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Synthesis and Preliminary *in-vitro* Cytotoxic Activity of Morpholino Propoxy Quinazoline Derivatives

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Abstract: Present research work is about the synthesis and biological evaluation of morpholino propoxy quinazoline derivatives where in 7-methoxy-6-(3-morpholinopropoxy)3,4-dihydro quinazoline-4-one was converted in to 4-chloro-7-methoxy-6-(3-morphholinopropoxy)quinazoline by treating with Phosphorus oxychloride and N,N-diethyl aniline. 4-chloro-7-methoxy-6-(3-morpholinopropoxy)quinazoline condensed with various substituted aromatic amines and thiol using methanol gives various derivatives of 4-substituted morpholino propoxy quinazoline. Synthesized compounds were tested for their physicochemical properties and were further characterized by spectral analysis using FTIR, NMR and Mass spectroscopy. All synthesized compounds (compound I to VIII) were tested for their cytotoxicity by MTT assay. Among all the synthesized derivatives, compound-II was shown promising anticancer activity as compared to other synthesized derivatives. This was indicated by the IC_{50} value of (5.5 and 7.1) respectively for the synthesized derivatives (compound- I and II). And gefitinib shown experimental IC_{50} value 4.9µM. Study concluded efficient synthesis of the stated derivatives with the scheme and also prepared derivatives were with promising cytotoxic effect.
Keywords: anti-cancer, benzo[d]thiazole, $IC_{50,}$ 7-methoxy-6-(3-morpholinopropoxy)3,4-dihydroquinazoline-4-one, MTT assay.

Introduction and Experimental:

The chemistry of quinazoline compounds has more than centuries old history; however the intense search for biologically active substances in this series began only in the last few decades. Evolution of quinazolines began only with discovery of febrifuge, a quinazolinone alkaloid, possessing anti-malarial potential from the Chinese plant aseru (Dichroa febrifuga Lour), which served as an impetus for initiation of the research on quinazolines. Quinazoline is a compound made up of two fused six-membered simple aromatic rings, a benzene ring and a pyrimidine ring. It is also called benzopyrimidine. It has the molecular formula $C_8H_6N_2$ and molecular mass 130.15 g/mol. It is isomeric with quinoxaline, phthalazine and cinnoline[1-4].

In the last 10-15years the search for quinazoline compounds has been characterized by significant advances. They have been reported to posses wide spectrum of biological activities like analgesic and anti-inflammatory, antimicrobial, anti-tubercular, antihistaminic, antitussive, bronchodilator, antidiabetic, antidiuretic, antihypertensive, sedative-hypnotic activity, antidepressant, antiparkinsonian, Phosphodiesterase inhibition and anticancer. It has been shown that they act as antifolate synthase inhibitors, EGFR tyrosine kinase inhibitors, and inhibitors of dihydrofolate reductase and tyrosine kinase. Some quinazolines interact with cytoskeleton, induce apoptosis, affect DNA topoisomerases and potentiate the efficacy of chemotherapeutic [5-7].

Traditional anticancer treatments have targeted the inhibition of DNA synthesis and function. Alternative approaches selectively targeting inhibition of signaling pathways that mediate proliferation are currently the subject of research. Epidermal growth factor receptor (EGFR) is important for growth signaling and is over-expressed in a significant number of human tumours. 4-Anilinoquinazolines such as gefitinib and erlotinib represent a potent and selective class of EGFR inhibitors which act via competitive binding at the ATP binding site of EGFR tyrosine kinase. Both compounds have recently been approved for the treatment of non-small cell lung cancer refractory to chemotherapy [8-11].

Materials and Methods:

Materials: 7-Methoxy-6(3-morpholinopropoxy)-3, 4-dihydro quinazoline-4-one was purchased from sigma Aldrich and rest of the chemicals and solvents used in the synthesis were of analytical grade.

