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Synthesis and Screening of Quinazoline Analogues as Cytotoxic Agents

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ABSTRACT

Synthesis of novel quinazoline derivatives was performed from reaction of N-benzoyl substituted piperazine-1-carbothioamide with 4-(2-chlormethyl) quinazoline-4-yloxy phenyl benzamide derivatives and screened for their in vitro cytotoxic activity by MTT assay. The cell lines used were MCF 7 (Breast cancer cell), HCT 15 (Colon cancer cell line), and VERO (Normal epidermal kidney cell). Result of screening on cell line showed moderate to good cytotoxic activity for all the compounds. Compound GNU 02 (IC_{50} = 7.94 µM, MCF), GNU 12 (IC_{50} = 8.43 µM, HCT) and GNU 25 (IC_{50} = 9.41 µM, HCT) was found to be most active compared to standard gefitinib (IC_{50} = 1.51 µM HCT, IC_{50} =22.37 µM MCF). Structure activity relationship of synthesized analogs suggested that the attachment of electron withdrawing groups like Nitro, Chloro gave better cytotoxic activity then unsubstituted phenyl and electron donating groups. Activity by substituted piperazine at 2nd position of thiazole linked with quinazoline scaffold gave better activity in the order of H > CH_3 > C_6H_5. Our findings may impart new direction to medicinal chemists and biochemists for further investigations of quinazoline-thiazole containing cytotoxic agents.

Keywords: Quinazoline, Thiazole, Cytotoxic, Piperazine, MTT assay.

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Number of cancer patients is increasing rapidly from day to day and good protection from cancer and with reduced adverse effects is the requirement of present scenario. Quinazolines are among the most useful heterocyclic compound from both synthetic and medicinal chemistry aspects. Most of researcher focused on synthesis of quinazoline and their anticancer property. [1] The structural design of quinazoline have attracted a great deal of attention because of their ready accessibility, diverse chemical reactivity, and biological activities like anti-inflammatory [2], antimalarial [3], anthelmintic [4], muscle relaxant [5], antihyperlipidemic [6], antitubercular [7], antimicrobial [8], and antihypertensive activities. [9] The synthetic flexibility of quinazoline scaffold led to the synthesis of variety of its substituted analogs. Raltitrexed and thymitaq, Gefitinib are now clinically used as anticancer drugs having quinazoline moiety.

Quinazoline moiety was envisaged as having purpose like affording two interactions with the hinge backbone, one due to the 1-NH bond acceptor and the other via 3-NH donor and due to rigidity of quinazoline and reduced number of rotatable bonds can desirable for improved pharmacokinetic properties. Thiazole is also good anticancer agents by linking of piperazine as spacer which occupies narrow tubular pocket produce cery good potency. Most kinase inhibitors bind into adenosine triphosphate and form

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interaction with the hinge separating the two lobes of domain. The hinge binds to adenine group of ATP, through generation of hydrogen bonds. This motif has been targeted in the design of most kinase inhibitors which contain hydrogen bond accepting and donating groups that can form noncovalent interaction with the hinge essential for the potency of inhibition. Number of heterocyclic group has been used as hinge-binding moiety to generate interaction with the backbone of hinge, for example 2-carboxamidopyridine group in sorafenib. [10]

Quinazoline hinge binder connected through oxygen atom to the phenyl ring that is expected to occupy DFG out pocket. Amide is linker frequently used in this inhibitor. E.g. In sorafenib where it interact with glu501 and backbone of asp594. We hypothesized that the designing of molecule with quinazoline moiety as molecular scaffold by using strategies like linking with thiazole and by varying chain length using piperazine as linker and substituted phenyl group attach at position the fourth of quinazoline ring in the target molecules for better anticancer activity.

