

Synthesis of Some Novel Quinazoline Derivatives Having Anti-Cancer Activity



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Submission: June 19, 2017; Published: July 21, 2017

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Abstract

Cancers are the leading causes of morbidity and mortality worldwide. There are an estimated 14.1 million new cases of cancer in the world and the childhood cancers represent a small fraction of total cancers with the estimated values of 150,000 diagnosis each year. Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. Hence, some new semi carbazides containing quinazoline moieties have been synthesized and their ability to inhibit growth of human cancer cell lines has been evaluated. The compound S1 and S3 has shown remarkable anticancer activity. The structures of all the compounds have been confirmed by FT-IR, NMR, and mass spectral analysis.

Keywords: Semicarbazides; Quinazoline; Anticancer activity; Cancer; Morbidity

Abbreviations: NCCS: National Center for Cell Science; FBS: Fetal Bovine Serum; DMEM: Dulbeccos Modified Eagles Medium; TLC: Thin Layer Chromatography; H1NMR: H1Nuclear Magnetic Resonance; UV: Ultra Violet

Introduction

Cancer is the uncontrolled growth and spread of cells. It can affect any part of the body. The growths often invade surrounding tissue and can metastasize to distant sites [1]. Majority of the cancer (90%-95%) are caused by frequent use of tobacco, obesity or over weight, chronic infections and prolonged exposure to radiations and a notable percentage (5%-10%) are due to heredity. Most cancer can be treated by radiology, chemo-therapy and surgery [2]. According to WHO, in 2012 approximately 14 million new cases and 8.2 million cancer related deaths worldwide. The number of new cases is projected to upsurge by about 70% over the next 2 decades. Among men, the most common sites of cancer diagnosed in 2012 were lung, prostate, colorectum, stomach and liver cancer; among women the most common sites diagnosed were breast, colorectum, lung, cervix and stomach cancer.

Tobacco use is the most important cause for around 20% of global cancer deaths and around 70% of global lung cancer deaths. Viral infections caused by HBV/HCV and or HPV are responsible for around 20% of cancer deaths in low- and middle-income countries. Over and above 60% of world's total new annual cases occur in Africa, Asia and Central and South America, these regions

account for 70% of the world's cancer deaths. The annual cancer cases expected to rise from 14 million in 2012 to 22 within the next 2 decades [3].

Material and methods

General scheme [4]

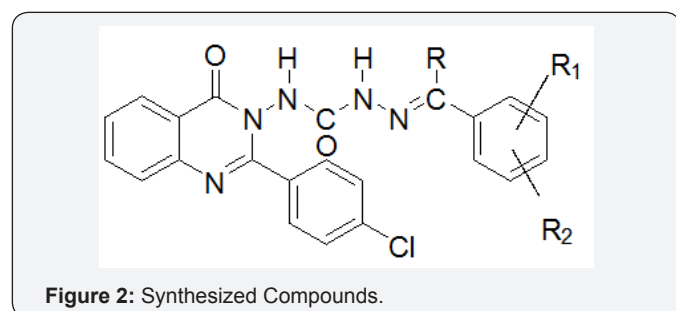
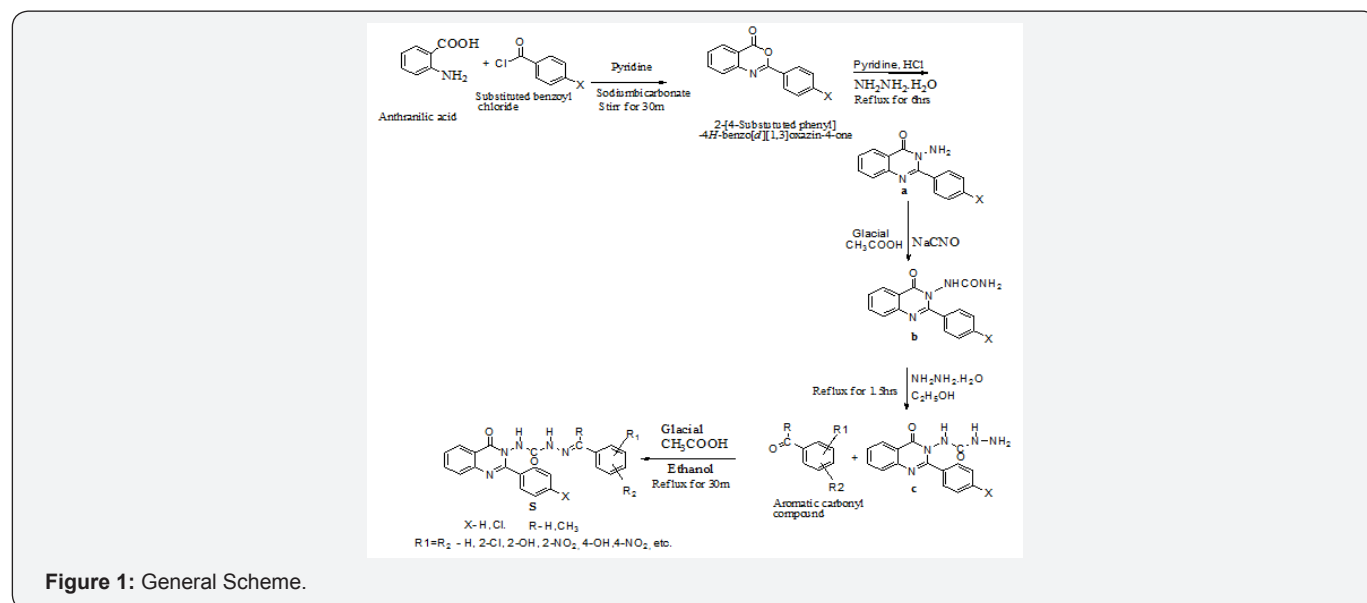
- 2-aryl-3-amino-4(3H) quinazolinone
- 1-(4-oxo-2-arylquinazolin-3(4H)-yl) urea
- 1-(4-oxo-2-arylquinazolin-3(4H)-yl) semicarbazide (Figure 1 & 2)