Instruments:

Synthesized derivatives were predicted for their structure using FTIR, MASS and 1H NMR. Molecular vibrations of individual derivatives were recorded using Shimadzu FTIR 8400 S spectrometer from Shree Dhanvantary Pharmaceutical Analysis and Research Centre (SDPARC), Kim. KBr pellet technique was used in FTIR spectral studies. Fragmentation pattern useful in structural determination was done in a Perkinelmer model Clarus 600 mass spectrometer from Oxygen Healthcare Research P. Ltd, Ahmadabad. Relative environment proton and their number in each environment in a molecule with respect to TMS as internal standard was calculated using Varian model B-NMR 400Hz NMR spectrometer from Sun Pharma, Baroda.

Method used for the synthesis:

Initially 7-Methoxy-6(3-morpholinopropoxy)-3, 4-dihydro quinazoline-4-one was taken as starting material and was chlorinated at 4^{th} position as per stated procedure. Chloro substituted morpholino propoxy quinazoline was further condensed with different aromatic amines/thiols to form novel substituted derivatives. (Figure 1)

Step: 1. Chlorination of 7-Methoxy-6(3-morpholinopropoxy)-3, 4-dihydro quinazoline-4- one.

a) A mixture of 7-Methoxy-6(3-morpholinopropoxy)-3,4-dihydro quinazoline-4-one (4.08 g) and *N*,*N*-diethyl aniline 2.85 ml in Phosphoryl chloride 11.4 ml was immersed in a preheated oil bath (100°C) and at this temperature stirred for 3 hours.
b) The reaction mixture was cooled to 80°C and stirred for further 3 hours chloride was removed and the obtained crude material triturated with toluene (3 x 150 ml).
c) The brownish precipitate was dried over P_2O_5 to give the product.

Step: 2. Preparation of 4-substituted aromatic amines/thio-7-methoxy-6-(3-morpholinopropoxy)quinazoline.

a) Methanol (120 ml) and 4-chloro-6-(3-morpholino propoxy)-7-methoxy quinazoline (0.01mol) were stirred for 15 minutes at 25-30^{0}C.
b) Then a solution of substituted aromatic amines/thiol in methanol (0.01mol) was added.
c) The reaction mixture was refluxed for around 4-6 hrs until the reaction has complete.
d) The reaction was monitored by TLC.
e) The reaction mass was cooled to 15-20^{0}C.
f) Hydrochloric acid was added drop wise and stirred at 5-10^{0}C for 30 minutes.
g) The product obtained was collected and washed with methanol.

