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SYNTHESIS, ANTI MICROBIAL, ANTI CANCER EVALUTION AND MOLECULAR DOCKING STUDIES OF QUINAZOLINYL THIADIAZOLES DERIVATIVES

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Abstract:
Quinazoline derivatives are reported to have anti microbial, anti inflammatory, analgesic and anti cancer activities. The incorporated oxymethyl carbamide at 4th position of the quinoline ring was found to influence the biological activities of the molecules with this some of new Quinolinyl- oxymethyl -thiadiazoles synthesized from 4-hydroxy quinoline through (quinolin-4yloxy) acetyl hydrazide intermediates. All the synthesized compounds were characterized by IR, H^1NMR spectral data and carried out molecular docking studies against calcium/calmoduline dependent protein kinase (ID:2JC6), based on these studies we have selected the anti microbial, anti cancer, activities for screening of biological activities of synthesized derivatives.

Keywords: *Anti microbial activity, Thiadiazoles, Quinazoline derivatives, docking studies.*

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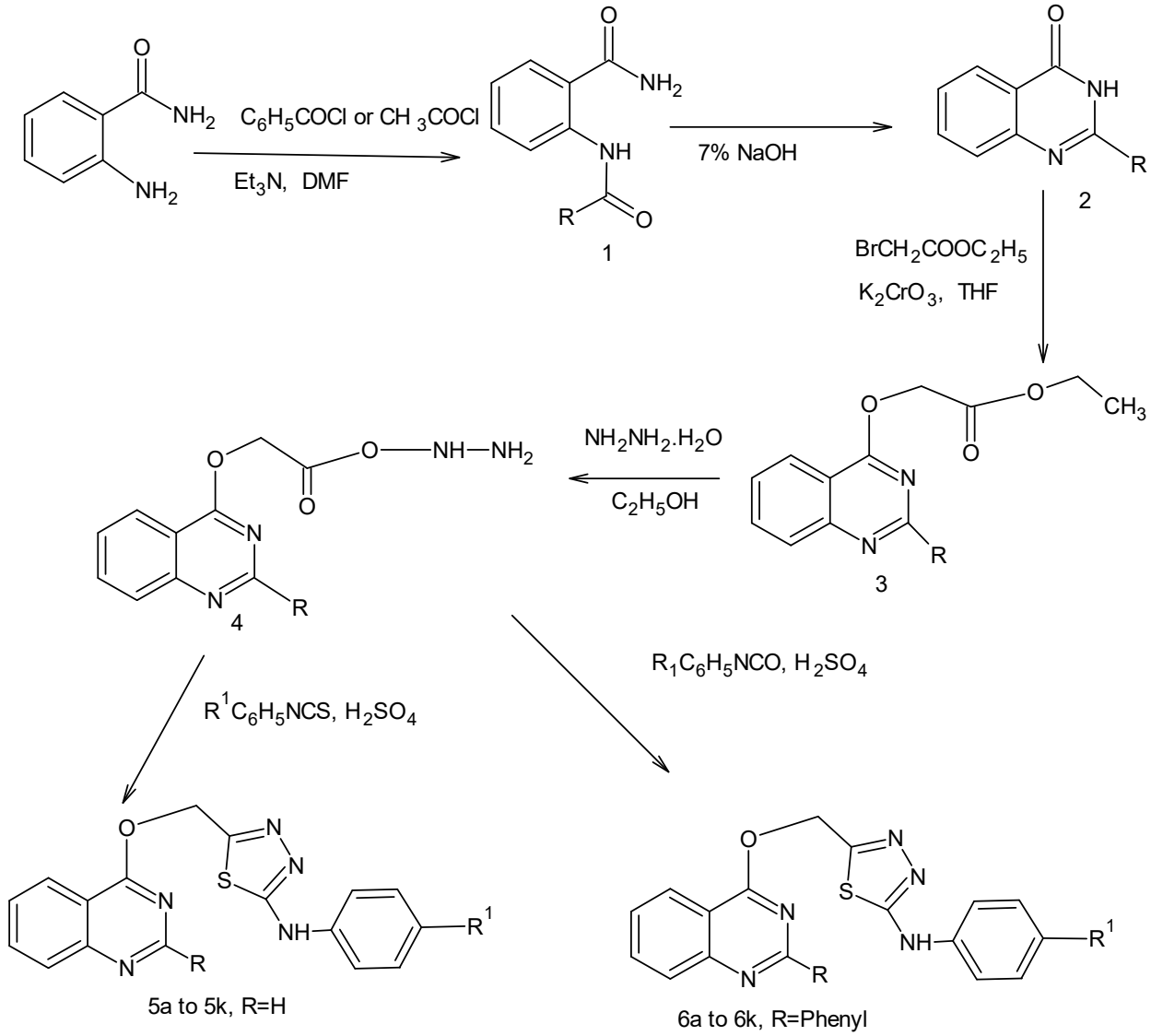
INTRODUCTION:

Quinazoline and its derivatives are found to posses anti microbial[1], anti inflammatory[2], Cytotoxicity activity. Thiadiazoles are found to posses significant anti bacterial, antifungal and anti inflammatory activities[1-3]. The incorporation of (quinazolin-4-yl)-oxy)-methyl]-1, 3, 4-thiadiazol moieties to quinoline via (OCH_2CONH) linkage was through to enhance the biological activities. Hence in the present study the 4th position of Quinazoline was used as target for chemical modification by incorporation thiadiazoles. 4(substituted benzylidine hydrazido methyloxy) quinoline (schiff's bases) were synthesized by the condensation of 2-quinazoline 4yloxy acetyl hydrazide with different substituted benzaldehydes, reaction of these schiff's bases with chloroacetyl chloride and mercapto acetic acid yielded 3 chloro (4quinazolinyl-oxy acetamidyl)-4(substituted aryl) thiazolidinones. These synthesized compounds characterized by IR, H^1NMR spectral data and carried out molecular docking studies against calcium/calmoduline dependent protein kinase (ID: 2JC6) and evaluated for their antimicrobial, anti cancer activities.

MATERIALS AND METHODS:

Melting points were determined with open capillary and are uncorrected. IR spectra were recorded on a PerkinElmer FTIR240 spectrophotometer using KBr optics. H^1-NMR spectra were recorded on 300MHz, bruker spectrometer in DMSO or $CDCl_3$ using TMS as an internal standard. All reactions were monitored by TLC on precoated silica gel $60F_{254}$ (mesh), spots were visualized with UV light.



Scheme: Synthesis of quinazolinyl thiadiazoles.

Reagents conditions: a) Benzoyl chloride & pyridine, b) $HCONH_2$, c) Ethyl chloroacetate and dry acetone d) $NHNH_2.H_2O$/ ethanol, e) CS_2/KOH, f) HCHO, N-alkyl amine, g) p- substituted phenyl isothiocynate, h)H_2SO_4 (AR).

CHEMISTRY:

Method of synthesis of ethyl (Quinazoline 4 yl oxy) acetate:

An equimolar mixture of 4 hydroxy **Quinazoline**, ethyl chloro acetate and anhydrous potassium carbonate in dry acetone was refluxed on water both for 24hrs. The solid was filtered and the excess solvent was removed on a rotavapour.

Synthesis of 2 (Quinazoline 4 yl oxy) acetohydrozide:

A mixture of compound 1 in absolute ethanol, hydrozenehydrate was added and the reaction mixture was refluxed for 15 hrs. The solution was concentrated and the solid that separate out on cooling was filtered at pump and re crystallized from absolute alcohol.