General procedures

Synthesis of 2-aryl-3-amino-4(3H) quinazolinone from anthranilic acid: Anthranilic acid (0.1mol, 13.71gm) was dissolved in 30ml of dry pyridine by stirring slowly at room temperature. The solution was cooled to 0 °C and a solution of an aromatic acid chloride (4-Chlorobenzoyl chloride) (0.02mole) in 30ml of dry pyridine was added slowly with constant stirring [4,5]. After this addition the reaction mixture was further stirred for half an hour at room temperature and set aside for 1h.

The pasty mass obtained was diluted with 50ml of water and treated with aqueous sodium bicarbonate solution. When the effervescence ceased, the precipitate obtained was filtered and

washed with water. The crude benzoxazine obtained was dried and recrystallized from diluted ethanol [4-6].



To a cold solution of 2-[4-Chloro phenyl]-4H-benzo[d][1,3]oxazin-4-one(0.05mol) in anhydrous pyridine, (20ml) added a solution of hydrazine hydrate (0.1mol) in anhydrous pyridine (25ml) drop wise with constant stirring. When the addition was complete, the reaction mixture was stirred vigorously for 30min at room temperature and subsequently heated under reflux for 6h under anhydrous reaction conditions. The reaction mixture was allowed to cool at room temperature and poured into ice cold water containing dilute hydrochloric acid. On standing for 1h solidification occurred which was allowed to settle down. It was filtered off, washed repeatedly with water and dried, recrystallized from dilute ethanol [4,6].

Synthesis of 1-(4-oxo-2-arylquinazolin-3(4H)-yl) urea from 2-aryl-3-amino-4(3H) quinazolinone: 3-Amino-2-(4-chlorophenyl)-4(3H) quinazolinone (0.1 mole) was dissolved in 10-30ml of glacial acetic acid and diluted to 100ml with water. To this equimolar quantity of sodium cyanate (0.1 mol, 6.5gm) in 50ml of warm water was added with constant stirring. The solution was allowed to stand for 30min. and then cooled in ice for further 30min. The precipitate obtained was filtered with suction, washed with water and dried. The precipitate was recrystallized

from boiling water and ethanol mixture [5,6].

Synthesis of 1-(4-oxo-2-arylquinazolin-3(4H)-yl) semicarbazide from 1-(4-oxo-2-arylquinazolin-3(4H)-yl) urea: To a solution of 1-(4-oxo-2-(4-chloro phenyl) -quinazolin-3(4h)-yl) urea (0.1 mole) in 200ml of water, an equimolar quantity of hydrazine hydrate (0.1mol, 5gm) was added. The reaction mixture was made alkaline by adding 4gm of sodium hydroxide and added 30ml of ethanol to get a clear solution. The reaction mixture was refluxed for 3h with stirring. Excess of ethanol was distilled off under vacuum and then poured in to ice. The obtained precipitate was filtered and recrystallized from 95% ethanol [4,7].

