SYNTHESIS OF 2-{4-[(Z) (BENZYLIDENE) AMINO] PHENYL}-3-[(DIMETHYLAMINO) METHYL] QUINAZOLIN-4(*3H*)-ONE DERIVATIVES AS ANTI CANCER AGENTS.

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ABSTRACT

For the last few years, the heterocyclic fused nucleus quinazoline have drawn an immense attention owing to its diversified application in the field of medicinal chemistry research. Being considered as a privileged scaffold, the modification made with different substituents around the centroid paved the researchers a way to deal with at ease. This review is an attempt to magnify the immense potentiality of this ring system. This study may also accelerate the designing process to generate more number of therapeutically viable clinical candidates.

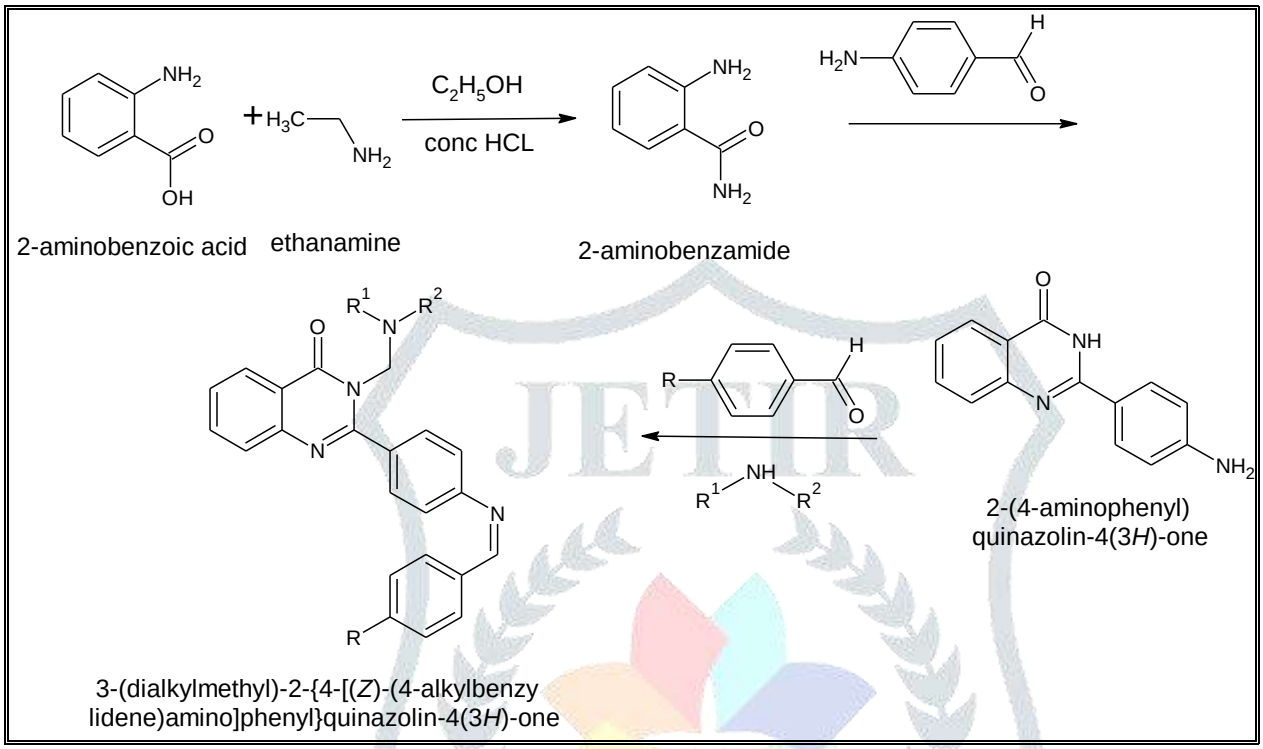
Key words: Benzilidine, Quinazoline, Anti cancer activity, SRB assay.

INTRODUCTION:

Reactions associated with tautomeric nature of the quinazolinones are often quite complex and generally unpredictable.[1] The recorded chemical investigation on the subject is voluminous. The amide linkages in quinazolinones should not be looked on as predominantly the keto or the enol form but as true keto-enol tautomers, showing reaction characteristic of both the forms.Quinazolinones are always high melting crystalline solids, insoluble in water and in most organic solvents but soluble in aqueous alkali. They are generally insoluble in dilute acids but are sometimes soluble in concentrated acids.[2-4] Simple 4-(3H) quinazolinones, although insoluble in dilute acids, are soluble in 6N hydrochloric acid. 4-(3H) quinazolinones form stable monohydrochlorides, chloroplatinate, chloroaurates and picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium.[4]Stability of the ring system The ring system in quinazolinone is exceedingly stable in oxidation, reduction, hydrolysis reactions and other treatment designed to break the ring.[5] There is no report of degradation of quinazolinone by simple chemical oxidation. Aromatisation When a simple and 2-substituted 4-(3H) quinazolinone is heated with an equivalent amount of phosphorous pentachloride in phosphorous oxychloride, the corresponding 4-chloroquinazoline is