IR (KBr, cm^{-1}): 1762(C=O), 1616(CONH-), 3216(-NH-), 3056-2916(C-H); ^{1}H-NMR ($CDCl_3$, ppm)δ: 7.3-7.9,(m, 4H, AR-H), 8.84(s, 1H, AR-H), 4.83(s,2H,OCH_2), 8.0(s,1H, CONH),2.0(s,2H,-NH_2).

N-phenyl-5-[(quinazolin-4-yl)-oxy)-methyl]-1, 3, 4-thiadiazol-2-amine (5a-5k):

To the boiling solution of compound IV (0.001 moles) in absolute ethanol (25 ml) phenyl iso thiocynate (0.01 moles) was added with stirring and solution heated for 5 hrs, to this add chiled H2SO4 (20 ml) gradually constant stiring after addition was over poured on crushed ice (100 gr) and kee in refrigiration over night, basified with 5% sodium hydroxide and extracted with ether and purified by column chromatography (chloroform: methanol) similarly the compound 5b to 5k were prepared by using para substituted phenyl isothiocynates and their physical data are given table 1.

IR(KBr,cm-1) : 1700(C=O),1672(C=N),3423(N-H),3329-1872 (C-H),578(C=S).

H1NMR(DMSO δppm): 7.0-8.3(m, 9H, Ar-H), 9.1(s, 1H, Ar-H), 4.9(s, 2H, OCH2), 2.9 (s,1H, N-H).

***N*-phenyl-5-{[(2-phenylquinazolin-4-yl)-oxy]-methyl}-1, 3, 4-thiadiazol-2-amine (6a-6k):**

To the boiling solution of compound 4 (0.001 moles) in absolute ethanol (25 ml) phenyl iso thiocynate (0.01 moles) was added with stirring and solution heated for 5 hrs, to this add chiled H_2SO_4 (20 ml) gradually constant stiring after addition was over poured on crushed ice (100 gr) and kee in refrigiration over night, basified with 5% sodium hydroxide and extracted with ether and purified by column chromatography (chloroform: methanol) similarly the compound 6b to 6k were prepared by using para substituted phenyl isothiocynates and their physical data are given table 1.

IR(KBr,cm^{-1}) : 1632(C=O), 3369(N-H),3329 (C-H), 607(C=S).

H^{1}NMR(DMSO δppm): 6.5-8.1(m, 14H, Ar-H), 4.5(s, 2H, OCH_2), 3.0 (s,1H, N-H).

Molecular docking studies:

All the derivatives of quinazolinyl-thiadiazoles (5a-k, 6a-k) have selected for their molecular docking studies on selected X-ray crystal structure of human calcium/calmoduline dependent protein kinase1D (pdb code; 2jc6) was retrieved from protein data bank using glid (Schrodinger, OPLS-2005 software), the ligands preparation was done in ligprep modules on glide program and selected protein was minimized by performing the following steps: assigning of bond orders, addition of hydrogen's, and optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex, and docked with prepared ligands was carried out by using extra precision(XP) modules on glide software. The docking scores (XP) of all quinazolinyl-thiadiazoles (5a-k, 6a-k) was given in table 2.

ANTIMICROBIAL ACTIVITY:

All the compounds were screened for their in vitro anti bacterial activity against two gram positive strains i.e, bacillus subtilis (NCIM 2921) and Staphylococcus aureus (NCIM 2079) and two gram negative strains Escherichia coli (NCIM 2068) and Klebsialla Pneumonia (NCIM 2957) and their anti fungal activity against two fungal strains Candida albican (NCIM 3471) and aspergillus flavus (NCIM 555). The specified strain of organisms was procured from The National Chemical Laboratory, Pune, India, and was used for the evolution of the test compounds by broth dilution method. Culture of test organisms were inoculated on nutrient agar slants and were sub cultured in nutrient broth prior to testing the media used was nutrient agar for bacterial strain and Sabourand dextrose agar media for candida albicans and czapexs dox agar media for aspegillus flavus procured from Hymenia Laboratory media, India. All the test compounds were dissolved in DMSO to give a concentration of 1mg/ml. the test compound were prepared in different concentrations from 5mg/ml to 500mg/ml in DMSO. Ciprofloxacin was used as standard for antibacterial activity and Amphotericin-B for antifungal activity, whole keeping DMSO as control the MIC value of all tests and standard compound are given in table 3.

