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SYNTHESIS, CHARACTERIZATION AND *In vitro* CYTOTOXICITY STUDY OF SOME NOVEL QUINAZOLIN - 4 (3*H*) - ONE DERIVATIVES

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Abstract:

A series of some novel 2,3-disubstituted quinazolin-4(3*H*) ones were synthesized by condensing 2-substituted-4*H*-3,1-Benzoxazin-4-one with Lamivudine to yield the title compounds. The starting material 2-substituted-4*H*-3,1-Benzoxazin-4-one was synthesized from anthranilic acid and substituted benzoyl chloride. The structures of the synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C- NMR, Mass and Elemental Analysis. The synthesized compounds were screened for their *in vitro* cytotoxic activity.

Key words: Quinazolinone, Lamivudine, DLA Cell Line model, Anticancer and *In vitro* cytotoxic activity.

1. INTRODUCTION

Quinazolinone and their derivatives have been found to possess potent wide spectrum of activities like antibacterial [1-5], antifungal [6-9], anticancer [10,11], antiviral [12-15], Cytotoxic activity [12-15&21], antiinflammatory [16,17], antihistaminic [17], anthelmintic [18], antitubercular [19] and anticonvulsant activity etc [20]. Considering the biological significance of them, quinazolinone nucleus was synthesized. In the present research study a series of some novel 2,3-disubstituted Quinazolin-4-(3*H*)-one derivatives were synthesized and screen them for their cytotoxic activity against DLA cell line at the different concentrations of 10, 20, 50, 100 and 200 µg/ml.

2. MATERIALS AND METHODS

The reaction condition was optimized by using thin layer chromatography on readymade silica gel plates (Merck) using chloroform-methanol (9.5:0.5) and n hexane-ethyl acetate (9:1) as solvent system. Iodine was used as developing agent. Melting point determination was carried in capillary tubes on melting point apparatus which are uncorrected. IR spectrum was recorded by KBr disc method in Thermo Nicolet 6700 FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded with 400 MHz and 100 MHz Bruker Advance-II NMR instrument. Elemental analysis of all the compounds was performed on Elementar Vario EL-II CHNS analyzer. Mass spectra (MS) were recorded on a Thermo Scientific High Resolution Magnetic Sector MS DFS by chemical ionization (CI) or negative-ion electro spray ionization (ESI) method.

Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by microanalysis. Elemental analysis (C, H, N) indicated that the calculated and observed values were within the acceptable limits (±0.4 %).

Step 1 - Synthesis of 2-substituted-4H-3,1-benzoxazin-4-one

A solution of substituted benzoyl chloride (0.01 mole) was slowly added to a solution of anthranilic acid/substituted anthranilic acid (0.01 mole) in anhydrous pyridine (15 ml) at 0 ^{o}C with constant stirring. The reaction mixture was stirred for 30 minutes with magnetic stirrer at room temperature and set aside for one hour. The stirred solution was treated with aqueous sodium bicarbonate to remove the unreacted acid until the effervescence ceases. The solution was filtered and washed with water to remove the inorganic materials and adhered pyridine. The crude benzoxazine thus obtained was dried and recrystallized from absolute ethanol.

Step 2 - Synthesis of 2,3 disubstituted quinazolin-4-(3H)-one

A cold solution of Lamivudine (0.05 mole) in anhydrous pyridine (10 ml) was added drop wise with constant stirring to 10 ml of cold solution of 2-substituted-4(H)-3,1-benzoxazine-4-one (0.05 mole) in glacial acetic acid. The resultant reaction mixture was stirred vigorously for 30 minutes at room temperature and subsequently heated under reflux for 36 - 48 hours under anhydrous reaction condition. It was allowed to cool at room temperature and poured to ice cold water. On standing for 12 hours, solidification occurred which was allowed to settle down. It was filtered off, dried in vacuum and purified by Column chromatography.

***In vitro* Cytotoxic Activity Assay Against Dalton's Lymphoma Ascites Cell Line (Trypan blue dye exclusion method)**

Dalton's Lymphoma Ascites (DLA) tumour cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, Kerala, India. These tumour cells are known to grow as uniform cell suspension in the peritoneal cavity of the mice. DLA was maintained by serial transplantation from mice to mice. The ascitic fluid of the DLA was drawn out from the donor mice carrying tumour for 7 to 9 days. The freshly drawn ascitic fluid from the peritoneal cavity was washed thrice with phosphate buffer saline (PBS, pH 7.4) and diluted in PBS to a concentration of 1×10^{6} cells/ml, and these cells were used for in vitro experiments. The tumour cells were aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with normal saline and checked for viability using trypan blue dye exclusion method. The cell suspension (1×10^{6} cells in 0.1 ml) was added to tubes containing various concentration of the test compounds (10, 20, 50, 100 and 200 µg/ml) and the volume was made upto 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension along with standard drug Methotrexate in the same concentrations. These assay mixtures were incubated for 3 hour at 37^{o} C. After incubation, 0.1 ml trypan blue was added and number of dead cells determined by using Haemocytometer. The cytotoxic data are given in Table - 1.