obtained.[6] If a methyl group is present at 3-position, prohibiting the usual tautomerism, the methyl group is lost during the chlorination.[7-8] Alkylation The position of alkylation of quinazolinones is similar to all the aromatic nitrogen heterocyclic systems in which a hydroxyl group is foundortho or para to the nitrogen position.[9] Such compounds exist in tautomeric mixture, the two structures being inter-convertible by the shift of one proton and one pair of electrons.[10]

SCHEME I



Experimental :

Synthesis of 2-amino benzamide:2-aminobenzoic acid (1.3 g, 1 mol), ethanamine (0.45, 1 mol) and ethanol (30ml) were taken in a round bottom flask fitted with a reflux condenser and calcium guard tube and refluxed for 1 h at 70 ^{o}C. The excess ethanol was distilled off from the reaction medium under negative pressure and the residue was added to crushed ice. The resulting precipitate (1) was washed with ice cold water, then filtered off, air dried and crystallized from chloroform. Mol formula $C_8H_{10}N_2O$, Mol Wt: 150.1, M.P 184^{0}C, Yield 90.40 %, R_f Value : 0.46, IR (Cm^{-1}) (KBr): 3442.7 (-NH, 2˚ amide); 2971.9 (-CH_2); 1693.4 (-C=O); 1594.1 (C=C, aromatic); MASS M/Z: 135 (M-H).

Synthesis of 2-(4-aminophenyl)quinazolin-4(3H)-one :2-aminobenzamide (1.3 g, 1 mol), 4-aminobenzaldehyde (1.2, 1 mol) and ethanol (30ml) and HCL as catalyst were taken in a round bottom flask fitted with a reflux condenser and calcium guard tube and refluxed for 2 h at 80 ^{o}C. The excess ethanol was distilled off from the reaction medium under negative pressure and the residue was added to crushed ice. The resulting precipitate (2) was washed with ice cold water, then filtered off, air dried and crystallized from ethanol. Mol formula $C_{14}H_{11}N_3O$, Mol Wt: 237.2, M.P 155^{o}C, Yield 86.10 %, R_f Value : 0.64, IR (Cm^{-1}) (KBr): 3444 (-NH, 2˚ amide); 3113 (NH 1^{O} amide) 3033 (-CH_2); 1735 (-C=O); 1594.1 (C=C, aromatic); 1220 C-N.MASS M/Z: 238 (M+H)

Synthesis of 2-{4-[(Z)-(4-chlorobenzylidene)amino]phenyl}-3-[(dimethylamino)methyl] quinazolin-4(3H)-one:2-(4-aminophenyl)quinazolin-4(3*H*)-one (3) (1.0 g, 40 mmol), and 4-chlorobenzaldehye (2.8 ml, 80 mmol) dimethyl amine (4.0 ml, 40 mmol) and dry toluene (30.0 ml) were taken in a round bottom flask and heated to reflux for 2 h at 80 ^{0}C. The excess toluene was distilled off from the reaction medium and the residue was added to crushed ice. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (3) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate. Mol formula $C_{24}H_{21}N_4OCL$, Mol Wt: 416, M.P 165^{o}C, Yield 67.10 %, R_f Value : 0.48, IR (Cm^{-1}) (KBr): 3451 (-NH, 2˚ amide); 3198 (NH 1^O amide) 3071 (-CH_2); 1591 (-C=O); 1488 (C=C, aromatic); 1229 C-N; 713 C-Cl; MASS M/Z: 417 (M+H)

Synthesis of 2-{4-[(Z)-(4-bromobenzylidene)amino]phenyl}-3-[(dimethylamino)methyl] quinazolin-4(3H)-one:(4)2-(4-aminophenyl)quinazolin-4(3*H*)-one (2) (1.0 g, 40 mmol), and 4-bromobenzaldehye (1.2 ml, 40 mmol) dimethyl amine (4.0 ml, 40 mmol) and dry toluene (30.0 ml) were taken in a round bottom flask and heated to reflux for 2 h at 80 ^{0}C. The excess toluene was distilled off from the reaction medium and the residue was added to crushed ice. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (4) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate.Mol formula $C_{24}H_{21}N_4OBr$, Mol Wt: 461, M.P 211 ^{0}C, Yield 75.62 %, R_f Value : 0.76, IR (Cm^{-1}) (KBr): 3451 (-NH, 2˚ amide); 3198 (NH 1^O amide) 3071 (-CH_2); 1591 (-C=O); 1488 (C=C, aromatic); 1229 C-N; 739 C-Br;MASS M/Z: 461 (M+H)