Synthesis of 1-(4-oxo-2-arylquinazolin-3(4H)-yl) aryl semicarbazones from 1-(4-oxo-2-arylquinazolin-3(4H)-yl) semicarbazide: 1-(4-oxo-2-arylquinazolin-3(4H)-yl) semicarbazide (0.01mol) was dissolved in ethanol (20ml) and added slowly to an ethanolic solution of aromatic carbonyl compound (0.01mole). The reaction mixture was acidified with 5ml of glacial acetic acid and refluxed for half an hour. The precipitate obtained was filtered and washed with the mixture of ether and water and dried. The product obtained was recrystallized from 95% ethanol [4-8].

In-Vitro anticancer screening

The human cervical cancer cell line (HeLa), NIH 3T3 mouse embryonic fibroblasts were obtained from National Center for cell science (NCCS), Pune. The HeLa cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and NIH 3T3 fibroblasts were grown in Dulbeccos Modified Eagles Medium(DMEM) containing 10%FBS [8-10]. For the screening experiment, the cells were seeded into 96-well plates

in 100µl of respective medium containing 10% FBS , at a plating density of 10,000 cells/well and incubated at 37 °C, 5%CO₂, 95% air and 100% relative humidity for 24hrs prior to addition of samples. The extracts were solubilised in DMSO and diluted in respective medium containing 1%FBS. After 24h, the medium was replaced with the respective medium containing the samples at various concentrations and incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 48h.

Triplicate was maintained and the medium without samples were served as control [8,9]. After 48h, 10µl of MTT (5mg/ml) in phosphate buffered saline was added to each well and incubated at 37 °C for 4h. The medium with MTT was then flicked off and the formed crystals were solubilised in 100 µl of DMSO and measured the absorbance at 570nm using microplate reader [10]. The percentage cell inhibition and IC50 were calculated and are given in Table 1.

Table 1: Physical data of the synthesized compounds.

Compd.	Substituent's		Yield (%)	Melting Point (°C)	Molecular Formula	Mol. Wt.	R _f	logP*
	R	R1, R2						
S1	CH ₃	4-OH	90	188 °C	C ₂₃ H ₁₈ ClN ₅ O ₃	447	0.62	1.88
S2	CH ₃	4-Cl	93	204 °C	C ₂₃ H ₁₇ Cl ₂ N ₅ O ₂	465	0.69	2.08
S3	H	4-OH	89	210 °C	C ₂₂ H ₁₆ ClN ₅ O ₃	433	0.68	2.14
S4	H	H	86	198 °C	C ₂₂ H ₁₆ ClN ₅ O ₂	417	0.52	2.18
S5	H	4-NO ₂	91	196 °C	C ₂₂ H ₁₅ ClN ₆ O ₄	462	0.58	1.89
S6	---	Isatin	88	203 °C	C ₂₃ H ₁₇ ClN ₆ O ₂	444	0.66	2.18
S7	----	5-Bromo isatin	92	214 °C	C ₂₃ H ₁₄ BrClN ₆ O ₃	537	0.56	3.87
S8	H	Furfural	96	232 °C	C ₂₁ H ₁₅ ClN ₄ O ₃	406	0.52	3.64
S9	H	2-OCH ₃	92	212 °C	C ₂₄ H ₁₉ ClN ₄ O ₃	446	0.42	4.24
S10	C ₆ H ₅	H	86	216 °C	C ₂₉ H ₂₁ ClN ₄ O ₂	492	0.54	4.56

*Log P was calculated by partition coefficient determination using Octanol and buffer system.

R_f- Solvent system- ethyl acetate-butanol:water (6:3:1)

Results and Discussion

The compounds were synthesized by various reaction intermediate such as:

- Synthesis of 2-(4-chloro phenyl)- 4H-3, 1- Benzoxazin-4 one through N-Benzoylation followed
- dehydrative cyclisation mechanism.
- Synthesis of 2-(4-chloro phenyl)-3-amino-4(3H)-Quinazoline.
- Synthesis of 2-(4-chloro phenyl)-3-amino-4(3H)-Quinazoline urea.
- Synthesis of 2-(4-chloro phenyl)-3-amino-4(3H)-Quinazoline semicarbazide.
- Semicarbazones reactions of 2-(4-chloro phenyl)-3-

amino-4(3H)-Quinazoline semicarbazide and aromatic aldehydes and ketones.

The synthesized compounds purity and its structural elucidation were done by various analytical techniques such as Melting point, Thin Layer Chromatography (TLC), Ultra Violet Spectroscopy (UV), Infrared Spectra (IR) and H¹ Nuclear magnetic resonance (H¹NMR).

