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Synthesis and *In Vitro* Anti Oxidant Activity Of Some Novel 2, 3-Disubstituted Quinazolin-4(3h)-Ones

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ABSTRACT

A series of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl)benzoyl] derivatives were synthesized. The synthesized compounds were characterized by IR, NMR and Mass spectral data. The synthesized compounds were screened for their antioxidant activity by DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging method and Ferric reducing antioxidant power (FRAP) assay. The synthesized 2,3-disubstituted-3H-Quinazolin-4-one derivatives exhibited moderate to good Anti oxidant activity. Among those Compounds C-GY,C-GU,C-TP, and C-TY were found to be the most potent compound with Promising Anti-Oxidant activity.

Keywords: Amino acids, Quinazolin 4(3H)ones, antioxidant, DDPH,FRAP method.

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INTRODUCTION

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species. Cancer 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. Tumors that stay in one spot and show limited growth are called benign which can often be removed, and, in most cases, they do not come back. Also, cells in benign tumors do not spread to other parts of the body. Cancer is the result of cells that uncontrollably grow and do not die.

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. [1-5].

Numerous research has shown that the Quinazoline nucleus possesses potent activity against human cancer particularly by killing the cells in a tumor-specific manner. The nucleus has also been reported to have potent antimicrobial and anti oxidant activities. 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 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,a structural feature which is believed to enhance anti Tumor activity were synthesized and its preliminary antioxidant activities are carried out and reported.

MATERIAL AND METHODS

All the chemicals were of synthetic grade and commercially procured from sigma aldrich Chemicals. 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- d6 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 QI:

A mixture of para chloro phenyl acetic acid (0.06 mol) 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 0^0 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 un reacted acid when effervescence ceased, The solid material was filtered off and washed with water to remove the inorganic material adhered pyridine. The crude benzoxazinone thus obtained was dried and re-crystallized from dilute ethanol[7].

Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl)benzoicacid Q2:

10gm of 2-chlorobenzyl 1,3-benzoxazin-4-one was added to a mixture of 4- amino benzoic acid (6.31gm) 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 Q3:

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[8]..

Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl)benzoyl]derivatives. Q4-Q11:

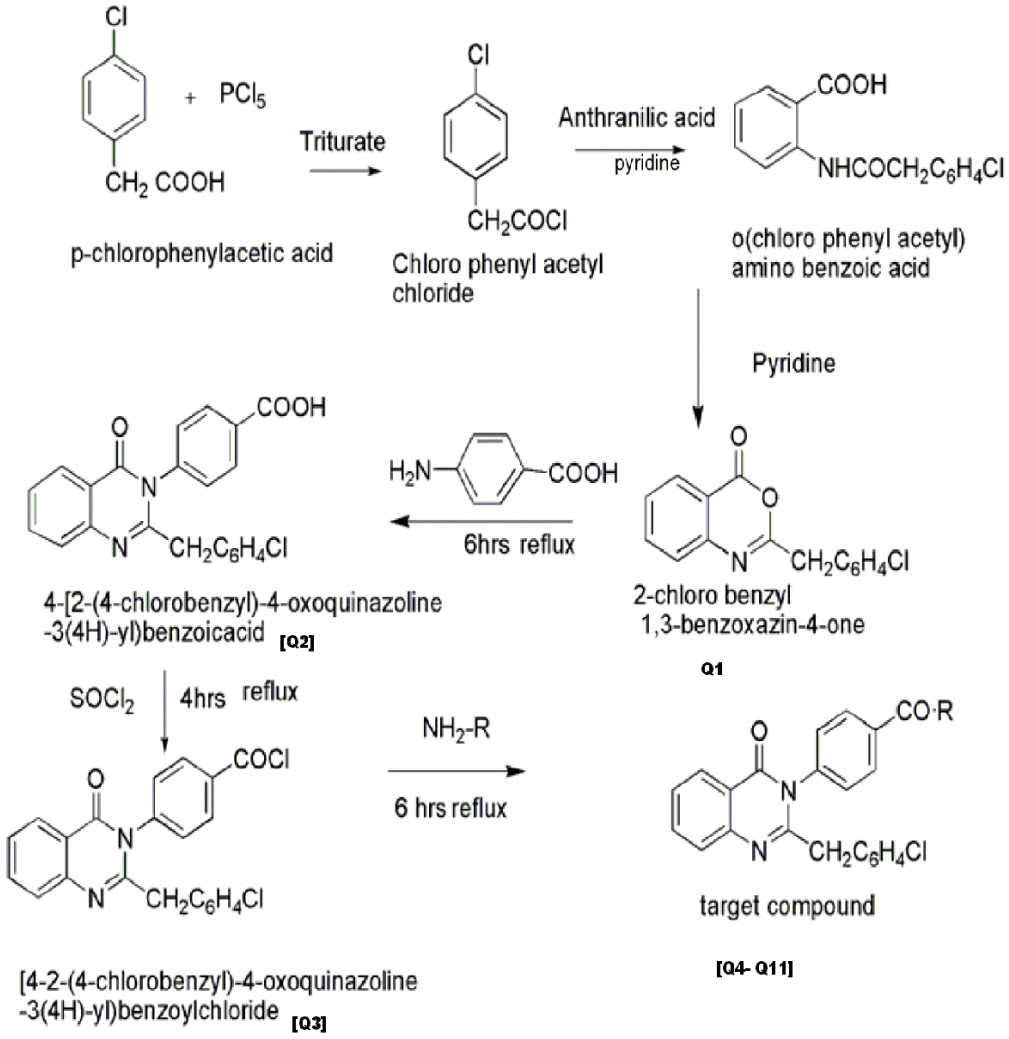
A solution of 4-[2-(4-chlorobenzyl)-4-oxoquinazoline3(4H)yl)benzoylchloride(4.1gm.0.01mol) in 1,4-dioxane was added to corresponding amino acid (0.01mol) in 0.1N sodium hydroxide (10ml) and the mixture was refluxed for 6hrs. The reaction mixture was then poured into 1N Hydrochloric acid (50ml) and the crude products were filtered and dried. The dried products were recrystallised from ethanol.