ANTI CANCER ACTIVITY:
The invitro anti cancer activity of synthesized compound was done by MTT assay
MTT solution preparation: 10 mg in 10 ml of Hank's balanced solution.

Maintenance of cell line:
The MCF- 7 breast adino carcinoma cancer cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0}C. For the MTT assay, MCF- 7 cells were plated in 96 well plate at 5.0 X 10^3 cells were per well in culture medium and incubated overnight at 37 ^{0}C.

MCF- 7 cells and Hep G2 cell viability:
Cell viability was evaluated by the MTT Assay with three independent triplicate experiments of three concentrations of compounds (50 75 and 100 µM). After 24 hrs of incubation, each treatment was withdrawn and MTT solution (0.5 mg / mL^{-1}) was added to each well and plates were incubated at 37 ^{0} C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a microplate reader.

Cytotoxicity Assay:
Invitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometrically determination of conversion of MTT into "Formazan blue" by living cells, remove the supernatant from the plate and add fresh Hank's balanced salt solution and treated with different concentration of compound appropriately diluted with DMSO. Control group contains only DMSO. After 24 hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4 hr incubation. The supernatant carefully aspirated the precipitated crystals of Formazan blue' were solubilized by adding DMSO (200µl) and optical density was measured at wavelength of 570nm, the result was represents the mean of three readings. The concentration at which the OD (optical density) of treated cells was reduced by 50% with respect to the untreated control. The Calculation of % of lyses of cells was done by comparing the O.D. of sample to that of the control and also by microscopic analysis and the IC_{50} values of tested compounds given in 4.

RESULT AND DISCUSSION:

MOLECULAR DOCKING:
To gain insight in to the molecular determinants that modulate the inhibitory activity of synthesized compounds (5a-K & 6a-K), the molecular docking simulations for these compounds to human calcium/calmoduline dependent protein kinase1D (pdb code; 2jc6) was performed using the glid (Schrodinger, OPLS-2005 software), based on the x-ray crystal structure of calcium/calmoduline dependent protein kinase1D was retrieved from protein data bank (pdb code; 2jc6). The docking and subsequent scoring were performed using the default parameters of the glid program demonstrated that all the molecules under study have a nice interaction with amino acids of calcium/calmoduline dependent protein kinase1D, and the dock scores (XP, kcal/moles) and predicted binding affinity (6j) of all compounds have shown in table-2. The compound *N*-(4-chlorobenzyl)-5-{[(2-phenylquinazolin-4-yl)-oxy]-methyl}-1,3, 4-thiadiazol-2-amine (6j) showed effective binding with docking score -10.342kcal/M and the interaction pose given in fig.no1: This compound was forming a hydrogen bond interaction of amino group substituted on 2nd position of thiadiazole ring with VAL101 residue. This compound was found to have hydrophobic interactions with TRP 306and PHE 310 residues. The 2nd position phenyl group play important role in biological activity of a compound it can forms hydrophobic interaction with TRP306 residue and the thiadiazole ring substituted at 4th position of quinazoline nucleus also forms a hydrophobic interaction with TRP306 amino residue. The phenyl group of quinazoline core nucleus hydrophobic interacts with PHE310 residues.

