derivatives *via* ionic liquid [Hmim]HSO_4 catalyzed reaction of 2-chloroacetophenone, substituted salicyaldehyde and 25% aqueous ammonia in solvent acetonitrile at 80 ^{o}C temperature. Initially, we studied the optimization of suitable solvent and catalytic concentration for the model reaction of 2-chloroacetophenone, 5-chloro-salicyaldehyde and 25% aqueous ammonia at 80 ^{o}C temperature. Various solvents were used to test the efficiency of ionic liquid [Hmim]HSO_4 (10 mol%) and the results are summarized in Table 1 (Entries 1-5). The optimum result was found in acetonitrile solvent, in which the catalyst worked most effectively (Table 1, Entry 4). Moreover the model reaction in solvent ethanol and methanol offered 72% and 64% yield respectively (Table 1, Entries 1 and 2). In the solvent dimethyl formamide and dichloromethane, the desired product was obtained in poor yields with extended reaction time (Table 1, Entries 5 and 6). We also examine the effect of catalytic loading on the reaction in solvent acetonitrile. On reducing the amount of catalytic [Hmim]HSO_4 to 5 mol%, the reaction afford the lower yield (69%) with extended reaction time (7 h) (Table 1, Entry 7). When catalytic amount was raised to 15 mol%, no improvement was observed with respect to the yield and reaction time (Table 1, Entry 8). Therefore the best optimized reaction condition was achieved at the catalytic amount 10 mol% of [Hmim]HSO_4 in acetonitrile at 80 ^{o}C. The model reaction afforded desired product in excellent yield (90%) within reaction time 6h (Table 1, Entry 4). In absence of catalyst, the model reaction showed poor results with respect to the yield and reaction time (Table 1, Entry 9). On increasing temperature up to 100 ^{o} C, the reaction did not show any improvement in the yield of desired product (Table 1, Entry 10). Moreover the reaction at room temperature condition offered 74 % yield with reaction time 12 hours (Table1, Entry 11).

Table 1: Optimization of the solvent and catalytic loading for the one-pot synthesis of 4-chloro-2-(4-methylquinazolin-2-yl)phenol derivatives[a]

Entry	Solvent	[Hmim]HSO_4 (mol %)	Temp (^{o}C)	Time (h)	Yield[b] (%)
1	Ethanol	10	80	8	72
2	Methanol	10	80	9	64
3	DMSO	10	80	10	48
4	**Acetonitrile**	**10**	**80**	**6**	**90**
5	DMF	10	80	10	34
6	DCM	10	80	14	28
7	Acetonitrile	5	80	7	69
8	Acetonitrile	15	80	6	85
9	Acetonitrile	-	80	15	20
10	Acetonitrile	10	100	6	88
11	Acetonitrile	10	RT	12	74

[a]Conditions: 2-Chloro acetophenone (1 mmol), 5-chloro salicyaldehyde (1mmol), 25% aqueous ammonia (1 ml), Solvent (15 ml), [Hmim]HSO_4 (10 mol %). Reaction was monitored by thin layer chromatography.

Table 2: Ionic liquid-catalyzed one-pot synthesis quinazoline derivatives[a]

Sr. No.	Products (3a-j)	Time (h)	Mp. (^{O}C)	Yield[a] (%)
1	 4a	7.00	160-162	91
2	 4b	7.30	198-200	85
3	 4c	6.50	214-216	89
4	 4d	6.30	178-180	88
5	 4e	6.00	182-184	90
6	 4f	6.30	191-193	88
7	 4g	7.00	198-200	87
8	 4h	6.50	174-176	93
9	 4i	8.00	168-169	85
10	 4j	8.30	155-157	82

[a]Conditions: 2-Chloro acetophenone (1 mmol), substituted salicyaldehyde (1mmol), 25% aqueous ammonia (1 ml), CH_3CN(15 ml), [Hmim]HSO_4 (10 mol %) at 80 ^{o}C. Reaction was monitored by thin layer chromatography.
[b]Isolated yield.

To observe the scope and generality of this methodology, we have used different substituted salicylaldehyde to afford corresponding quinazoline derivatives in excellent yields. The results are listed in Table 2 (Entries 1-10). Salicylaldehydes having both electron-donating and electron-withdrawing groups were used and afforded the desired products in high yields.

Based on the priority of known anticancer activity of quinazoline derivatives, we were interested to test anticancer properties in vitro. We evaluated our derivatives 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 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
4a	78.6	67.7	48.9	19.9	73.9	64.4	46	18.5	74.3	69.2	48.8	18.8	75.6	67.1	47.9	19.1
4b	80.1	69.2	50.3	21.5	76.1	66.3	48.1	20.2	76.3	71.2	50.4	20.2	77.5	70.6	56.5	20.6
4c	72.2	60.3	39.2	12.2	67.4	55.7	37.3	11.5	68.4	65.3	40.1	11.4	69.3	60.4	38.8	11.7
4d	65.4	53.2	32.9	06.3	61.3	48.7	31.2	05.4	61.2	58.3	33.2	04.3	62.6	53.4	32.4	5.3
4e	70.2	58.4	37.3	10.2	65.3	53.1	35.4	09.3	65.7	62.4	38.2	08.7	67.0	57.9	36.9	9.4
4f	68.3	55.8	35.4	08.7	63.3	51.2	33.4	07.6	63.2	60.3	35.5	06.3	64.9	55.7	34.7	7.5
4g	63.2	51.3	30.2	04.3	58.6	46.2	29.3	03.5	58.7	55.7	31.4	02.7	60.1	54.2	30.3	3.5
4h	74.4	62.8	41.8	14.4	69.4	58.8	39.8	13.7	71.8	68.4	42	13.4	71.9	63.3	41.2	13.8
4i	61.2	48.8	27.9	02.3	55.4	44.2	27.3	01.2	55.4	52.8	28.3	01.3	57.3	48.6	27.8	1.6
ADR	5.7	4.1	-0.8	-29.9	1.4	5.0	-2.2	-31.8	1.2	6.2	2.5	-36.4	2.8	5.1	-0.2	-32.7