Synthesis and characterization of individual compounds

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido) acetic acid

M.p-195, yield-62%, M.F-$C_{24}H_{18}ClN_3O_4$ M.W-447.87,IR- 2923.03(COOH) 1675.05(C=O)1592.91(C=N) 1299.79(C-N)789.70(C-Cl),1HNMR(δppm):7.4-7.9(d, 4H, ArH), 10.7(s,1H, COOH), 8.0(t, 1H, NH), 3.7(s, 2H,CH_2), MS (m/z) 446.98.

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)pentanedioicacid- M.p-205, yield-78%, M.F-$C_{27}H_{22}ClN_3O_4$,M.W-519.93,IR 1675.05(C=O), 3127.15(COOH), 1592(C=N), 1200.20(C-N)



Scheme 1

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)-3-phenylpropanoic acid - M.p-256,yield-82%,M.F-$C_{31}H_{24}ClN_3O_4$,M.W-537.99,IR-3142.15(COOH), 1592.91(C=N), 697.15(monosubstitutedbenzene),1616.20(C=C)

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)-3-(hydroxyphenyl)propanoic acid - M.p-256,yield-82%,M.F-$C_{31}H_{24}ClN_3O_5$,M.W553.99,IR-3565.15(OH), 1596.91(C=N), 827.17(P-Substituted benzene)

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)-3-mercaptopropanoic acid - M.p-225, yield-74%,M.F-$C_{25}H_{20}ClN_3O_4S$, M.W-493.96,IR-1550.25(C=N),1300.20(C-N),788.20(C-S)

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)-3-(1H-imidazol-4-yl)propanoic acid - M.p-198,yield-56%,M.F-$C_{28}H_{22}ClN_5O_4$,IR-1581.17(C=N), 1671.05(C=O), 3127.95(COOH), 1200.20(C-N),789.20(C-Cl),1438.85(C-H)

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) benzamido)-3-(1H-indole -3-yl)propanoicacid - M.p-270,yield-63%,M.F-$C_{33}H_{25}ClN_40_4$,M-W577.04,IR-1548(C=N), 1661.37(C=O), 3123.15(COOH), 815.42(C-Cl)

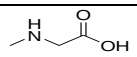
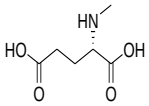
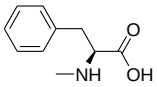
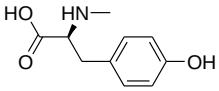
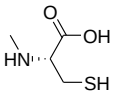
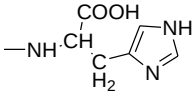
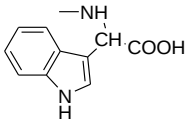
(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)-N'-tosylbenzohydrazide - M.p-246,yield-86%,M.F-$C_{29}H_{23}ClN_4O_4S$,M.W-559.04,IR-1159.01(SO2), 2923.56(CH_3), 788.74(C-Cl),7.4-7.9(d,4H,ArH),7.6(S,IH,NH),2.0(S,1H,CH_3), MS (m/z)559.73.

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)-2,3,4-trimethylsulfonohydrazide - M.p-298,yield-92%,M.F-$C_{22}H_{17}ClN_4O_2$,M.W-404.85,IR-2925(C-CH3),1503.24(C=N),1164.79(C-N) 1302.08(S=O),NMR-7.3-7.9(M,ArH),2.1(S,CH_2),2.2-2.6(S,CH_3)

(4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl) -benzohydrazide

M.p-185,yield-76%,M.F-$C_{22}H_{17}ClN_4O_2$,M.W-404.85,IR-3338.12(NH_2), 1503.24(C=N), 790.03(C-Cl),1164.79(C-N).