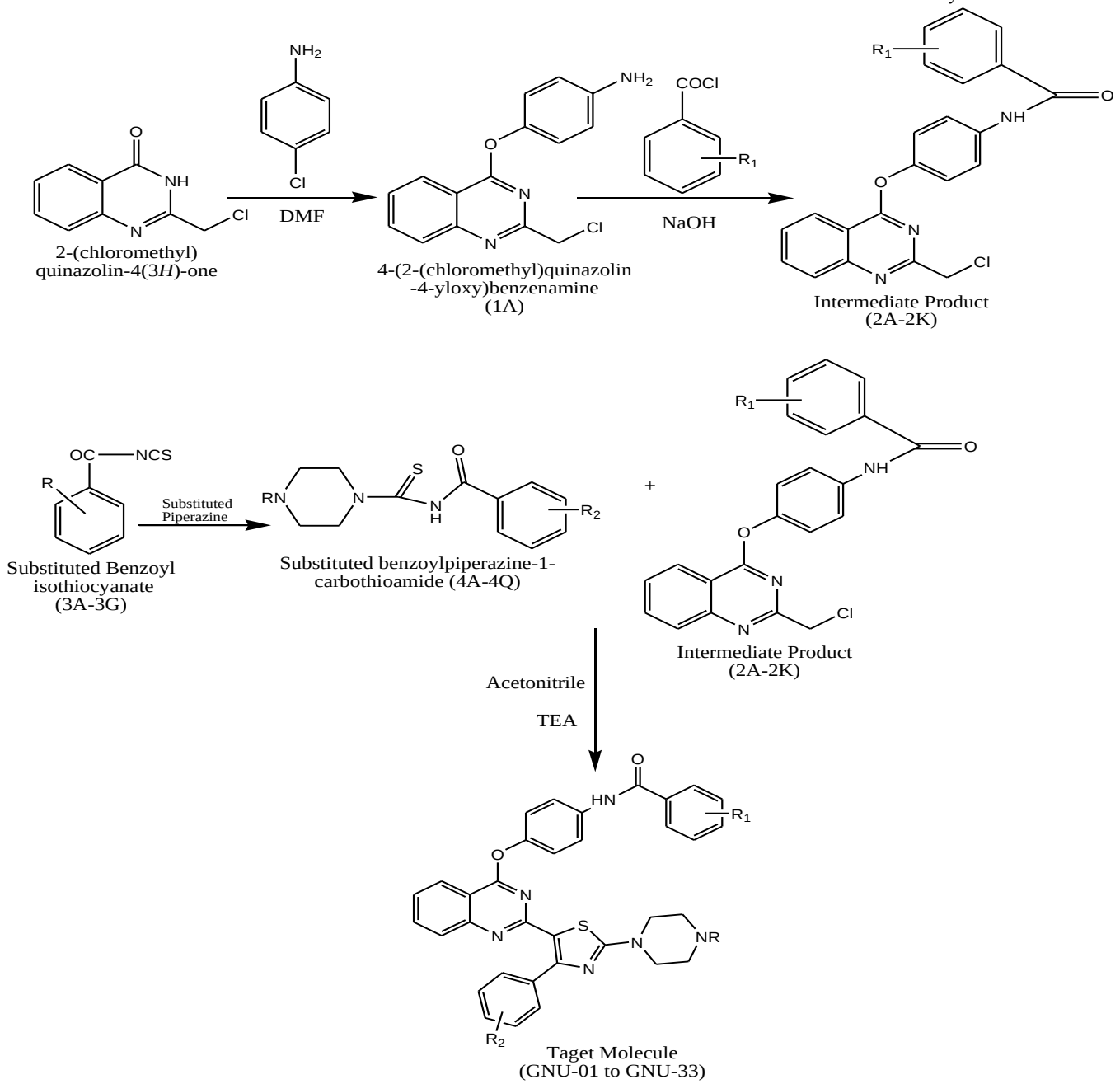


Fig. 1: Schematic Representation for the synthesis of quinazoline derivatives

MATERIAL AND METHODS

Melting points of all synthesized compounds were determined in open capillaries using Veego melting point apparatus, Model VMP-D (Veego India ltd., Mumbai, India) and were uncorrected. Infrared spectra were recorded using KBr pellets on SHIMADZU-FT-IR 8400S instrument. Mass spectra were recorded on PerkinElmer LC–MS PE Sciex API/65 Spectrophotometer. The ^{1}H NMR spectra were recorded on Brucker Avance-300 (300 MHz) model spectrophotometer in $CDCl_3$ using DMSO as solvent and TMSi as internal standard with ^{1}H resonant