Synthesis of 2-{4-[(Z)-(4-iodobenzylidene)amino]phenyl}-3-[(dimethylamino)methyl] quinazolin-4(3H)-one:(5)2-(4-aminophenyl)quinazolin-4(3*H*)-one (2) (1.0 g, 40 mmol), and 4-iodobenzaldehye (2.3 ml, 40 mmol) dimethyl amine (4.0 ml, 40 mmol) and dry toluene (30.0 ml) were taken in a round bottom flask and heated to reflux for 3 h at 80 ^{0}C. The excess toluene was distilled off from the reaction medium and the residue was added to crushed ice. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (5) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate.Mol formula $C_{24}H_{21}N_4OI$, Mol Wt: 508, M.P 196^{0}C, Yield 64.5%, R_f Value : 0.58, IR (Cm^{-1}) (KBr): 3446 (-NH, 2˚ amide); 3230 (NH 1^O amide) 3087 (-CH_2); 1585 (-C=O); 1488 (C=C, aromatic); 1227 C-N; 951C-I;MASS M/Z: 509 (M+H)

Synthesis of 2-{4-[(Z)-(benzylidene)amino]phenyl}-3-[(dimethyl amino)methyl]quinazolin-4(3H)-one:(6)2-(4-aminophenyl)quinazolin-4(3*H*)-one (6) (1.0 g, 40 mmol), and benzaldehye (1.0 ml, 40 mmol) dimethyl amine (4.0 ml, 40 mmol) and dry toluene (30.0 ml) were taken in a round bottom flask and heated to reflux for 3 h at 80 ^{0}C. The excess toluene was distilled off from the reaction medium and the residue was added to crushed ice. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (6) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate.Mol formula $C_{24}H_{21}N_4O$, Mol Wt: 382, M.P 236^{0}C,

Yield 74.5 %, R_f Value : 0.36, IR (Cm^{-1}) (KBr): 3441 (-NH, 2˚ amide); 2917 (NH 1^O amide) 2860(-CH_2); 1570 (-C=O); 1433 (C=C, aromatic); 1232 C-N; MASS M/Z: 382 (M+H)

Synthesis of 2-{4-[(Z)-(4-nitrobenzylidene)amino]phenyl}-3-[(dimethyl amino)methyl]quinazolin-4(3H)-one:(7)2-(4-aminophenyl)quinazolin-4(3*H*)-one (2) (1.0 g, 40 mmol), and 4-nitrobenzaldehye (1.5 ml, 40 mmol) dimethyl amine (4.0 ml, 40 mmol) and dry toluene (30.0 ml) were taken in a round bottom flask and heated to reflux for 1 h at 80 ^{0}C. The excess toluene was distilled off from the reaction medium and the residue was added to crushed ice. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (7) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate. Mol formula $C_{24}H_{21}N_5O_3$, Mol Wt: 382, M.P 428 ^{0}C, Yield 84.5 %, R_f Value : 0.54, IR (Cm^{-1}) (KBr): 3444 (-NH, 2˚ amide); 3218 (NH 1^O amide) 1589 (-C=O); 1491 (C=C, aromatic); 1215 C-N; 1491 (C-N=O);MASS M/Z: 428 (M+H)

BIOLOGICAL SCREENING

SULFORHODAMINE B ASSAY:

The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein dye sulforhodamine B to bind electrostatically. The activity is pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement. The signal-to-noise ratio is favorable and the resolution is 1000-2000 cells/well. Its performance is similar when compared to other cytotoxicity assays such as MTT or clonogenic assay. The SRB assay possesses a colorimetric end point and is nondestructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity even at large-scale application.

Parameters reported: GI_{50}, TGI, and LC_{50}

GI_{50}: Growth inhibition of 50 % (GI_{50}) calculated from drug concentration resulting in a 50 % reduction in the net protein increase.

TGI: Drug concentration resulting in total growth inhibition (TGI).

LC_{50}: Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment (concentration of drug causing lethality to 50 % of the cells as compared to that at the beginning) indicating a net loss of cells following treatment.

The *in vitro* testing for anticancer activity was carried out in Tata Memorial Centre [Advanced Centre for Treatment Research and Education in Cancer (ACTREC)], Mumbai.