The percent viability was calculated by using formula:

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells + No. of dead cell}} \times 100$$

Table - 1: Cytotoxic properties of synthesized compounds on DLA cell line

S. No	Compound	% Cytotoxicity (cell death)				
		10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
1	L8	0	4	11	20	42
2	L10	0	2	5	11	20
3	L11	0	0	8	16	32
4	L12	8	12	24	46	60
5	L16	2	8	14	28	42
6	L17	7	18	36	52	68
7	L20	22	38	54	72	80
8	L24	0	9	18	30	52
9	L27	0	4	12	28	40
10	L28	0	8	12	20	35
11	Methotrexate	52	64	96	100	100

Table – 2: IC_{50} value for the Synthesized compounds

S.No	Compound	IC_{50} (µg/ml)
1	L8	238.10
2	**L10**	**500.00**
3	L11	312.50
4	L12	108.70
5	L16	238.10
6	L17	96.15
7	**L20**	**46.30**
8	L24	192.31
9	L27	250.00
10	L28	285.71
11	Methotrexate	9.62

Figure 1:Graphical representation of cytotoxic activity of synthesized compounds on DLA cell lines

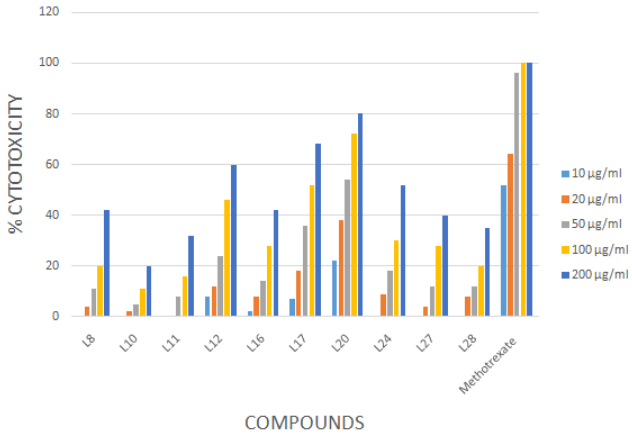
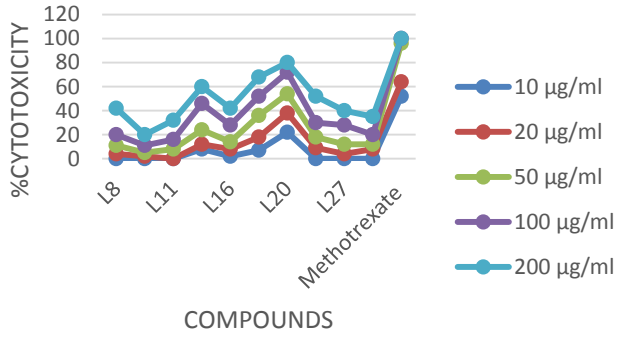


Figure 2:Line graph showing cytotoxic activity of synthesized compounds on DLA cell lines



Compound RajL1: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(o-tolyl)quinazolin-4(3H)-one

Yield: 68 %; m.p 192 - 194 °C; TLC R_f = 0.73; Log P: 4.31; IR (KBr) cm^{-1}: 1671.32 (C=O str.), 1597.97 (ring C=N str.), 3108.85 (O-H str. for –OH); Anal. Calcd. for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49; S, 7.15; Found: C, 61.63; H, 4.51; N, 12.47; S, 7.14; MS (m/z): 448.12 (M^+)

Compound RajL2: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydro pyrimidin-4-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one

Yield: 66 %; m.p 202-204 °C; TLC R_f = 0.74; IR (KBr) cm^{-1} :1671.32(C=O str.), 1594.28 (ring C=N str.), 3108.85 (O-H str. for –OH);^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.46 (s, 1H), 8.29 – 8.12 (m, 2H), 8.05 (d, *J* = 31.1 Hz, 2H), 7.93 – 7.75 (m, 2H), 7.56 (s, 1H), 7.51 (s, 1H), 7.41 (s, 1H), 5.82 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.93 (d, *J* = 7.4 Hz, 2H), 3.45 (s, 1H), 2.71 (s, 1H); MS (m/z): 480.09 (M^+ +1);Anal.Calcd. for $C_{22}H_{17}N_5O_6S$: C, 55.11; H, 3.57; N, 14.61; S, 6.69;Found : C, 55.15; H, 3.59; N, 14.59; S, 6.65