frequency of 300 MHz. The TLC was performed on precoated alumina silica gel 60 F_{254} (Merck). The mobile phase was benzene: methanol (9: 1) and detection was made using UV light. The resulting compounds were purified by recrystallization using suitable solvent. The elemental analyses were done on elementar Vario EL 3 Carlo erba 1108 and were well in accordance with the structures assigned to the compound. Synthetic grade chemicals procured from SD fine chemicals, Baroda, India were used for the synthesis of the target compounds. All the compounds were prepared according to the literature procedures with some minor modifications [11-12] General synthetic procedures used for the preparation of the target compound are as follows:

Synthesis of 4-(2-(chloromethyl) quinazolin-4-yloxy) benzenamine (1a)

Equimolar quantity of 2-(chloromethyl)-quinazoli-4(3H)-one, p-chloro aniline and potassium carbonate were dissolved in dimethylformamide. The mixture was refluxed for 3 hours with stirring. The resulting mixture was cooled to room temperature and poured into ice water. The precipitate were collected by filtration and washed with water to give compound 4-(2-(chloromethyl) quinazolin-4-yloxy) benzenamine. The crude product was recystilized with toluene to yield product. [13]

Synthesis of N-(4-(2-(chloromethyl) quinazolin-4-yloxy) phenyl)benzamide derivatives (2a-2k)

4-(2-(chloromethyl) quinazoline-4-yloxy) benzenamine in 5 % aqueous NaOH in conical flask. Add substituted benzoyl chloride dropwise with constant shaking. Shake vigorously for 5-10 min. Ensure that the mixture remains alkaline. Filter off the solid mass, wash with cold water and recrystallized from hot water. [14-15]

Synthesis of Substituted benzoyl isothiocynate (3a-3g)

A RBF equipped with a dropping funnel, mechanical stirrer, drying tube is charged with substituted benzoyl chloride in benzene, few drops of PEG-400 and 33% of potassium thiocynate solution was added from the dropping funnel over 25 min period. Stirring was continued for 4 hours at room temperature. The layers then separated and combine organic layer was dried with sodium sulphate. The solution was filtered and the solvent was evaporated under reduced pressure to yield substituted benzoyl isothiocynate. [14]

Synthesis of substituted benzoylpiperazine-1-carbothioamide (4a-4q)

RBF equipped with a dropping funnel, mechanical stirrer, is charged with substituted piperazine, toluene and substituted benzoyl isothiocynate was added from the dropping funnel. Stirring was continued for 2 hours at room temperature. Crude solid product of was precipitate out. The precipitate was collected by suction and repeatedly washed with small portions of toluene and recrystallised with ethyl acetate to yield product. [15]

Synthesis of target molecules (GNU-01 to GNU-33)

Equimolar quantity of N-(4-(2-(chloromethyl) quinazolin-4-yloxy) phenyl)benzamide derivatives (2a-2k) and substituted benzoylpiperazine-1-carbothioamide (4a-4q) in acetonitrile were reacted in presence of triethylamine. The reaction mixture was allowed to reflux and completion of reaction was checked by TLC. Precipitate was obtained after pouring the reaction mixture to crushed ice and finally compounds were recrystallized using methanol. [16]