	Drug concentrations mg/ml calculated from graph		
MCF7	**LC 50**	**TGI**	**GI50**
4a	>80	>80	43.7
4b	>80	>80	47.1
4c	>80	48.87	26.7
4d	>80	21.1	12.1
4e	>80	37.5	20.5
4f	>80	31.3	17.1
4g	>80	14.6	8.0
4h	>80	57.6	31.5
4i	>80	06.5	3.6
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.
ADR	Adriamycin (Doxorubicin). Known drug.

The good results however were obtained using derivatives 4d, 4e, 4f, 4g, 4i (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 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 4i, 4g showed 57.3, 60.1 where as for concentration 80 mg/ml for 4i and 4g showed 1.6 and 3.5 inhibition, respectively. In compared to Adriamysin 2.8 and -32.7 inhibition indicating that the anticancer properties of these molecules are possibly due to their sirtuin inhibiting properties. The compound 4i shows good inhibition activities against human breast cancer cell (MCF7) in **Figure 1**.

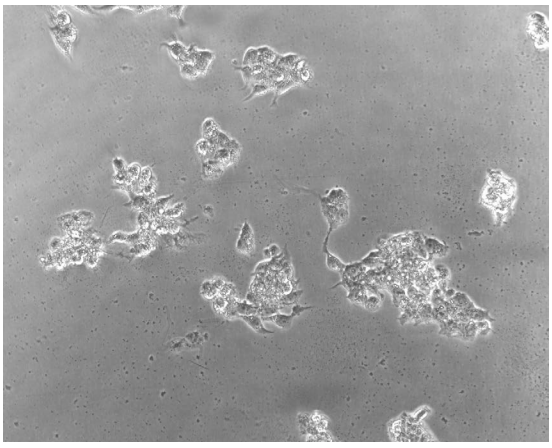


Figure 1: MCF7 of 4i

Conclusion

In conclusion, we have developed a convenient method for the synthesis of quinazoline derivatives by the one-pot reaction of a 2-chloroacetophenone, substituted salicylaldehyde and aqueous ammonia in presence of ionic liquid [Hmim]HSO_4 as catalyst. This modified method offers enhanced performance over the many conventional methods. The delightful features of this protocol are use of environmentally benign catalyst and excellent yields of quinazoline derivatives. All the synthesized derivatives were evaluated for their anticancer activities. The initial assays indicated 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.

Acknowledgment

We are thankful to Dr. P. L. More, Principal, Dnyanopasak College, Parbhani and Dr. balasaheb chavan, Principal, yogeshwari mahavidyalaya ,Ambajogai for providing necessary facilities to the research work and the for Tata Memorial Centre Advanced Centre for Treatment Research and Education in Cancer (Actrec) Anti Cancer Drug Screening Facility (Acdsf), Sector-22, Kharghar, Navi Mumbai, providing anticancer activity data.

Reference:

1. S. Mehta, N. Swarnkar, M. Vyas, J. Vardia, P. B. Punjabi, S. C. Ameta, Bull. Korean Chem. Soc., **2007**, 28, 2338.
2. J. Vijaynathappa, S. Bhojraj, Journal of Health Sciences, **2008**, 54, 524.
3. V. Alagarsamy, V. Raja Solomon, K. Dhanabal, Bioorg. Med. Chem., **2007**, 15, 235.
4. R. S. Giri, H. M. Thaker, T. Giordano, Eur. J. Med. Chem. **2009**, 44, 2184.
5. A. M. Al-Obaid, S. G. Abdel-Hamide, H. A. El-Kashef, Eur. J. Med. Chem., **2009**, 44, 2379.
6. V. Chandregowda, A. K. Kush, G. Chandrasekara Reddy, Eur. J. Med. Chem., **2009**, 44, 3046.
7. R. S. Giri, H. M. Thaker, T. Giordano, Bioorg. Med. Chem.

2010, 18, 2796.
8. D. Vachala, H. Unnissa, Indian J. Heterocycl. Chem., **2008**, 17, 347.
9. V. Jatav, P. Mishra, S. Kashaw, J. P. Stables, Eur. J. Med. Chem., **2008**, 43, 1945.
10. A. Subramaniam, E. R. Faaleolea, R. C. Goldman, Tuberculosis, **2009**, 89, 334.
11. Y. Kabri, A. Nadine, A. L. Dume tre, Eur. J. Med. Chem. **2010**, 45, 616.
12. Xu. Guang-Fang, S. Bao-An, P. S. Bhadury, Bioorgan. Med. Chem. **2007**, 15, 3768.
13. J. Guan, Q. Zhang, M. O'Neil, N. Obaldia, A. Ager, L. Gerena, A. Lin, J. Antimicrob. Agents Chemother. **2005**, 49, 4928.
14. A. A. Khalil, S. G. Abdel Hamide, A. M. Al-Obaid, H. I. El-Subbagh, Arch. Pharm. Med. Chem., **2003**, 2, 95.
15. I. Ugi, Pure Appl. Chem., **2001**, 73, 187.
16. G. Pandey, R. P. Singh, A. Garg, V. K. Singh, Tetrahedron Lett. **2005**, 46, 2137.
17. S. C. Jadhvar, H. M. Kasraliker, S. R. Bhusare, Am. J. PharmTech Res. **2015**; 5(2), 477.