Compound RajL3: 2-(4-fluorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 62 %; m.p 204-206 °C; TLC R_f = 0.67; Log P: 3.98 IR (KBr) cm^{-1} : 1683.48 (C=O str.), 1606.95 (ring C=N str.);^{1}H NMR ((DMSO-*d6*, δ in ppm): δ 8.47 (s, 1H), 8.08 (s, 1H), 7.87 (s, 1H), 7.57 (t, *J* = 4.5 Hz, 3H), 7.50 (s, 1H), 7.40 (s, 1H), 7.06 – 6.99 (m, 2H), 5.84 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.93 (d, *J* = 7.6 Hz, 2H), 3.46 (s, 1H), 2.71 (s, 1H); MS (m/z): 452.10 (M^+); Anal.Calcd. for $C_{22}H_{17}FN_4O_4S$: C, 58.40; H, 3.79; N, 12.38; S, 7.09; Found: C, 58.42; H, 3.81; N, 12.36; S, 7.11.

Compound RajL4: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydro pyrimidin -4-yl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one

Yield: 63 %; m.p 222-224 °C; TLC R_f = 0.73; Log P: 3.7; IR (KBr) cm^{-1} :1686.27(C=O str.), 1608.55 (ring C=N str.), 3125.92 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.47 (s, 1H), 8.08 (s, 1H), 7.80 (s, 1H), 7.66 – 7.53 (m, 3H), 7.50 (s, 1H), 7.39 (s, 1H), 6.99 – 6.81 (m, 2H), 5.84 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.93 (d, *J* = 7.5 Hz, 2H), 3.81 – 3.76 (m, 3H), 3.46 (s, 1H), 2.71 (s, 1H); MS (m/z): 465.12 (M^+ +1); Anal.Calcd. for $C_{23}H_{20}N_4O_5S$: C, 59.47; H, 4.34; N, 12.06; S, 6.90; Found: C, 59.51; H, 4.38; N, 12.04; S, 6.92

Compound RajL5: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydro pyrimidin-4-yl)-2-(p-tolyl)quinazolin-4(3H)-one

Yield:74 %; m.p:182-184 °C; TLC R_f = 0.71; Log P:4.31; IR (KBr) cm^{-1} :1696.19(C=O str.), 1610.59 (ring C=N str.), 3122.10 (O-H str. for –OH);^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.32 (s,

1H), 8.08 (s, 1H), 7.83 (s, 1H), 7.64 – 7.46 (m, 4H), 7.40 (s, 1H), 7.25 – 7.07 (m, 2H), 5.67 (s, 1H), 4.34 (s, 1H), 4.17 (d, *J* = 34.7 Hz, 2H), 3.91 (s, 1H), 3.45 (s, 1H), 2.71 (s, 1H), 2.35 – 2.30 (m, 3H); MS (m/z): 449.12 (M^++1); Anal.Calcd. for $C_{23}H_{20}N_4O4S$: C, 61.59; H, 4.49; N, 12.49; S, 7.15; Found: C, 61.61; H, 4.53; N, 12.47; S, 7.11

Compound RajL6: 2-(4-(chloromethyl)phenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 68 %; m.p 196-198 ^oC; TLC R_f = 0.74; Log P:4.48; IR (KBr) cm^{-1} :1671.32(C=O str.), 1594.28 (ring C=N str.), 3108.85 (O-H str. for –OH);^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.37 (s, 1H), 8.08 (s, 1H), 7.64 (s, 1H), 7.61 – 7.53 (m, 3H), 7.50 (s, 1H), 7.41 (s, 1H), 7.36 – 7.18 (m, 2H), 6.35 (s, 1H), 4.52 – 4.47 (m, 2H), 4.35 (d, *J* = 10.6 Hz, 2H), 4.15 (s, 1H), 3.94 (s, 1H), 3.43 (s, 1H), 3.18 (s, 1H);MS (m/z): 482.08 (M^+); Anal.Calcd. for $C_{23}H_{19}ClN_4O_4S$: C, 57.20; H, 3.97; N, 11.60; S, 6.64; Found: C, 57.22; H, 3.95; N, 11.62; S, 6.62