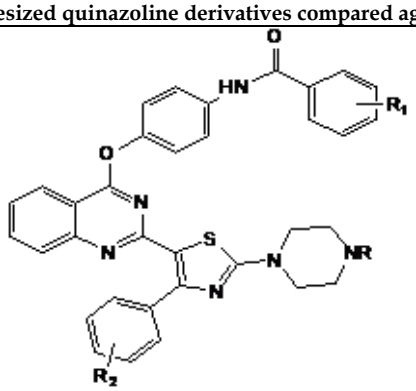
Cytotoxic Study

All the synthesized compounds were screened for their cytotoxic activity on MCF-7 (human mammary gland adrenocarcinoma cell line), HCT-15 (colon cancer cell line) and VERO (Normal kidney cancer cell line) by MTT assay. MCF-7, NCI and VERO cell culture were procured from national center for cell sciences, Pune, India. The screening experiments were carried out at Department of Biotechnology, Faculty of pharmacy, Dharamsinh Desai University, Nadiad. Cultures were observed using an inverted microscope to assess the degree of viability, and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS without Ca^{++}/Mg^{++} using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1 ml per 25 cm^2 of surface area. Flask was rotated to the cover monolayer with trypsin and moved to the incubator and left for 2-4 minutes. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were resuspended in a small volume of fresh serum containing medium and 100-200 µl was removed to perform cell count. The required number of cells were transferred to a new labeled flask containing prewarmed HCT-15 and incubated as appropriate for the cell line. All the cytotoxicity experiments were carried out in 96 well plates. Gefitinib was used as a reference standard for cytotoxic activity. All solutions of test compound were prepared using DMSO. IC_{50} values were calculated. It is a drug concentration causing 50% inhibition of cell proliferation [17-18] Statistical significance of the data (expressed as mean ± SEM) was demonstrated by performing one-way ANOVA test followed by Dennett comparison of IC_{50} of the entire compounds against gefitinib using Graph pad Prism (Version 5.0) software. Results of the biological screening and statistical analysis are shown in Table 1.

RESULTS

The target molecules were designed by joining two different moieties i.e., quinazolinone and thiazole. These novel quinazolinone-thiazole derivatives were synthesized with different aryl substitution at 4^{th} position of quinazolinone ring and different substituted aryl at 4^{th} position of thiazole and different piperazine at 2^{nd} position of thiazole. Synthesis of target molecule was carried out as per scheme.

Table 1: ***In vitro*** **cytotoxic screening data of synthesized quinazoline derivatives compared against the standard drugs**



Compound Code	R	R_1	R_2	Mean IC_{50}(μM)		
				MCF 7	HCT 15	VERO
GNU-01	H	3,5-Cl	3,5-NO_2	12.43	141.1	127.7
GNU-02	H	2,4-Cl	3,5-NO_2	7.94	35.97	208.56
GNU-03	H	3,5-NO_2	3,5-NO_2	52.28	6.56	66.38
GNU-04	H	H	4-NO_2	73.25	44.28	55.76
GNU-05	H	4-OCH_3	4-OCH_3	>100	>100	107.7
GNU-06	H	4-NO_2	4-NO_2	34.62	3.22	>100
GNU-07	H	4-Cl	4-OCH_3	>100	3.14	104.12
GNU-08	H	3-OCH_3	2-Cl	35.01	39.59	59.49
GNU-09	H	2-F	3,5-Cl	81.00	32.53	141.1
GNU-10	H	2-Cl	4-NO_2	83.55	10.42	>100
GNU-11	H	2,3-Cl	4-OCH_3	>100	>100	>100
GNU-12	CH_3	3,5-NO_2	2-Cl	10.93	8.43	151.68
GNU-13	CH_3	3-OCH_3	3,5-NO_2	13.45	71.47	54.42
GNU-14	CH_3	2-F	3,5-NO_2	27.70	70.48	179.7
GNU-15	CH_3	H	4-NO_2	>100	50.23	6.56
GNU-16	CH_3	4-OCH_3	4-NO_2	>100	25.74	8.40
GNU-17	CH_3	4-NO_2	2-Cl	87.22	31.89	47.90
GNU-18	CH_3	4-Cl	4-OCH_3	>100	65.96	8.43
GNU-19	CH_3	3,5-Cl	4-NO_2	>100	18.48	10.42
GNU-20	CH_3	2-Cl	4-NO_2	>100	55.76	8.83
GNU-21	CH_3	2,4-Cl	4-OCH_3	>100	8.83	7.94
GNU-22	CH_3	2,3-Cl	3,5-Cl	66.75	>100	>100
GNU-23	C_6H_5	2,3-Cl	3,5-NO_2	16.33	12.74	74.28
GNU-24	C_6H_5	2-Cl	3,5-NO_2	>100	30.34	25.76
GNU-25	C_6H_5	H	3,5-NO_2	25.76	9.41	110.41
GNU-26	C_6H_5	4-OCH_3	4-NO_2	9.13	>100	13.45
GNU-27	C_6H_5	4-NO_2	H	13.45	47.90	>100
GNU-28	C_6H_5	4-Cl	4-OCH_3	80.86	36.30	9.13
GNU-29	C_6H_5	3-OCH_3	2-F	26.93	20.54	105.1
GNU-30	C_6H_5	3,5-NO_2	2-Cl	19.83	60.72	>100
GNU-31	C_6H_5	3,5-Cl	2-F	>100	8.40	31.23
GNU-32	C_6H_5	2-F	4-NO_2	31.23	25.12	>100
GNU-33	C_6H_5	2,4-Cl	3,5-Cl	18.61	66.38	22.37
Std (Gefitinib)				22.37	1.51	>100