Melting point

The melting point is an important physical property of a compound and it can be used to identify a substance and its purity. The melting point of solid is the temperature at which the solid exists in equilibrium with its liquid under an external pressure of one atmosphere [11]. A synthesized compounds melting point and its reactants melting point were recorded by open capillary tube method [4,12], which are uncorrected. A reactant and products

melting point were differing from each other. It clearly indicates that the formation of a new chemical entities. The melting point values of the synthesized compounds are presented in Table 2.

Table 2: In-vitro Anti-cancer activity of compounds.

Compound	HeLa (IC50)
Compound S1	93.30mM
Compound S3	6.90mM

Data are the mean of three or more experiment and are reported as mean±standard error of the mean (SEM).

Thin layer chromatography

Thin layer chromatography (TLC) is a very expedient and effective technique for the separation and identification of inorganic ions. It permits selective separations, simple detection and easy manipulation of the mobile phase. As a result, numerous sorbents and even better number of mobile phases have been developed for attaining the improved chromatographic performance in terms of selectivity, resolution, rapidity and reproducibility [13]. Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance [14]. Thin layer chromatography techniques were performed for all synthesized compound as well as the parent compounds, all synthesized compounds gave a single spot whose Rf values are different from their reactants. It ultimately shows that the compound's purity and completion of the reaction. The Rf value are presented in Table 3.

Table 3: In-vitro cytotoxicity of compound against human cervical cancer cell line (HELA).

Code	Cell line	Concentration (µM)	% Cell inhibition
S1	HeLa	19	2.1
		37.5	4.7
		75	16.3
		150	52
		300	76.4
		400	99.4
S1	NIH3T3	50	22.3
		100	32.6
		200	38.3
		300	42.7
		400	48.5
		500	65.3
S3	HeLa	1.2	3
		2.4	14.5
		4.8	36.8
		9.6	56.9
		19.2	70.1
		38.4	96.6

S3	NIH3T3	0.9	7.8
		1.8	19.5
		3.75	20
		7.5	34.4
		15	51.6
		30	65.7

Infra-red spectra

Infrared spectroscopy is one of the classical methods for structure determination of small molecules. This standing is due to its sensitivity to the chemical composition and architecture of molecules [15]. Infra-Red spectroscopy was taken for all the synthesized compounds. The characteristic absorption peaks were observed for all relevant groups. Aromatic acid chlorides strongly absorb at 1800-1770cm⁻¹ which was not observed in spectrum, it clearly gives a chemical entity in step I. The absorption peaks around 1600-1500cm⁻¹ indicates that the formation of C=N Schiff bases. C=O stretching vibration around 1870cm⁻¹ was not appeared in spectrum. It shows that there was no impurities like aldehyde/ketone., C-C, C=C & C=N ring stretching vibration at 1050-1200,754cm⁻¹ also appeared and aromatic amide N-H was observer at 3100-3500cm⁻¹ and all other relevant groups absorption were observed for all the synthesized compounds.

¹H Nuclear magnetic resonance

NMR or nuclear magnetic resonance spectroscopy is a technique used to determine a compound's unique structure. It identifies the carbon-hydrogen framework of an organic compound. Using this method and other instrumental methods including infrared and mass spectrometry, scientists are able to determine the entire structure of a molecule [16]. ¹H nuclear magnetic spectra were taken for all the synthesized compounds. Aromatic protons were observed 6.68-8.13δppm, Amide N-H proton was observed at 6.05-6.40δppm, for all the synthesized compounds.

Anti-cancer activity

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors, which play a direct and/or indirect role in the induction and deterioration of cancers [17]. Cancer has a major impact on society in the United States and across the world. Cancer statistics describe what happens in large groups of people and provide a picture in time of the burden of cancer on society [18]. Hence, some of the synthesized compounds were tested for *in-vitro* anticancer activity. Among the synthesized derivatives compound S1 and S3 were screened for *in-vitro* cytotoxicity effect. The results indicate that the synthesized compound possesses cytotoxicity against both cancer cells as well as normal cells. It proves that suitable structural modifications will have to be carried out to get novel compounds having potent anticancer activity with least effect on normal cells [19-21].

Conclusion

In summary, a new series of quinazoline derivatives (s1-s10) have been synthesized and evaluated for anti-cancer as well as anti-bacterial activities. Among the synthesized compounds S1, S3 were the potent compounds with good results for anti-cancer activity. These quinazoline derivatives S1, S3 represent promising structures for the development of new compounds with better activity results and for future *in-vivo* analysis.

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