Compound RajL7: 2-(2-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 68 %; m.p 199-201^{o}C; TLC R_f = 0.67; Log P: 4.38;^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.44 (s, 1H), 8.09 (s, 1H), 7.94 (s, 1H), 7.58 (d, *J* = 16.9 Hz, 2H), 7.50 (s, 1H), 7.41 (s, 1H), 7.30 – 7.18 (m, 3H), 5.79 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.92 (d, *J* = 11.6 Hz, 2H), 3.46 (s, 1H), 2.71 (s, 1H); MS (m/z): 468.07 (M^+); Anal. Calcd. for $C_{22}H_{17}ClN_4O_4S$: C, 56.35; H, 3.65;N,11.95; S, 6.84; Found: C, 56.33; H, 3.67;N,11.93; S, 6.86

Compound RajL8: 2-(2,3-dichlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 64 %; m.p 186-188 ^{o}C; TLC R_f = 0.67; Log P: 4.94; ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.37 (s, 1H), 8.07 (d, *J* = 8.3 Hz, 2H), 7.56 (s, 1H), 7.50 (d, *J* = 3.5 Hz, 2H), 7.41 (s, 1H), 7.28 (s, 1H), 7.16 (s, 1H), 5.68 (s, 1H), 4.40 (s, 1H), 4.34 (s, 1H), 4.14 (s, 1H), 3.92 (s, 1H), 3.40 (s, 1H), 3.20 (s, 1H); MS (m/z): 504.02 (M^+ + 2); Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_4S$: C, 52.49; H, 3.20; N, 11.13; S, 6.37; Found: C, 52.47; H, 3.22; N, 11.15; S, 6.36

Compound RajL9: 2-(furan-2-yl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 66 %; m.p 224-226 ^{o}C; TLC R_f = 0.68; Log P:2.24;^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.46 (s, 1H), 8.11 (s, 1H), 7.95 (s, 1H), 7.74 (d, *J* = 19.7 Hz, 2H), 7.58 (s, 1H), 7.53 (s, 1H), 7.43 (s, 1H), 6.79 (s, 1H), 5.93 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 4.11 (s, 1H), 3.94 (s, 1H), 3.46 (s, 1H), 2.71 (s, 1H); MS(m/z): 424.08 (M^+); Anal. Calcd. for $C_{20}H_{16}N_4O_5S$: C, 56.60; H, 3.80; N, 13.20; S, 7.55; Found: C, 56.62; H, 3.82; N, 13.18; S, 7.57

Compound RajL10: 7-chloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(p-tolyl)quinazolin-4(3H)-one

Yield: 61 %; m.p 182-184 ^{o}C; TLC R_f = 0.68; Log P: 4.87; MS (m/z): 482.08 (M^+); Anal. Calcd. for $C_{23}H_{19}ClN_4O_4S$: C, 57.20; H, 3.97; N, 11.60; S, 6.64; Found: C, 57.18; H, 3.96; N, 11.63; S, 6.62;

Compound RajL11: 7-chloro-2-(2-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 64 %; m.p 196-198 ^{o}C; TLC R_f = 0.62; Log P:4.94; IR (KBr) cm^{-1} :1670.88(C=O str.), 1577.17 (ring C=N str.), 3165.90 (O-H str. for –OH); MS (m/z): 502.03 (M^+) ; Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_4S$: C, 52.49; H, 3.20; N, 11.13; S, 6.37; Found: C, 52.47; H, 3.22; N, 11.15; S, 6.35

Compound RajL12: 7-chloro-2-(2,3-dichlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 64 %; m.p 188-190 ^{o}C; TLC R_f = 0.67; Log P:5.5; IR (KBr) cm^{-1} :1678.66(C=O str.), 1577.08 (ring C=N str.), 3150.64 (O-H str. for –OH); ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.20 (s, 1H), 7.99 (s, 1H), 7.60 (s, 1H), 7.47 (d, *J* = 5.4 Hz, 2H), 7.38 (s, 1H), 7.26 (s, 1H), 7.14 (s, 1H), 5.60 (s, 1H), 4.34 (s, 1H), 4.27 (s, 1H), 3.97 (s, 1H), 3.56 (s, 1H), 3.16 (s, 1H), 0.84 (s, 1H); MS (m/z): 535.99 (M^+); Anal. Calcd. for $C_{22}H_{15}Cl_3N_4O_4S$: C, 49.13; H, 2.81; N, 10.42; S, 5.96; Found: C, 49.15; H, 2.83; N, 10.40; S, 5.94