The structure of all synthesized compound were confirmed by physical characterization i.e., melting point, R_f value, IR, MASS and NMR spectroscopy which are as belows.

3, 5-dichloro-N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-01)

Light yellow product; R_f value 0.61, Yield 49%; M.p. 203-207°C; IR (KBr, υ_{max}, cm^{-1}): 1760 (C=O), 3496(NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 10.62 (s, 1H, NH), 7.54-7.31 (m, 14H, Ar-H), 5.54 (s, 1H, NH), 3.03-2.92 (m, 4H, Piperazine), 2.67 (s, 4H, Piperazine) ppm; and MS: m/z 742.1 (M)

2, 4-dichloro-N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-02)

Light Brown product; R_f value 0.61, Yield 78%; M.p. 177-178°C; IR (KBr, υ_{max}, cm^{-1}): 1642 (C=O), 3393(NH); MS: m/z 743.5 (M + 1)

N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3, 5-dinitrobenzamide (GNU-03)

Light brown product; R_f value 0.62, Yield 46%; M.p. 171-175°C; IR (KBr, υ_{max}, cm^{-1}): 1738 (C=O), 3424(NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 10.23 (s, 1H, NH), 9.29-7.22 (m, 14H, Ar-H), 3.66 (s, 1H, NH), 3.19-2.93 (m, 4H, Piperazine), 2.41 (s, 4H, Piperazine) ppm; and MS: m/z 765.2 (M + 1)

N-(4-(2-(4-(4-nitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-04)

Light Yellow product; R_f value 0.67, Yield 74%; M.p. 198-199°C; IR (KBr, υ_{max}, cm^{-1}): 1683 (C=O), 3200(NH); MS: m/z 630.2 (M + 1)

4-methoxy-N-(4-(2-(4-(4-methoxyphenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-05)

Yellow product; R_f value 0.71, Yield 59%; M.p. 188-192°C; IR (KBr, υ_{max}, cm^{-1}): 1685 (C=O), 3050(NH), 2923 (CH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.21 (s, 1H, NH), 9.47-7.20 (m, 16H, Ar-H), 3.37 (s, 1H, NH), 3.17-2.91 (m, 4H, Piperazine), 2.79 (s, 3H, methoxy), 2.37 (s, 4H, Piperazine), 2.23 (s, 3H, methoxy) ppm; and MS: m/z 744.1 (M + 1)

4-nitro-N-(4-(2-(4-(4-nitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-06)

Light Brown product; R_f value 0.72, Yield 80%; M.p. 121-123°C; IR (KBr, υ_{max}, cm^{-1}): 1697 (C=O), 3080(NH); MS: m/z 675.2 (M + 1)

4-chloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-07)

Light Brown product; R_f value 0.69, Yield 45%; M.p. 189-192°C; IR (KBr, υ_{max}, cm^{-1}): 1683 (C=O), 3010 (CH), 3100(NH); MS: m/z 648.2 (M)

N-(4-(2-(4-(2-chlorophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3-methoxybenzamide (GNU-08)