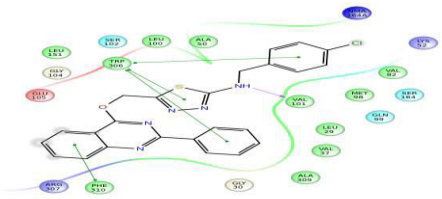


Fig No1: interaction image of compound 6j

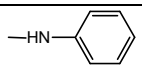
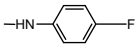
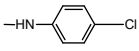
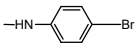
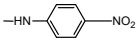
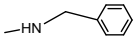
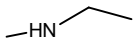
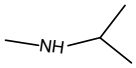
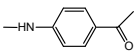
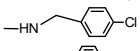
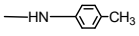
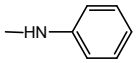
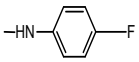
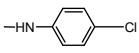
ANTIMICROBIAL ACTIVITIES:

The antibacterial activity of test compounds shows that the newly synthesized quinazolinyl-thiadiazole derivatives (5a-5k, 6a-6k) are exhibits mild to moderate antibacterial activity against the test organisms employed in the present investigation. However, the degree of inhibition varied with the test compound with respect to the test organisms used in this study. All the test compounds i.e., 5a-6k showed a varied degree of antibacterial activity against the test organisms employed. However, among this series of compounds 5a and 6j shows similar and high activity against *E.coli*, where as the test compounds 5j, 6j, and 6e exhibited more activity against *B. subtilis* and *Staphylococcus aureus*. Among the test compounds employed 5a, 6i, 6j were having relatively moderate activities against. *Klebsiella pneumonia.* Antifungal activity of test compounds shows that the newly synthesized quinazolinyl-thiadiazole derivatives (5a-5k, 6a-6k) 5a, 6j exhibiting high activity against *Candida albicans* and *Aspargillus flavum* whereas the test compounds i.e., 5c, 6e, moderate more activity against *Aspargillus flavum*, whereas 5b exhibited mild to moderate activity against *Candida albicans*.

ANTI CANCER ACTIVITY:

Based on docking studies the compounds were selected for their anticancer activity against adino carcinoma of breast **(MCF-7)** and Hepatocellular carcinoma **(HepG2)** cell lines by MTT assay method. The percent inhibition and IC_{50} values for the tested compounds were calculated, and the results are given in the Table. No 4. The compound **6j** showed highest activity against adino carcinoma of breast **(MCF-7)** and Hepatocellular carcinoma **(HepG2)** cell lines with IC_{50} values at **48.11** and **43.76** µg/ml respectively. The compound 5a showed moderate activity against adino carcinoma of breast **(MCF-7)** and Hepatocellular carcinoma **(HepG2)** cell lines with IC_{50} values at **62.68** and **70.3** µg/ml respectively due to lack of substitution on 2^{nd} position of quinazoline pharmacophore. While the compounds **5f, 5i, 5j, 6a, 6b, 6c, 6h and 6i**showed moderate activity and other compounds did not show cytotoxic activity against **MCF-7** and **HepG2** cell lines. Among the all synthesized compounds of thiadiazole (5a-5k & 6a-6k) substitution of 4^{th} position of quinazoline skeleton the thiadiazole derivatives (6a-6k) exhibits more anti cancer activity. The compounds without substitution on 2^{nd} position of quinazoline skeleton exhibits less anticancer activity (5a-5k) than phenyl substitution on2^{nd} position of quinazoline skeleton.

Table 1: Physical data of quinazolinyl thiadiazole derivatives

S.no.	R	R^1	m.p.^{oc}	%Yield	Molecular formula	Molecular .wt	R_f (H:E)
5a	H		112-114	95	$C_{17}H_{13}N_5OS$	335	0.69
5b	H		122-124	89	$C_{17}H_{12}FN_5OS$	353	0.59
5c	H		130-132	80	$C_{17}H_{12}ClN_5OS$	369	0.54
5d	H		120-124	85	$C_{17}H_{12}BrN_5OS$	414	0.6
5e	H		140-142	79	$C_{17}H_{12}N_6O_3S$	380	0.5
5f	H		174-176	75	$C_{18}H_{15}N_5OS$	349	0.55
5g	H		190-192	69	$C_{13}H_{13}N_5OS$	287	0.62
5h	H		220-224	65	$C_{14}H_{15}N_5OS$	301	0.59
5i	H		184-186	69	$C_{19}H_{15}N_5O_2S$	377	0.5
5j	H		152-154	74	$C_{18}H_{14}ClN_5OS$	383	0.52
5k	H		130-132	70	$C_{18}H_{15}N_5OS$	349	0.54
6a	C_6H_5		124-126	85%	$C_{23}H_{17}N_5OS$	411	0.65
6b	C_6H_5		146-148	84%	$C_{23}H_{16}FN_5OS$	429	0.6
6c	C_6H_5		152-154	87%	$C_{23}H_{16}ClN_5OS$	445	0.68