Table 1: 4-[2-(4-chlorobenzyl)-4-oxoquinazoline-3(4H)-yl)benzoyl]derivatives

Compound code	R
Q4	
Q5	
Q6	
Q7	
Q8	
Q9	
Q10	
Q11	

BIOLOGICAL EVALUTION[8-9]:

***In vitro* anti-oxidant screening: Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging activity:**

The free radical-scavenging activity of the synthesized compounds were measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 0.1 mM solution

of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1–5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0 \times 100$$

where A_0 was the absorbance of the control (blank, without compounds) and A_t was the Absorbance in the presence of the compounds. All the tests were performed in triplicate and the graph was plotted with the mean values.

Ferric reducing antioxidant power (FRAP) assay :

FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ), forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content. 0.2 ml of the compound is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM FeCl3. 6H2O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox
can be used as a positive control.

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-benzoxazine-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. This was subsequently reacted with thionyl chloride, to give 4-[2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl] benzoyl derivativies. Then the reaction compounds were added with corresponding amino acids to get the target compounds. The physicochemical parameters are given in Table:2. The structure of the synthesized compound was confirmed by IR and NMR

spectral analysis.

Table: 2 Physicochemical parameters

S.no	Compound Code.	Molecular formula	Molecular weight	Melting point	Rf value	solubility	Percentage yield
1.	C-GY	$C_{24}H_{18}ClN_3O_4$	447.87	195	0.34	Ethanol/DMSO	72%
2.	C-GU	$C_{27}H_{22}ClN_3O_6$	519.93	205	0.58	Ethanol/DMSO	78%
3.	C-PA	$C_{31}H_{24}ClN_3O_4$	537.99	246	0.38	Ethanol/DMSO	69%
4.	C-TY	$C_{31}H_{24}ClN_3O_5$	553.99	256	0.72	Ethanol/DMSO	82%
5.	C-CY	$C_{25}H_{20}ClN_3O_4S$	493.96	225	0.68	Ethanol/DMSO	74%
6.	C-HS	$C_{28}H_{22}ClN_5O_4$	527.96	198	0.44	Ethanol/DMSO	86%
7.	C-TP	$C_{33}H_{25}ClN_4O_4$	577.03	270	0.32	Ethanol/DMSO	93%
8.	C-PT	$C_{29}H_{23}ClN_4O_4S$	559.04	246	0.62	Ethanol/DMSO	86%
9.	C-2	$C_{31}H_{27}ClN_4O_4S$	587.06	298	0.74	Ethanol/DMSO	92%
10.	C-HH	$C_{22}H_{17}ClN_4O_2$	404.85	185	0.46	Ethanol/DMSO	76%

Antioxidant activity

The synthesized compounds were tested for anti-oxidant activity by DPPH and FRAP assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml in DMSO. Ascorbic acid was used as standard. Compounds C-TY, C-GU, C-GY and C-TP were shown good anti oxidant activity with EC- 50 value of 35, 27, 28 and 26 µg (Table 3-4)..

Antioxidant screening

Table: 3 - DPPH method

Percentage of Inhibition					
Concentration	**C-TY**	**C-GY**	**C-GU**	**C-TP**	**Ascorbic Acid**
25µ	35.59	44.72	45.17	43.76	56.7
50µ	44.49	45.71	53.00	46.56	68.9
75µ	49.69	47.46	97.12	48.87	89.0
100µ	50.51	48.37	98.36	49.07	96.0
EC_{50} (µg)	35	27	28	26	22

Table 4: FRAP Method

Absorbance					
Concentration	**C-TY**	**C-GY**	**C-GU**	**C-TP**	**Ascorbic Acid**
25µ	0.0274	0.1021	0.1568	0.0038	0.9504
50µ	0.2055	0.3565	0.2743	0.1366	1.0020
75µ	0.4974	0.5612	0.4733	0.4328	1.1843
100µ	0.6940	0.9269	0.6853	0.5398	1.2743
R^2	0.95	0.95	0.96	0.95	0.98

CONCLUSION

Some novel 2,3-disubstituted-3H-Quinazolin-4-one derivatives with the aim and to get more potent anti oxidant were synthesised. 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 oxidant among those Compounds C-GY,C-GU,C-TP, and C-TY were found to be the most potent compound with Promising activity of Anti-Oxidant. Amino acids incorporation into pharmacologically active Quinazolinone moiety 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. Further studies on its possible anti cancer activity, its mechanism of action and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue as an anti tumor agent.

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