Synthesis and in Vitro Anti Tumor Activity of Some Novel 2, 3-Disubstituted Quinazolin 4(3H)-one Derivatives

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ABSTRACT
A series of 4-[2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl]benzoyl] derivatives were synthesized. The synthesized compounds were characterized by IR, NMR and Mass spectral data and were screened for their anti cancer activity. The in vitro anticancer studies were performed on 4 selected compounds using MTT assay against HeLa cell line (NCCS). The synthesized 2,3-disubstituted-3H-Quinazolin-4-one derivatives exhibited significant Anti oxidant and anti tumor activities.

INTRODUCTION
Normal cells in our body follow an orderly path of growth, division and death. Cancer is a class of diseases characterized by out-of-control cell growth which harms the body by forming lumps or masses of tissue called tumors. Tumors are invasive, aggressive and mostly metastatic. The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species. These Free radicals cause irreversible damage to the DNA and other molecules may lead to cancer. Antioxidants or “mopping up” free radicals interact with and stabilize free radicals and may prevent some of the damage caused by free radicals. Considerable laboratory evidence from chemical, cell culture and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer. Amino acids will minimize the side effects of the metabolite of the parent compound upon metabolism in the body and enhance the solubility, when it is incorporated into pharmacologically active moiety. Quinazoline ring is a versatile lead molecule which has been investigated widely which posses analgesic, anti-inflammatory, antihypertensive, sedative, and hypnotic, antihistaminic, antitumor, antimicrobial, anticonvulsant, enzyme inhibition activity and many other activities (Kumar et al., 1981; Rekha et al., 2010; Hithuri et al., 1995; Rivero et al., 1998; Lakhan et al., 1998; Cao et al., 2009). Numerous research has shown that the Quinazoline nucleus possesses potent activity against human cancer particularly by killing the cells in a tumor-specific manner(Malleshappa et al., 2011; Dinakaran et al., 2003; Raff et al., 2004; Girija et al., 2005). The nucleus has also been reported to have potent anti oxidant activity (Rajasekharan et al., 1998). Amino acids will minimize the side effects of the metabolite of the parent compound upon metabolism in the body and enhance the solubility of the synthesised candidates, when it is incorporated into pharmacologically active moiety (Meyyanathan et al., 1998). These observations gave us a great impetus to the search for potential biological active drugs carrying 2,3-disubstituted Quinazoline-4(3H)-one in combination with amino acids and sulfonyl hydrazides, hoping to add some synergistic biological significance to the target molecules. In order to produce potent new leads for anticancer drugs, a new series of Quinazoline analogue have been designed by fusing with some biologically friendly amino acids and some sulphonyl hydrazides(Raja reddy et al., 1998; Yuefen Zhou et al., 2004), the structural features which are believed to enhance anti tumor activity.

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The synthesized analogs have been screened for antioxidant activity and have been reported in our previous study (Hurmath et al., 2012).

**EXPERIMENTAL**

**Material and methods**
All the chemicals were of synthetic grade procured from Sigma Aldrich Chemicals Ltd. Melting points were recorded on a Buchi capillary melting point apparatus. IR spectra were recorded on Fourier Transform (SHIMADZU) Infrared spectrophotometer, using KBr disc method.

The 1H-NMR spectra were recorded in DMSO- $\delta$ on Perkin Elmer NMR Spectrophotometer-300 MHz, using TMS as an internal standard. Thin layer chromatography analyses were performed on pre-coated silica gel plates.

**Synthesis of 2-Chloro benzyl 1, 3-benzoxazin-4-one (Mishra et al., 1997)**
A mixture of Para Chloro phenyl acetic acid (0.06mol) and phosphorous penta chloride (0.06 mol) was triturated to get chloro phenyl acetyl chloride. Anthranilic acid (0.06 mol) was dissolved in 30ml of anhydrous pyridine by stirring slowly at room temperature, cooled to $0^\circ$ C and a solution of chloro phenyl acetyl chloride in anhydrous pyridine 30ml was added to this solution slowly with constant stirring for half an hour mechanically at room temperature and set aside for 1 hr.

The pasty mass obtained was diluted with water and treated with aqueous sodium bicarbonate to remove the unreacted acid. The solid material was filtered off and washed with water to remove the inorganic material adhered pyridine. The crude benzoazinone thus obtained was dried and re-crystallized from dilute ethanol.

**Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl]benzoicacid**
10gm of 2-chlorobenzyl 1,3-benzoxazin-4-one was added to a mixture of 4- amino benzoic acid (6.31 gm) and glacial acetic acid(30ml) and the mixture was refluxed under anhydrous condition for 6hrs. After then added ice cold water into it and the crude the product was filtered and dried. The crude product was recrystallised from ethanol.
Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl]benzoylchloride

A solution of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl]benzoicacid (7.03gm) in 1,4-dioxane (20ml) was placed in a 250ml flask fitted with a condensor. Thionyl chloride (2ml) was then added drop wise to the flask using dropping funnel. The mixture was refluxed under anhydrous condition for 4hrs. The excess of thionyl chloride was removed by distillation. The reaction mixture was poured into the 100ml ice cold water and the crude product was filtered and dried. The dried crude product was recrystallised from 1,4-dioxane.

Table 1  Physico chemical parameters of synthesized compounds.

<table>
<thead>
<tr>
<th>Comp code</th>
<th>R</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting point °c</th>
<th>Rf value</th>
<th>% yield</th>
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<tr>
<td>Q1</td>
<td></td>
<td>C_{24}H_{18}ClN_{3}O_{4}</td>
<td>447.87</td>
<td>195</td>
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<tr>
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<td>C_{27}H_{20}ClN_{3}O_{6}</td>
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<td>Q3</td>
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<td>C_{30}H_{22}ClN_{3}O_{4}</td>
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<td>0.38</td>
<td>69%</td>
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<tr>
<td>Q4</td>
<td></td>
<td>C_{30}H_{22}ClN_{3}O_{4}</td>
<td>553.99</td>
<td>256</td>
<td>0.72</td>
<td>82%</td>
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<tr>
<td>Q5</td>
<td></td>
<td>C_{25}H_{20}ClN_{3}O_{4}S</td>
<td>493.96</td>
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<td>0.68</td>
<td>74%</td>
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<tr>
<td>Q6</td>
<td></td>
<td>C_{28}H_{22}ClN_{3}O_{4}</td>
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<td>0.44</td>
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<tr>
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<td></td>
<td>C_{33}H_{20}ClN_{4}O_{4}</td>
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<td>76%</td>
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</table>
BIOLOGICAL EVALUATION (Gaidelis et al., 1990; Denny et al., 1996)

Anticancer screening method by MTT assay procedure

For screening experiment, the cells were seeded into 96-well plates in 100µl of medium containing 5% FBS, at plating density of 10,000 cells/well and incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of samples. The samples of synthesized compounds were solubilized in Dimethylsulfoxide and diluted in serum free medium. After 24h, 100µl of the medium containing the samples at various concentration (6.25, 12.5, 25, 50mM etc...) was added and incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 48h. Triplicate was maintained and the medium containing without samples were served as control.

After 48h, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then clicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell inhibition = 100 - Abs (sample)/Abs (control) x 100.

Non-linear regression graph was plotted between % cell inhibition and Log10 concentration and IC50 was determined using Graph Pad Prism Software.

RESULT AND DISCUSSION

Interaction of p- chloro phenyl acetic acid with phosphorous pentachloride afforded phenyl acetyl chloride which on reaction with anthranilic acid and pyridine yields corresponding 2-chloro benzyl 1,3-benzoazine-4-one derivative. Reaction of compound with para amino benzoic acid in glacial acetic acid afforded 4-[2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl] benzoic acid by typical Niementowski cyclocondensation. This was subsequently reacted with thionyl chloride, to give 4-[2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl] benzoyl derivaties. Then fused with amino acids and sulfonohydrazide compounds. The structure of the synthesized compound was confirmed by IR and NMR spectral analysis.

Anticancer activity

The in vitro anticancer studies were performed on compounds using MTT assay against HeLa cell line (NCCS). The results indicated that among the four compounds tested, the compound Q2-Q9 inhibited the proliferation of cancer cells (Table: 2).

CONCLUSION

Some novel 2,3-disubstituted-3H-Quinazolin-4-one derivatives with the aim and to get more potent drug for the treatment of Anti tumor and microbial infectious diseases. The structure of the compounds was confirmed by spectral analysis. The synthesized 2, 3-disubstituted-3H-Quinazolin-4-one derivatives exhibited moderate to good Anti tumor activity. Among those Compounds Q2, Q9 were found to be good Anti-Cancer activity due to incorporation of Amino acids into pharmacologically active Quinazolone moiety which will minimize the side effects of the metabolite of the parent compound upon metabolism in the body and enhance the solubility and fusion of sulphonyl hydrazine group in Q6 shows anti tumor activity. Further studies on its possible mechanism and in vivo trials in experimental animals to broaden their Pharmacological assessment may provide a new analogue.

Table : 2 In vitro Anticancer screening.

<table>
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<tr>
<th>Conc (µM)</th>
<th>% cell Inhibition</th>
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<td>0.1</td>
<td>2.00</td>
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<tr>
<td>1</td>
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<tr>
<td>10</td>
<td>68.37</td>
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REFERENCES


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