Table 1.Reports of *in vitro* testing for anticancer activity

Compound No.	Human Skin Cancer Cell Line G361 % Growth* Molar Drug Concentration			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IA	100.0	100.0	**90.9**	**-59.3**
IB	100.0	100.0	99.7	48.5
IC	100.0	100.0	100.0	87.7
ID	100.0	99.6	100.0	75.1
IE	100.0	100.0	99.7	56.5
IF	99.7	97.8	94.3	47.6
ADR	46.9	-76.1	-73.6	-76.4

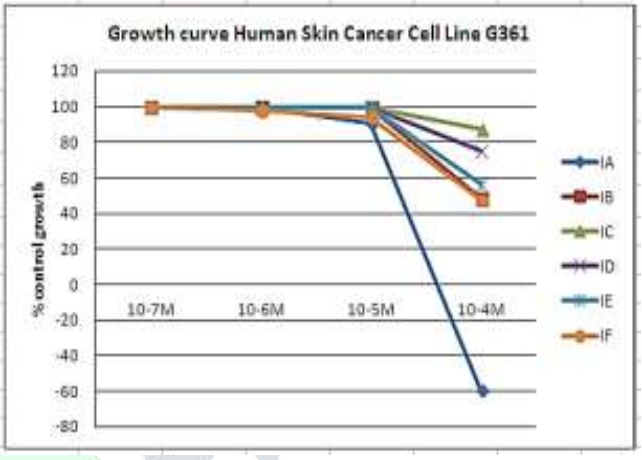
*Average values of 3 experiments

Table 2. Parameters calculated from Graph

G 361	Molar drug concentration		
	LC 50*	TGI*	G150*
IA	$>10^{-4}$	$3.32*10^{-5}$	$2.08*10^{-6}$
IB	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IC	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
ID	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IE	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IF	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
	$2.03*10^{-6}$	$1.27*10^{-7}$	$<10^{-7}$

*Average of 3 experiments

Figure 1. Growth Curve G361 Cell line



DISCUSSION

The synthesis of 2-{4-[(*Z*)-(4-chlorobenzylidene)amino]phenyl}-3-[(dimethylamino) methyl]quinazolin-4(3*H*)-one were synthesized using the appropriate synthetic procedures.

STEP:1 Synthesis of 2-amino benzamide:The reactants 2-aminobenzoic acid, ethanamine and ethanol were taken and heated at refluxing temperature for 1 h. The excess solvent was distilled off at negative pressure and finally, crushed ice was added to precipitate the product to give the yield of 90.40%. This results in liberation of two molecules of ethylalcohol giving 2-amino benzamide. The resulting precipitate (1) was washed with ice cold water, then filtered off, air dried and crystallized from chloroform.STEP:2 2-(4-aminophenyl)quinazolin-4(3*H*)-one: The synthesis of the above mentioned compound is carried out as per the reported procedure. 2-aminobenzamide was treated with 4-aminobenzaldehyde in solvent ethanol at refluxing temperature for 80 ^{0}C to give a yield of 86.10%. The primary aldehydes attacks 2-amino benzamide liberating one molecule of ethylalcohol. Finally, 2-(4-aminophenyl)quinazolin-4(3*H*)-one was

formed by internal cyclisation. STEP:3 2-{4-[(*Z*)-(4-chlorobenzylidene)amino]phenyl}-3-[(dimethylamino)ethyl]quinazolin-4(3*H*)-one:The 2-(4-aminophenyl)quinazolin-4(3*H*)-one (2) was stirred at room temperature with dimethylamines in presence of formaldehyde and substituted benzaldehydes for 2 hr. The excess toluene was distilled off from the reaction medium under reduced pressure. The mixture was added to crushed ice. It was neutralized with dilute hydrochloric acid. The mixture was extracted with ethyl acetate. The excess solvent was evaporated at room tempaerature. The resulting precipitate, (3) was washed with ice cold water, then filtered off, air dried and crystallized from ethyl acetate.

ANTICANCER ACTIVITY

The newly synthesized compounds were screened for their anticancer activity against Human Skin Cancer Cell Line G361 by Sulforhodamine B assay. Doxorubicin was used as a standard reference drug and the results obtained.All compounds (**1-6)** showed low antiproliferative activity. The % Growth inhibition of the compound **(5)** was found to be considerable at a concentration of 10^{-4}M. TGI_{50} (Growth inhibition of 50 % cells, calculated from drug concentration resulting in a 50 % reduction in the net protein increase) value of **(5)** is $3.32*10^{-5}$. As derivative is the most active compound, it serves as a lead to further optimization in drug discovery process.

SUMMARY AND CONCLUSION

2-(4-Methylphenyl)quinazolin-4(3*H*)-one were treated with different diamines and aryl substituted aldehydes under mild conditions to give corresponding diamino benzylidine derivatives, the title compounds in good yields. A facile method under mild conditions has been developed for the synthesis of the title compounds. All the compounds synthesized were characterized by physical (R_f values, Melting point, Molecular weight, Molecular formula) and spectral data (^{1}H NMR, IR, Mass spectra).The title compounds were screened for anticancer activity against Human Skin Cancer Cell LinesG361, by sulforhodamine B assay and analyzed statistically. This acts as a lead for further optimization.

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