Compound RajL13: 7-chloro-2-(4-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 66 %; m.p 190-192 ^{o}C; TLC R_f = 0.74; Log P:4.94; IR (KBr) cm^{-1} :1659.45(C=O str.), 1607.66 (ring C=N str.), 3115.28 (O-H str. for –OH);^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.20 (s, 1H), 7.99 (s, 1H), 7.60 (s, 1H), 7.59 – 7.48 (m, 2H), 7.32 (dd, *J* = 30.3, 7.3 Hz, 4H), 5.59 (s, 1H), 4.34 (s, 1H), 4.27 (s, 1H), 3.97 (s, 1H), 3.55 (s, 1H), 3.15 (s, 1H), 0.83 (s, 1H); MS (m/z): 502.03 (M^+); Anal.Calcd. for $C_{22}H_{16}Cl_2N_4O_4S$: C, 52.49; H, 3.20;N, 11.13; S, 6.37; Found: C, 52.47; H, 3.22;N, 11.15; S, 6.3

Compound RajL14: 6,8-dibromo-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(o-tolyl)quinazolin-4(3H)-one

Yield: 63 %; m.p 212-214 ^{o}C; TLC R_f = 0.66; Log P:5.97; IR (KBr) cm^{-1} :1649.48(C=O str.), 1612.90 (ring C=N str.), 3281.12 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.29 (d, *J* = 23.1 Hz, 2H), 7.82 (d, *J* = 11.5 Hz, 2H), 7.60 (s, 1H), 7.23 (d, *J* = 14.6 Hz, 2H), 7.12 (s, 1H), 5.51 (s, 1H), 4.52 (s, 1H), 4.34 (s, 1H), 4.13 (s, 1H), 3.92 (s, 1H), 3.39 (s, 1H), 3.19 (s, 1H), 2.24 – 2.19 (m, 3H); MS (m/z): 605.94 (M^+); Anal.Calcd. for $C_{23}H_{18}Br_2N_4O_4S$: C, 45.56; H, 2.99; N, 9.24; S, 5.29

Compound RajL15: 6,8-dibromo-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(p-tolyl)quinazolin-4(3H)-one

Yield: 63 %; m.p 208-210 ^{o}C; TLC R_f = 0.68; Log P:5.97; IR (KBr) cm^{-1} :1650.49(C=O str.), 1608.26 (ring C=N str.), 3108.85 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.33 (s, 1H), 8.26 (s, 1H), 7.83 – 7.77 (m, 2H), 7.64 – 7.46 (m, 2H), 7.25 – 7.07 (m, 2H), 5.67 (s, 1H), 4.34 (s, 1H), 4.20 (d, *J* = 18.4 Hz, 2H), 3.92 (s, 1H), 3.45 (s, 1H), 2.71 (s, 1H), 2.35 – 2.30 (m, 3H); MS (m/z): 605.94 (M^+ + 2); Anal.Calcd. for $C_{23}H_{18}Br_2N_4O_4S$: C, 45.56; H, 2.99; N, 9.24; S, 5.29; Found: : C, 45.58; H, 2.98; N, 9.22; S, 5.31

Compound RajL16: 7-chloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one

Yield: 64 %; m.p 222-224 ^{o}C; TLC R_f = 0.67; Log P:4.26; IR (KBr) cm^{-1} :1656.94(C=O str.), 1607.07 (ring C=N str.), 3173.51 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.76 (s, 1H), 8.41 (s, 1H), 7.99 (s, 1H), 7.69 (s, 1H), 7.66 – 7.51 (m, 2H), 7.42 (s, 1H), 7.01 – 6.83 (m, 2H), 5.94 (s, 1H), 4.35 (d, *J* = 5.8 Hz, 2H), 4.18 (s, 1H), 3.95 (s, 1H), 3.82 – 3.77 (m, 3H), 3.42 (s, 1H), 2.71 (s, 1H); MS (m/z): 498.08 (M^+); Anal.Calcd. for $C_{23}H_{19}Cl N_4O_5S$: C, 55.37; H, 3.84; N, 11.23; S, 6.43; Found: : C, 55.39; H, 3.86; N, 11.21; S, 6.41

Compound RajL17: 7-chloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(o-tolyl)quinazolin-4(3H)-one