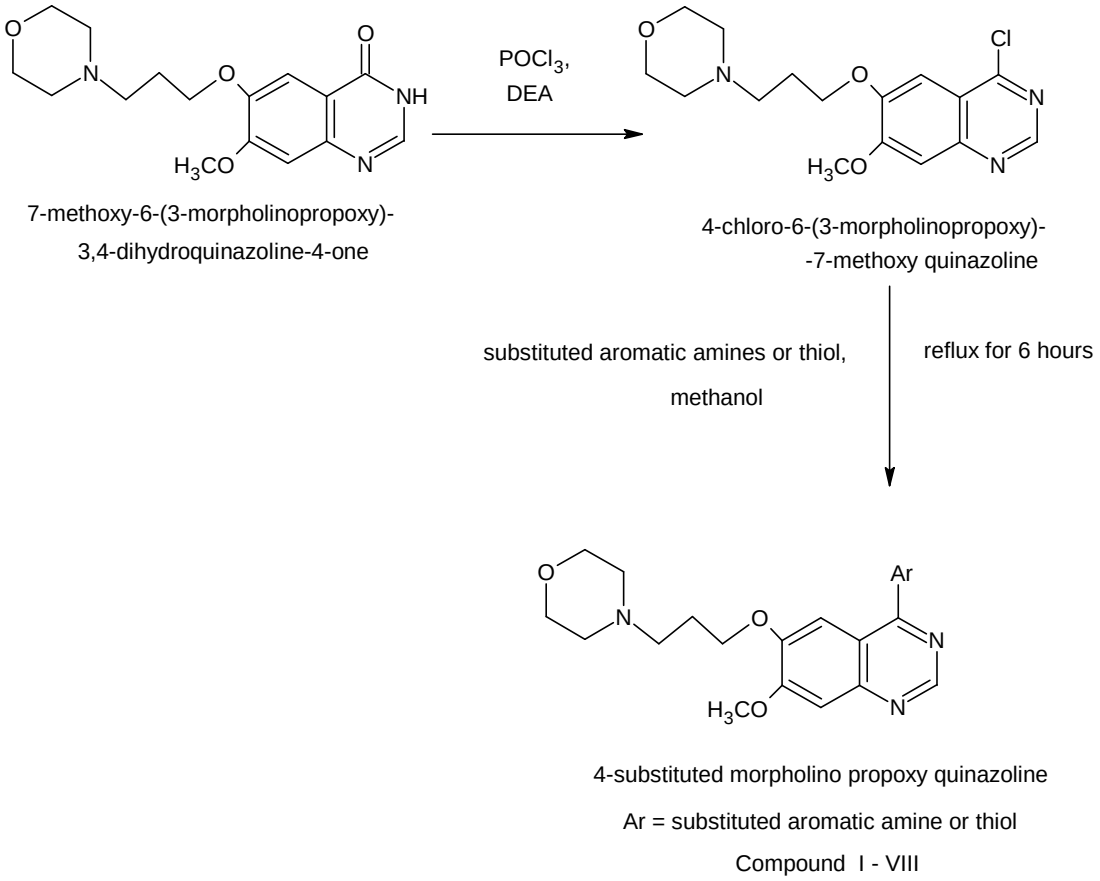


Figure 1: Scheme for synthesis of various novel derivatives of 4-substituted morpholino propoxy quinazoline

Results and Discussion:

Identification of Physico Chemical Properties:

All the derivatives synthesized were tested for their physico-chemical properties such as Melting point, Boiling point, Solubility, Crystalinity, pKa, TLC, etc.

Different substitution at 4th position of morpholino propoxy quinazoline resulted in 8 different derivatives (compound I-VIII). (Table no.1)

Physico-chemical and characteristic spectral properties of novel derivatives:

Compound-I

4-(7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-yl amino) benzene sulfonamide:

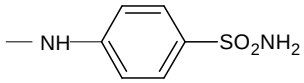
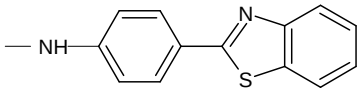
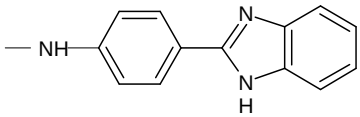
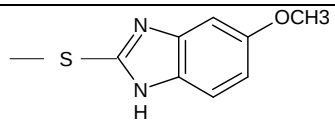
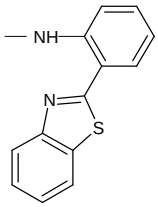
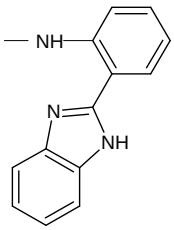
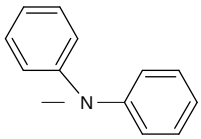
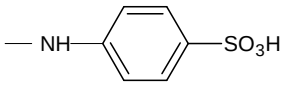
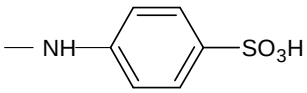
Yield: 31%; Melting point: 156-159°C ; IR(KBr)cm^{-1} -NH(str) 3488.74,-SO_2(sym str)1289.96, -SO_2(asym str)1314.53, MS (m/z) 471.9(M^{-1}) ^{1}H NMR(DMSO): 7.51-7.94δ (7H Ar-H), 3.22-3.65δ (8H, morpholino CH_2), 3.67δ (2H,NH_2), 3.96δ (1H,NH), 3.64δ (6H,propoxyH)

Compound-II

N-(4-(benzo[d]thiazole-2-yl) phenyl) 7-methoxy-6-(3-morpholinopropoxy)quinazoline:

Yield 38%; Melting point: 130-138°C; IR (KBr) cm^{-1}:-NH (str) 3448.84, -C-O-C-(str) 1286.56; MS (m/z) 528.0(M^{+1})