White product; R_f value 0.67, Yield 76%; M.p. 175-177°C; IR (KBr, υ_{max}, cm^{-1}): 1679 (C=O), 2952 (CH), 3120(NH); MS: m/z 649.1 (M + 1)

N-(4-(2-(4-(3, 5-dichlorophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-2-fluorobenzamide (GNU-09)

Light Yellow product; R_f value 0.70, Yield 38%; M.p. 177-181°C; IR (KBr, υ_{max}, cm^{-1}): 1733 (C=O), 2895 (CH), 3096(NH); MS: m/z 670.1 (M)

2-chloro-N-(4-(2-(4-(4-nitrophenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-10)

Light Brown product; R_f value 0.72, Yield 74%; M.p. 151-152°C; IR (KBr, υ_{max}, cm^{-1}): 1712 (C=O), 3049(NH); MS: m/z 663.2 (M)

2, 3-dichloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(piperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)benzamide (GNU-11)

Light Brown product; R_f value 0.63, Yield 75%; M.p. 140-14°C; IR (KBr, υ_{max}, cm^{-1}): 1693 (C=O), 3150(NH), 2952 (CH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.20 (s, 1H, NH), 9.28-7.21 (m, 14H, Ar-H), 3.18 (s, 1H, NH), 3.05-2.92 (m, 4H, Piperazine), 2.37 (s, 4H, Piperazine), 2.23 (s, 3H, methoxy) ppm; and MS: m/z 683.1 (M + 1)

N-(4-(2-(4-(2-chlorophenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3, 5-dinitrobenzamide (GNU-12)

Dark Brown product; R_f value 0.71, Yield 40%; M.p. 159-162°C; IR (KBr, υ_{max}, cm^{-1}): 1683 (C=O), 2964 (CH), 3049(NH); MS: m/z 724.1 (M + 2)

N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3-methoxybenzamide (GNU-13)

Brown product; R_f value 0.67, Yield 73%; M.p. 122-123°C; IR (KBr, υ_{max}, cm^{-1}): 1670 (C=O), 2998 (CH), 3178(NH); ^1H NMR (300 MHz, δ ppm, DMSO): 9.79 (s, 1H, NH), 7.51-6.96 (m, 15H, Ar-H), 3.97-3.09 (m, 4H, Piperazine), 2.11-1.51 (m, 4H, Piperazine), 1.39-1.37 (m, 3H, CH_3), 1.30-1.25 (m, 3H, CH_3) ppm; and MS: m/z 719.2 (M + 1)

N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-2-fluorobenzamide (GNU-14)

Yellow product; R_f value 0.65, Yield 31%; M.p. 166-169°C; IR (KBr, υ_{max}, cm^{-1}): 1635 (C=O), 2910 (CH), 3030(NH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.71 (s, 1H, NH), 7.63-7.21 (m, 14H, Ar-H), 3.33-3.04 (m, 4H, Piperazine), 2.68 (s, 4H, Piperazine), 2.24 (s, 3H, CH_3) ppm; and MS: m/z 706.1 (M)

N-(4-(2-(2-(4-methylpiperazin-1-yl)-4-(4-nitrophenyl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-15)

Light Brown product; R_f value 0.68, Yield 68%; M.p. 166-167°C; IR (KBr, υ_{max}, cm^{-1}): 1670 (C=O), 2950 (CH), 3193(NH); MS: m/z 644.3 (M + 1)

4-methoxy-N-(4-(2-(2-(4-methylpiperazin-1-yl)-4-(4-nitrophenyl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-16)

Light Yellow product; R_f value 0.70, Yield 70%; M.p. 171-173°C; IR (KBr, υ_{max}, cm^{-1}): 1650 (C=O), 2916 (CH), 3010(NH); MS: m/z 674.2 (M + 1)

N-(4-(2-(4-(2-chlorophenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-4-nitrobenzamide (GNU-17)

Light Yellow product; R_f value 0.75, Yield 79%; M.p. 189-190°C; IR (KBr, υ_{max}, cm^{-1}): 1610 (C=O), 2904 (CH), 3031(NH); MS: m/z 679.2 (M + 1)