Yield: 63 %; m.p 196-198 ^{o}C; TLC R_f = 0.68; Log P: 4.87; IR (KBr) cm^{-1} :1672.83(C=O str.), 1603.89 (ring C=N str.), 3170.10 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.32 (s, 1H), 8.02 (s, 1H), 7.84 (s, 1H), 7.64 (s, 1H), 7.59 (s, 1H), 7.41 (s, 1H), 7.23 (d, *J* = 15.3 Hz, 2H), 7.12 (s, 1H), 5.50 (s, 1H), 4.52 (s, 1H), 4.34 (s, 1H), 4.13 (s, 1H), 3.92 (s, 1H), 3.39 (s, 1H), 3.19 (s, 1H), 2.24 – 2.19 (m, 3H); MS (m/z): 482.08 (M^+) ; Anal. Calcd. for $C_{23}H_{19}ClN_4O_4S$: C, 57.20; H, 3.97; N, 11.60;S, 6.64; Found: C, 57.18; H, 3.99; N, 11.62; S, 6.63.

Compound RajL18: 2-cyclohexyl-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 58 %; m.p 208-210 ^{o}C; TLC R_f = 0.67; Log P: 3.9; IR (KBr) cm^{-1}:1698.73.(C=O str.), 1611.53 (ring C=N str.), 3182.40 (O-H str. for –OH);

Compound RajL19: 2-(4-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 68 %; m.p 182-184 ^{o}C; TLC R_f = 0.64; Log P:4.38; IR (KBr) cm^{-1} :1662.56(C=O str.), 1607.38 (ring C=N str.), 3314.73 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.58 (s, 1H), 8.39 (s, 1H), 8.04 (s, 1H), 7.68 – 7.49 (m, 4H), 7.42 (s, 1H), 7.39 – 7.24 (m, 2H), 5.96 (s, 1H), 4.35 (d, *J* = 7.0 Hz, 2H), 4.18 (s, 1H), 3.95 (s, 1H), 3.42 (s, 1H), 2.71 (s, 1H); Ms (m/z): 468.07 (M^+) ; Anal.Calcd. for $C_{22}H_{17}ClN_4O_4S$: C, 56.35; H, 3.65; N, 11.95;S, 6.84

Compound RajL20: 6,8-dibromo-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-phenylquinazolin-4(3H)-one

Yield: 56 %; m.p 202-204 ^{o}C; TLC R_f = 0.72; Log P: 5.48; IR (KBr) cm^{-1} :1671.32(C=O str.), 1610.02 (ring C=N str.), 3283.36 (O-H str. for –OH); ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.29 (d, *J* = 23.7 Hz, 2H), 7.82 (d, *J* = 6.4 Hz, 2H), 7.68 – 7.54 (m, 2H), 7.29 (t, *J* = 4.8 Hz, 3H), 5.67 (s, 1H), 4.34 (s, 1H), 4.21 (s, 1H), 3.86 (d, *J* = 41.5 Hz, 2H), 3.48 (s, 1H), 2.71 (s, 1H);

MS (m/z): 591.92 (M^+ + 2); Anal.Calcd. for $C_{22}H_{16}Br_2N_4O_4S$: C, 44.61; H, 2.72; N, 9.46; S, 5.41; Found: C, 44.63; H, 2.76; N, 9.42; S, 5.40

Compound RajL21: 6,8-dibromo-2-(4-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 56 %; m.p 202-204 ^{o}C; TLC R_f = 0.65; Log P: 6.04; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.21 (d, *J* = 3.5 Hz, 2H), 7.76 (s, 1H), 7.60 – 7.42 (m, 2H), 7.30 (t, *J* = 9.1 Hz, 3H), 5.59 (s, 1H), 4.34 (s, 1H), 4.28 (s, 1H), 3.97 (s, 1H), 3.51 (s, 1H), 3.15 (s, 1H), 1.17 (s, 1H); MS (m/z): 625.88 (M^+ + 2); Anal.Calcd. for $C_{22}H_{15}Br_2ClN_4O_4S$: C, 42.16; H, 2.41;N, 8.94; S, 5.12; Found: C, 42.19; H, 2.44;N, 8.91; S, 5.11

Compound RajL22: 6,8-dichloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(p-tolyl)quinazolin-4(3H)-one

Yield: 68 %; m.p 198-200 ^{o}C; TLC R_f = 0.71; Log P: 5.43; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.21 (s, 1H), 8.01 (s, 1H), 7.61 – 7.46 (m, 2H), 7.43 (s, 1H), 7.19 (t, *J* = 32.9 Hz, 2H), 7.12 (s, 1H), 5.59 (s, 1H), 4.34 (s, 1H), 4.27 (s, 1H), 3.97 (s, 1H), 3.55 (s, 1H), 3.15 (s, 1H), 2.36 – 2.31 (m, 3H), 0.83 (s, 1H); MS (m/z): 516.04 (M^+); Anal.Calcd. for $C_{23}H_{18}Cl_2N_4O_4S$: C, 53.39; H, 3.51; N, 10.83;S, 6.20; Found: C, 53.41; H, 3.50; N, 10.81;S, 6.22