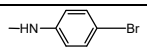
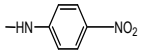
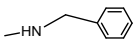
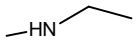
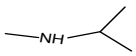
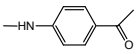
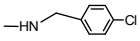
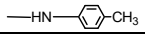
6d	C_6H_5		162-164	79%	$C_{23}H_{16}BrN_5OS$	490	0.54
6e	C_6H_5		172-174	75%	$C_{23}H_{16}N_6O_3S$	456	0.59
6f	C_6H_5		184-186	74%	$C_{24}H_{19}N_5OS$	425	0.64
6g	C_6H_5		224-226	65%	$C_{19}H_{17}N_5OS$	363	0.6
6h	C_6H_5		242-244	69%	$C_{20}H_{19}N_5OS$	377	0.59
6i	C_6H_5		230-232	64%	$C_{25}H_{19}N_5O_2S$	453	0.54
6j	C_6H_5		170-172	70%	$C_{24}H_{18}ClN_5OS$	459	0.62
6k	C_6H_5		140-142	74%	$C_{24}H_{19}N_5OS$	425	0.56

Table2: Docking scores of quinazolinyl thiadiazole derivatives (5a-5k, 6a-6k)

S.NO	Docking score(kcal/moles)	XP G Score (kcal/moles) kcal/moles	Glide energy (kcal/moles)	XP H Bond (Å)
5a	-7.06	-7.06	-37.516	-0.7
5b	-6.107	-6.107	-36.716	-0.35
5c	-5.774	-5.774	-39.556	-0.35
5c	-5.761	-5.761	-39.062	-0.35
5d	-7.254	-7.254	-41.641	-0.7
5e	-6.959	-6.959	-43.103	-0.539
5f	-7.855	-7.855	-41.648	-0.88
5g	-7.521	-7.521	-34.69	-0.7
5h	-6.261	-6.261	-35.221	-0.7
5i	-7.799	-7.799	-43.992	-0.35
5j	-8.167	-8.167	-43.293	0
5k	-6.868	-6.868	-41.577	-0.656
6a	-6.237	-6.237	-41.33	-0.285
6b	-6.855	-6.855	-43.185	-0.283
6c	-6.498	-6.498	-46.039	-0.302
6d	-6.906	-6.906	-49.633	-0.304
6e	-9.458	-9.458	-47.024	-0.558
6f	-8.901	-8.901	-47.889	-0.7
6g	-6.702	-6.702	-41.389	-0.313
6h	-7.045	-7.045	-42.678	-0.35
6i	-9.24	-9.24	-47.077	-0.7
6j	-9.568	-9.568	-50.957	-0.7
6k	-7.429	-7.429	-49.107	-0.316

Table 3: Antimicrobial activity of quinazolinyl thiadiazole derivatives (5a-5k, 6a-6k)