Table1: Table represents the structure and name of synthesized derivatives morpholino propoxy quinazoline:

Derivatives	**Ar**	IUPAC name
I		4-(7-methoxy-6-(3-morpholinopropoxy)quinazoline-4-yl amino)benzenesulfonamide
II		N-(4-(benzo[d]thiazole-2-yl)phenyl)7-methoxy-6-(3-orpholinopropoxy)quinazoline
III		N-(4-(1H-benzo[d]imidazole-2-yl)phenyl)7-methoxy-6-(3-orpholinopropoxy)quinazoline
IV		7-methoxy-4-(6-methoxy-1H-benzo[d] imidazole-2-ylthio)6-(3-morpholinopropoxy) quinazoline
V		N-(2-(benzo[d]thiazole-2-yl)phenyl)7-methoxy-6-(3-morpholinopropoxy)quinazoline
VI		N-(2-(1H-benzo[d]imidazole-2-yl)phenyl)7-methoxy-6-(3-morpholinopropoxy)quinazoline
VII		7-methoxy-6-(3-morpholinopropoxy)-N,N-diphenyl quinazolin-4-amine
VIII		4-(7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-yl amino)benzenesulfonic acid
VIII		4-(7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-yl amino)benzenesulfonic acid

Compound-III

N-(4-(1H-benzo[d]imidazole-2-yl) phenyl) 7-methoxy-6-(3-morpholinopropoxy) quinazoline:

Yield 25%; Melting point: 154-157°C; IR (KBr) cm^{-1}:-NH (str) 3439.19

Compound-IV

7-methoxy-4-(6-methoxy-1H-benzo[d]imidazole-2-yl thio) 6-(3 morpholinopropoxy) quinazoline:

Yield 40%; Melting point: 155-158 °C IR (KBr) cm^{-1}:–C-S (str) 684, -C-O-C-(str) 1286

Compound-V

N-(2-(benzo[d]thiazole-2-yl) phenyl) 7-methoxy-6-(3-morpholinopropoxy) quinazoline:

Yield 18%; Melting point: 170-172°C; IR (KBr) cm^{-1} -NH (str) 3455

Compound-VI

N-(2-(1H-benzo[d]imidazole-2-yl) phenyl) 7-methoxy-6-(3-morpholinopropoxy) quinazoline:

Yield 22%; Melting point: 179-182°C; IR (KBr) cm^{-1} -NH (str) 3380

Compound-VII

7-methoxy-6-(3-morpholinopropoxy)-N, N-diphenyl quinazolin-4-amine:

Yield 38%; Melting point: 165-168°C; IR (KBr) cm^{-1} –C=N (str) 1452.45, -C-O-C-(str) 1285.50

Compound-VIII

4-(7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-yl amino) benzenesulfonic acid:

Yield 35%; Melting point: 190-194°C

Evaluation of cytotoxic activity by MTT assays method[12]:

1st day

1. Harvest cells (leukemia blood) by centrifugation at 1800 rpm for 20 minutes. Discard supernatant.
2. Wash cells by resuspending in 5 ml sterile PBS (Phosphate Buffer Solution) or cell culture medium. Pellet cells by centrifugation at 1800 rpm for 20 minutes at 2 - 8° C. Discard supernatant.
3. Calculate the proper number of cells by neubar chamber and Thoma's WBC pipette.
4. Dilute a cell by PBS and Seed cells at 5000 cells/well (80 µl) in a 96 well plate. Leave at least three wells without cells. These wells serve as a control for the minimum absorbance. And covered the plate with aluminum foil.
5. Incubate the plate overnight at 37°C in an incubator.

2nd day

6. Add 10 µl /well test compounds (Drug samples) to the plate. Include replicates for a range of concentrations. Gefitinib is taken as standard drug. Include negative controls and a positive control.
7. Incubate the plate overnight at 37°C in an incubator.