4-chloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)benzamide (GNU-18)

Light Yellow product; R_f value 0.68, Yield 46%; M.p. 160-162°C; IR (KBr, υ_{max}, cm^{-1}): 1616 (C=O), 2921 (CH), 3010(NH); ^1H NMR (300 MHz, δ ppm, DMSO): 9.83 (s, 1H, NH), 7.53-6.96 (m, 16H, Ar-H), 3.79-3.19 (m, 4H, Piperazine), 2.11-1.53 (m, 4H, Piperazine), 1.38-1.24 (m, 6H, methoxy) ppm; and MS: m/z 664.2 (M + 2)

3, 5-dichloro-N-(4-(2-(2-(4-methylpiperazin-1-yl)-4-(4-nitrophenyl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-19)

Light Brown product; R_f value 0.70, Yield 70%; M.p. 171-173°C; IR (KBr, υ_{max}, cm^{-1}): 1673 (C=O), 2960 (CH), 3240(NH); MS: m/z 711.1 (M)

2-chloro-N-(4-(2-(2-(4-methylpiperazin-1-yl)-4-(4-nitrophenyl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-20)

Light Yellow product; R_f value 0.75, Yield 79%; M.p. 189-190°C; IR (KBr, υ_{max}, cm^{-1}): 1706 (C=O), 2920 (CH), 3030(NH); MS: m/z 677.1 (M)

2, 4-dichloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)benzamide (GNU-21)

Light Yellow product; R_f value 0.70, Yield 70%; M.p. 171-173°C; IR (KBr, υ_{max}, cm^{-1}): 1698 (C=O), 2950 (CH), 3045(NH); MS: m/z 696.1 (M)

2, 3-dichloro-N-(4-(2-(4-(3, 5-dichlorophenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-22)

Light Brown product; R_f value 0.75, Yield 79%; M.p. 189-190°C; IR (KBr, υ_{max}, cm^{-1}): 1693 (C=O), 2955 (CH), 3020(NH); MS: m/z 734.1 (M)

4-chloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-23)

Light Yellow product; R_f value 0.66, Yield 68%; M.p. 188-189°C; IR (KBr, υ_{max}, cm^{-1}): 1670 (C=O), 3056(NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 10.69 (s, 1H, NH), 8.69-6.96 (m, 19H, Ar-H), 1.53-1.38 (m, 4H, Piperazine), 1.36-1.24 (m, 4H, Piperazine) ppm; and MS: m/z 818.3 (M)

2-chloro-N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-24)

Light Brown product; R_f value 0.69, Yield 63%; M.p. 166-167°C; IR (KBr, υ_{max}, cm^{-1}): 1693 (C=O), 3030 (NH); MS: m/z 784.2 (M)

N-(4-(2-(4-(3, 5-dinitrophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-25)

Light Brown product; R_f value 0.71, Yield 80%; M.p. 190-193°C; IR (KBr, υ_{max}, cm^{-1}): 1673 (C=O), 3108(NH); MS: m/z 751.1 (M)

4-methoxy-N-(4-(2-(4-(4-nitrophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-26)

Light Yellow product; R_f value 0.73, Yield 76%; M.p. 125-127°C; IR (KBr, υ_{max}, cm^{-1}): 1650 (C=O), 2917 (CH), 3020(NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 10.25 (s, 1H, NH), 8.68-6.96 (m, 21H, Ar-H), 2.11-1.95 (m, 4H, Piperazine), 1.68-1.37 (m, 4H, Piperazine) ppm; and MS: m/z 735.2 (M - 1)

4-nitro-N-(4-(2-(4-phenyl-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-27)

Light Brown product; R_f value 0.68, Yield 69%; M.p. 122-123°C; IR (KBr, υ_{max}, cm^{-1}): 1660 (C=O), 3100(NH); MS: m/z 706.3 (M + 1)

4-chloro-N-(4-(2-(4-(4-methoxyphenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-28)

Light Brown product; R_f value 0.71, Yield 47%; M.p. 171-175°C; IR (KBr, υ_{max}, cm^{-1}): 1670 (C=O), 2912 (CH), 3058(NH); MS: m/z 724.3 (M)