Compound RajL23: 6,8-dichloro-2-(2-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan -5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 62 %; m.p 192-194 ^{o}C; TLC R_f = 0.69; ; Log P: 5.5; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.24 (s, 1H), 8.01 (s, 1H), 7.58 (s, 1H), 7.44 (d, *J* = 2.0 Hz, 2H), 7.27 – 7.17 (m, 3H), 5.75 (s, 1H), 4.34 (s, 1H), 4.19 (s, 1H), 3.98 (s, 1H), 3.47 (s, 1H), 3.27 (s, 1H), 1.53 (s, 1H); MS (m/z): 535.99 (M^+); Anal.Calcd. for $C_{22}H_{15}Cl_3N_4O_4S$: C, 49.13; H, 2.81; N, 10.42; S, 5.96; Found: C, 49.15; H, 2.80; N, 10.40; S, 5.95

Compound RajL24: 6,8-dichloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one

Yield: 56 %; m.p 224-226 ^{o}C; TLC R_f = 0.72; ; Log P:4.82; ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.53 (s, 1H), 7.70 (s, 1H), 7.69 – 7.50 (m, 3H), 7.41 (s, 1H), 7.00 – 6.82 (m, 2H), 5.82 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.93 (d, *J* = 8.0 Hz, 2H), 3.80 – 3.75 (m, 3H), 3.51 (s, 1H), 2.71 (s, 1H); MS (m/z): 532.04 (M^+); Anal.Calcd. for $C_{23}H_{18}Cl_2N_4O_5S$: C, 51.79; H, 3.40; N, 10.50; S, 6.01; Found: C, 51.77; H, 3.41; N, 10.51; S, 6.03

Compound RajL25: 6,8-dichloro-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one

Yield: 64 %; m.p 224-226 ^{o}C; TLC R_f = 0.63; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.26 (s, 1H), 8.23 – 8.05 (m, 2H), 8.01 (s, 1H), 7.91 – 7.72 (m, 2H), 7.52 (s, 1H), 7.44 (s, 1H), 5.79 (s, 1H), 4.34 (s, 1H), 4.19 (s, 1H), 3.98 (s, 1H), 3.47 (s, 1H), 3.27 (s, 1H), 1.20 (s, 1H); MS (m/z): 547.01 (M^+); Anal.Calcd. for $C_{22}H_{15}Cl_2N_5O_6S$: C, 48.19; H, 2.76; N, 12.77; S, 5.85; Found: C, 48.21; H, 2.74; N, 12.79; S, 5.84

Compound RajL26: 6,8-dichloro-2-(2,3-dichlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)quinazolin-4(3H)-one

Yield: 59 %; m.p 202-204 ^{o}C; TLC R_f = 0.71; ; Log P: 6.06; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.26 (s, 1H), 8.01 (s, 1H), 7.53 (s, 1H), 7.46 (d, *J* = 16.2 Hz, 2H), 7.26 (s, 1H), 7.13 (s, 1H), 5.94 (s, 1H), 4.34 (s, 1H), 4.19 (s, 1H), 3.98 (s, 1H), 3.47 (s, 1H), 3.27 (s, 1H), 1.34 (s, 1H); MS (m/z): 569.95 (M^+); Anal.Calcd. for $C_{22}H_{14}Cl_4N_4O_4S$: C, 46.17; H, 2.47; N, 9.79; S, 5.60; Found: C, 46.20; H, 2.45; N, 9.77; S, 5.62

Compound RajL27: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydro pyrimidin-4-yl)-6,8-diiodo-2-(p-tolyl)quinazolin-4(3H)-one

Yield: 52 %; m.p 214-216 ^{o}C; TLC R_f = 0.63; ; Log P: 7.03; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.44 (s, 1H), 8.17 (s, 1H), 7.95 (s, 1H), 7.73 (s, 1H), 7.62 – 7.44 (m, 2H), 7.26 – 7.08 (m, 2H), 4.34 (s, 1H), 4.19 (s, 1H), 3.96 (s, 1H), 3.38 (d, *J* = 33.1 Hz, 2H), 3.16 (s, 1H), 2.39 – 2.34 (m, 3H), 1.72 (s, 1H); MS (m/z): 699.91 (M^+) ; Anal. Calcd. for $C_{23}H_{18}I_2N_4O_4S$: C, 39.45; H, 2.59; N, 8.00; S, 4.58; Found: : C, 39.46; H, 2.57; N, 8.02; S, 4.57