	Zone of inhibition (in mm) for the Quantity in 100 µg					
S.no	E. coli*	*S. a**	*B. s**	*K .p**	*C. a**	*A .f**
5a	**16**	**19**	**17**	**16**	**16**	**19**
5b	12	14	15	13	12	8
5c	11	12	15	10	8	10
5d	16	19	17	16	6	9
5e	3	6	2	3	2	6
5f	3	6	6	2	2	6
5g	3	2	3	6	3	2
5h	3	6	2	6	3	6
5i	16	19	**3**	**2**	6	9
5j	18	19	18	20	8	8
5k	3	6	2	6	3	1
6a	3	6	6	6	2	1
6b	3	6	5	6	2	6
6c	4	3	2	2	1	3
6d	5	4	3	3	5	4
6e	**22**	**19**	**23**	**18**	**8**	**9**
6f	16	19	17	16	6	9
6g	6	7	5	6	6	7
6h	**3**	**1**	**4**	**1**	**3**	**1**
6i	**19**	**19**	**11**	**19**	**9**	**8**
6j	**19**	**21**	**22**	**20**	**10**	**18**
6k	15	13	16	11	13	13
Std1	26	25	26	24	-	-
Std2	-	-	-	-	25	23

Table 4: Anti cancer activity of quinazolinyl thiadiazole derivatives (5a-5k, 6a-6k)

S.NO	**Percent inhibition(µg/mL)**	
	IC 50 (MCF- 7)	IC 50 (Hep G2)
5a	62.68	70.3
5b	189.15	156.62
5c	137.86	128.23
5d	117.12	93.75
5e	125.12	132.43
5f	95.4	110.5
5g	120.2	118
5h	112.5	110.1
5i	85.4	91.2
5j	86.5	94.5
5k	101.4	112.5
6a	75.5	80.2
6b	80.1	89.5
6c	92.4	100.5
6d	189.15	156.62
6e	140.86	130.23
6f	104.12	93.75
6g	120.12	118.43
6h	92.4	100.5
6i	85.4	91.2
6j	48.11	43.76
6k	189.15	156.62
Cisplatin	13.4	6.8

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REFERENCES:

1. Irfan A, Mohammed, E.V.S. Subramanyam. et al., Synthesis and antimicrobial activity of some novel 1-[(quinolinyl-8yloxy)- methyl urea, International Journal of advances in ph. science, vol (1), 2010: 38-42.
2. Panda S, Chowdary P.V.R and Rani S, Design and synthesis of novel quinazolin incorporated Azetidinones and thiazolidinones as sedative, antibacterial and anti fungal agent, Indian drugs, 45 (2) 2008: 84-89.
3. Shireesha B, Umasankar k, Srinivas S and Mallareddy V, Synthesis and anti microbial evaluation and docking studies of novel 4-substituted quinazoline derivative as DNA-gyrase inhibitors, Arch.pharm.chem.lifesci., volume 343, (10) 2010: 570-576.
4. Mohd amir and Rajesh Agarwal, Synthesis and anti-inflammatory activity of 5-(8-quinolinoxy methyl)-1,3,4- oxadiazole/ 1,3,4-thiadiazole and 1,2,4(H) triazole, Indian Journal of Heterocyclic Chemistry,1998: (7); 225-228.
5. Sharma S, Mehra A, Pandhi S, Studies on fused lactams; Synthesis and anti microbial activity of pyridyl and quinonyl-2-azetidinones, Indian Journal of Chemistry, Section B: Organic Chemistry including Medicinal Chemistry,1988: 27B (5),494-497.
6. Trivedi P.B, Undavia N.K, et.al., Synthesis and anti microbial activity of 4-oxothiazolidines, 4-oxoazetidinesmalonanilic acid hydrazine's and pyrazoline derivatives of phenothiazine, Indian Journal of Chemistry, Section B: Organic Chemistry including Medicinal Chemistry, 1993: 32B (7),760-765.
7. Hogale M.B, Uthale A.C, Nikam B.P, Synthesis and anti microbial activity of N10-arylidenehydrazido phenothiazine and their 4-thiazolinones and 2-azetidinones, Indian Journal of Chemistry, Section B: Organic Chemistry including Medicinal Chemistry, 1991: 30B (7), 717-720.