N-(4-(2-(4-(2-fluorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3-methoxybenzamide (GNU-29)

Light Brown product; R_f value 0.66, Yield 66%; M.p. 187-189°C; IR (KBr, υ_{max}, cm^{-1}): 1686 (C=O), 2918 (CH), 3030(NH); MS: m/z 708.1 (M)

N-(4-(2-(4-(2-chlorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl)-3, 5-dinitrobenzamide (GNU-30)

Light Yellow product; R_f value 0.67, Yield 46%; M.p. 168-172°C; IR (KBr, υ_{max}, cm^{-1}): 1693 (C=O), 2916(NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 9.83 (s, 1H, NH), 7.54-6.96 (m, 20H, Ar-H), 2.11-1.93 (m, 4H, Piperazine), 1.44-1.26 (m, 4H, Piperazine) ppm; and MS: m/z 785.2 (M + 1)

3, 5-dichloro-N-(4-(2-(4-(2-fluorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-31)

Light Brown product; R_f value 0.70, Yield 43%; M.p. 170-174°C; IR (KBr, υ_{max}, cm^{-1}): 1731 (C=O), 3040(NH); MS: m/z 746.1 (M)

2-fluoro-N-(4-(2-(4-(4-nitrophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-32)

Light Yellow product; R_f value 0.68, Yield 46%; M.p. 181-182°C; IR (KBr, υ_{max}, cm^{-1}): 1716 (C=O), 2956 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 10.29 (s, 1H, NH), 8.69-6.96 (m, 20H, Ar-H), 1.65-1.51 (m, 4H, Piperazine), 1.50-1.11 (m, 4H, Piperazine) ppm; and MS: m/z 723.1 (M)

2, 4-dichloro-N-(4-(2-(4-(3, 5-dichlorophenyl)-2-(4-phenylpiperazin-1-yl) thiazol-5-yl) quinazolin-4-yloxy) phenyl) benzamide (GNU-33)

Light Brown product; R_f value 0.69, Yield 73%; M.p. 119-120°C; IR (KBr, υ_{max}, cm^{-1}): 1731 (C=O), 2981(NH); MS: m/z 796.2 (M)

Cytotoxic Activity

All the synthesized compound and standard drugs gefitinib were subjected to cytotoxic activity on 3 different cell line like MCF 7 (Breast cancer cell line), HCT 15 (Colon cancer cell line) and VERO (Normal kidney cell line). In MFC 7 cell line, total six compounds GNU 02 (7.94), GNU 12 (10.93), GNU 13 (13.45), GNU 26 (9.13), GNU 30 (19.83), GNU 33 (18.61) were active which having high activity as compare to standard drug gefitinib (22.37). In HCT cell line, four compounds GNU 03 (6.56), GNU 21 (8.83), GNU 25 (9.41), GNU 31 (8.40) were active which having comparable activity as compare to standard drug (1.51). In VERO cell line which is normal cell line, seven compounds GNU 01 (127.7), GNU 02 (208.5), GNU 07 (104.12), GNU 12 (151.68), GNU 14 (179.7), GNU 25 (110.41), GNU 29 (105.1) have less affect to normal cell as compare to standard gefitinib. From above all cell line study, Compound GNU 02, GNU 12, and GNU 25 are very potent and good cytotoxic compounds.

DISCUSSION

A series of quinazoline derivatives was synthesized and screened for their in vitro cytotoxic activity. Results of assay indicated that all the compounds were found to have good to moderate activity. Compound GNU 02, GNU 12 and GNU 25 possess higher activity than the standard drug gefitinib. These compounds were particularly promising, since it was able to kill cancer

cells more effectively than the non-cancerous cell which was observed from the result of VERO cell line. Furthermore, it concluded that compound with unsubstituted piperazine and attachment of electron withdrawing group has been recognized as potent anticancer agent. Therefore, this type of compound may further be optimized and evaluated with enzymatic assay and in vivo animal models in the line of the development and also can serve as a prototype molecule of new class of anticancer agents.

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