3rd day

8. Add MTT reagent (10µl/100µl per well of the 96 well plate).
9. Incubate at 37°C for 3 hours
10. Add 1 volume (100µl) of the stop mix solution and rock the plate at room temperature for a minimum of 1 hour. (Allows time for the formazan precipitate to dissolve) The stop mix solution must be added

in a fume hood. A purple color should be visible at this stage and should deepen over the 1 hour incubation period.

11. After the 1 hour incubation, ensure the formazan precipitate is dissolved by pipetting each well up and down until not precipitate is visible.
12. Read the plate on a plate reader using 550nM as test wavelength and 650nM as the reference wavelength.
13. Record data in an excel spreadsheet, saved with a unique identifier.
14. Tabulate results and calculate the percentage viability.

Results of absorbance of cell measure by micro plate reader were tabulated in table no.2.

Cytotoxic activities exerted by all the derivatives are listed in the table 2. Results indicate good cytotoxic activity of the synthesized derivatives and results obtained were in comparable with the standard drug gefitinib.

For the cytotoxic activity determination gefitinib was taken as standard drug and activity of the test compound were determined on leukemia cell using standard MTT assay procedure and absorbance was recorded at 550nm.

Table no: 2. Results of absorbance

Conc.(µM)	Standard drug (Gefitinib)	Synthesized morpholino propoxy quinazoline derivatives (I-VIII)							
		I	II	III	IV	V	VI	VII	VIII
2	0.521	0.488	0.50	0.462	0.544	0.480	0.507	0.471	0.508
4	0.391	0.380	0.380	0.442	0.494	0.464	0.471	0.450	0.452
6	0.302	0.387	0.331	0.384	0.393	0.445	0.423	0.455	0.397
8	0.261	0.312	0.283	0.351	0.336	0.404	0.376	0.402	0.324
10	0.254	0.307	0.266	0.333	0.333	0.351	0.365	0.383	0.322

Absorbance of control = 0.681

Measurement of percentage viability:

Viability means in general "capacity for survival" and is more specifically used to mean a capacity for living, developing, or germinating under favorable conditions.

% Viability is measure by the results of absorbance. % viability is measure by the below formula.

Percentage Viability = (Mean Absorbance of Sample/ Mean Absorbance of Control) * 100 (Figure 2)

Results of percentage viability were tabulated in table no.3.

Measurement of percentage inhibition:

Inhibition**:-** The blocking or limiting of the activity of an organ, tissue, or cell of the body, caused by the action of a nerve or neuron or by the release of a substance such as a hormone or neurotransmitter or by the drug molecule.

Percentage inhibition = 100 – percentage viability. (Figure 3)

Results of percentage inhibition were tabulated in table no.4.

IC_{50} values of synthesized compounds were tabulated in table no.5.

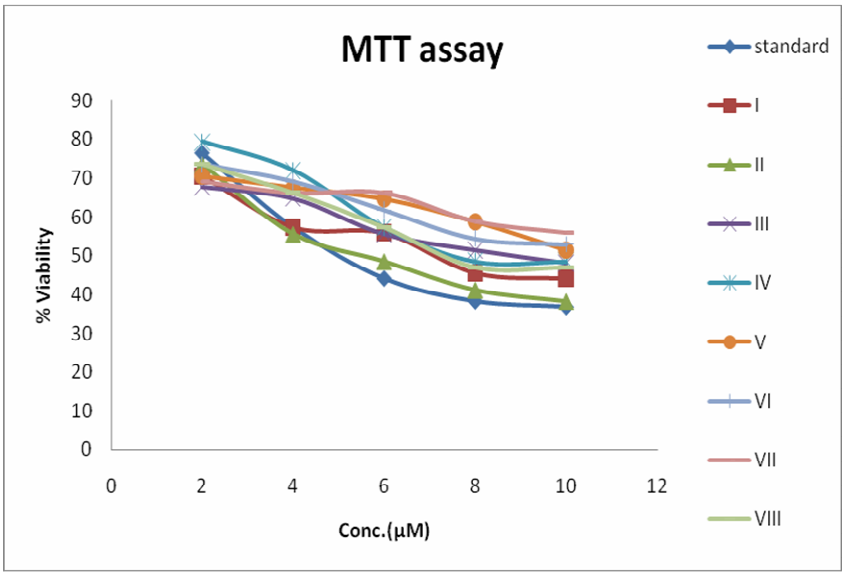


Figure 2: Graph represents cells percentage viability exerted by synthesized derivatives at different concentration