Compound RajL28: 3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-6,8-diiodo-2-(4-nitrophenyl)quinazolin-4(3H)-one

Yield: 52 %; m.p 224-226 ^{o}C; TLC R_f = 0.63; ^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.45 (s, 1H), 8.21 (d, *J* = 18.3 Hz, 2H), 8.16 – 8.02 (m, 2H), 7.85 – 7.68 (m, 2H), 7.45 (s, 1H), 5.64 (s, 1H), 4.34 (s, 1H), 4.29 (s, 1H), 3.98 (s, 1H), 3.72 (s, 1H), 3.19 (s, 1H), 2.70 (s, 1H); MS (m/z): 730.88 (M^+); Anal. Calcd. for $C_{22}H_{15}I_2N_5O_6S$: C, 36.13; H, 2.07; N, 9.58; S, 4.38; Found: C, 36.11; H, 2.09; N, 9.55; S, 4.39

Compound RajL29: 2-(2-chlorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-6,8-diiodoquinazolin-4(3H)-one

Yield: 52 %; m.p 220-222 ^{o}C; TLC R_f = 0.65; Log P:7.1; ^{1}H NMR (DMSO-*d6*, δ in ppm): δ 8.46 (d, *J* = 24.0 Hz, 2H), 8.23 (s, 1H), 7.93 (s, 1H), 7.61 (s, 1H), 7.30 – 7.20 (m, 3H), 5.79 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.92 (d, *J* = 11.6 Hz, 2H), 3.46 (s, 1H), 2.71 (s, 1H); MS (m/z): 719.86 (M^+); Anal.Calcd. for $C_{22}H_{15}ClI_2N_4O_4S$: C, 36.66; H, 2.10; N, 7.77; S, 4.45; Found: C, 36.69; H, 2.11; N, 7.73; S, 4.44

Compound RajL30: 2-(4-fluorophenyl)-3-(1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-6,8-diiodoquinazolin-4(3H)-one

Yield: 53 %; m.p 212-214 ^{o}C; TLC R_f = 0.65; Log P: 6.7;^{1}H NMR ($CDCl_3$, δ in ppm) δ 8.43 (s, 1H), 8.20 (d, *J* = 19.3 Hz, 2H), 7.59 – 7.54 (m, 2H), 7.33 (s, 1H), 7.04 – 6.97 (m, 2H), 5.59 (s, 1H), 4.34 (s, 1H), 4.27 (s, 1H), 3.97 (s, 1H), 3.51 (s, 1H), 3.15 (s, 1H), 1.14 (s, 1H); MS (m/z): 703.89 (M^+); Anal. Calcd. for $C_{22}H_{15}FI_2N_4O_4S$: C, 37.52; H, 2.15; N, 7.96; S, 4.55; Found: C, 37.54; H, 2.14; N, 7.94; S, 4.56

3. Results and Discussion

In the present study, thirty novel 2,3-disubstituted quinazolin-4(3*H*)one derivatives were synthesized, purified by column chromatography and characterized by using FT-IR, ^{1}H-NMR,

Mass spectra and Elemental analysis. The synthesized compounds were screened for their *in vitro* cytotoxic activity against DLA cell line at the different concentrations of 10, 20, 50, 100 and 200 µg/ml and the results were shown in Table 1. IC_{50} values were calculated by using Table - 1 and the results were shown in Table - 2. From the calculated IC_{50} values of the synthesized compounds, it was clear that the compound L20 was the most potent and the compound L10 was the least.

4. Conclusion

In the present study, thirty novel 2, 3-disubstituted quinazoline derivatives were synthesized and purified by column chromatography. The spectral data of the titled compounds were in correlation with the expected structure. All the studied compounds were shown to possess mild to moderate and high cyctotoxic activity in DLA cell line model. The compounds L12, L17, L20 and L24 were found to be more potent than the other compounds. The compounds L12, L17, L20 and L24 showed an IC_{50} value 108.70, 96.15, 46.30 and 192.31 µg/ml respectively and they also showed significant activity when compared to standard. From the calculated IC_{50} values of the synthesized compounds, it was clear that the compound L20 was the most potent and the compound L10 was the least. The cytotoxic nature also correlated with molecular docking studies for anticancer nature of nearly 75% of the compounds, which are found to show positive correlation. From the calculated IC_{50} values of the synthesized compounds, it was clear that the compound L20 was the most potent and the compound L10 was the least.

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