Table no: 3. Table represents the percentage viability of the cells at different concentration of test drug

Conc.(µM)	standard	I	II	III	IV	V	VI	VII	VIII
2	76.47	70.58	73.52	67.65	79.41	70.58	73.52	69.12	73.52
4	57.35	57.35	55.58	64.70	72.05	67.64	69.11	66.17	66.17
6	44.11	55.88	48.52	55.58	57.35	64.70	61.76	66.17	57.35
8	38.23	45.58	41.17	51.47	48.52	58.82	54.41	58.82	47.05
10	36.76	44.11	38.23	48.12	48.52	51.47	52.94	55.88	47.05

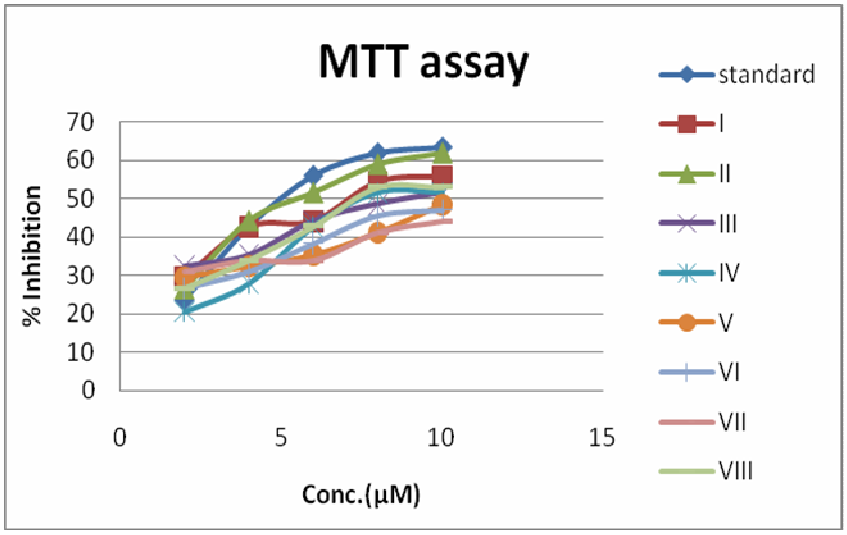


Figure 3: Graph represents the percentage inhibition of cell viability exerted by synthesized derivatives at different concentration

Table no: 4. Table represents the percentage inhibition of the cells at different concentration of test drugs

Conc.(µM)	standard	I	II	III	IV	V	VI	VII	VIII
2	23.53	29.42	26.48	32.35	20.59	29.42	26.48	30.88	26.48
4	42.65	42.65	44.19	35.30	27.95	32.36	30.89	33.83	33.83
6	55.89	44.12	51.48	44.12	42.65	35.30	38.24	33.83	42.65
8	61.77	54.42	58.83	48.53	51.48	41.18	45.59	41.18	52.95
10	63.24	55.89	61.77	51.48	51.48	48.53	47.06	44.12	52.95

Table no: 5. IC_{50} value of synthesized compound

DRUG	**Exp .IC50 VALUE**
Standard (Gefitinib)	4.9µM
Compound - I	7.1µM
Compound - II	5.5µM
Compound - III	8.6µM
Compound - IV	7.6µM
Compound - V	10.3µM
Compound - VI	11.8µM
Compound - VII	12µM
Compound - VIII	7.2µM

Conclusion:

Stated scheme of 4-substituted morpholino propoxy quinazoline derivative synthesis was effective in terms of good yield and quality of the end product and was quiet simple method of synthesis. When synthesized derivatives of the morpholino propoxy quinazoline were tested for cytotoxic activity on leukemic cells using MTT assay method, all the derivatives exhibited good IC_{50} value indicating the cytotoxic potential. Among the other derivatives compound II and I were with promising cytotoxic activity threshold.

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