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RESEARCH ARTICLE

Novel Hybrid Molecules of Quinazoline Chalcone Derivatives: Synthesis and Study of *In Vitro* Cytotoxic Activities

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Abstract: ***Background*:** A new series of quinazoline linked chalcone conjugates were synthesized and evaluated for their *in vitro* cytotoxicity.

***Methods*:** The quinazoline-chalcone derivatives (**13a-r**) have been prepared by the Claisen-Schmidt condensation of various substituted benzaldehydes (**12a-r**) with substituted l-(4-(3,4-dihydroquinazolin-4-ylamino)phenyl)ethanone **(11a-b)** in the presence of aqueous NaOH. Three potential compounds **13f, 13g** and **13h** exhibited cytotoxicity against leukemia (GI_{50} value of 1.07, 0.26 and 0.24 μM), Non-small lung (GI_{50} values of 2.05,1.32 and 0.23 μM), colon (GI_{50} values of 0.54, 0.34 and 0.34 μM) and breast (GI_{50} values of 2.17, 1.84 and 0.22 μM) cell line, respectively.

***Results and Conclusion*:** Based on these biological results, it is evident that compound **13h** has the potential to be considered for further detailed studies either alone or in combination with existing therapies as potential anticancer agents.

Keywords: *In vitro*, cytotoxic studies, hybrid molecules, quinazolines, chalcone derivatives, potential anticancer agents.

1. INTRODUCTION

Cancer is a leading cause of death worldwide, and has been recognized as a disease of uncontrolled cell proliferation. Chemotherapy is currently one of the most effective ways to treat metastatic cancers and has achieved significant success through the discovery of new drugs. The genes that regulate cell proliferation have been the target of cancer chemotheraphy [1]. Among the currently identified antimitotic agents, chalcones represent an important class of molecules that are abundant in edible plants. A number of chalcones (**1**, **2**; Fig. **1**) have been reported to be active antimitotic agents, inhibiting tubulin polymerization [2].

Systematic studies on chalcones have been carried out [3-5] in order to understand the molecular mechanism. Isoliquiritigenin (**3**, Fig. **2**) and licochalcone (**4**) are potent inhibitors of skin carcinogenesis and induce apoptosis through cell cycle arrest in various cancer cells by promotion of Bax protein expression and activation of caspases [6, 7] Xanthoangelol (**5**) has been reported to induce apoptosis and inhibit tumour promotion and metastasis in several cancer cell lines [8, 9] Flavokawain A (**6**) suppressed bladder tumour growth at a dose of 50 mg/kg of body weight in a mouse xenograft model [10].

Chalcones shown in (Fig. **1**) have been reported to be active antimitotic agents inhibiting tubulin polymerization [2].

Literature survey shows that quinazoline derivatives function as anticancer agents as well as multitargetagents. In recent days, synthesis of antitumour agents having quinazoline backbone has been one of the primary concerns.

In recent days, the model of "hybrid drugs" has acquired recognition in medicine and this concept was originated from combination therapies which were conventionally applied to cure unresponsive patients. A review article was published on mathematical modelling approaches to design hybrid molecules for tumour growth inhibition. Another review article was published on the role of hybrid molecules in the treatment of breast cancer [11]. Hybrid molecules may also exhibit synergetic effect compared to the individual pharmacophores.

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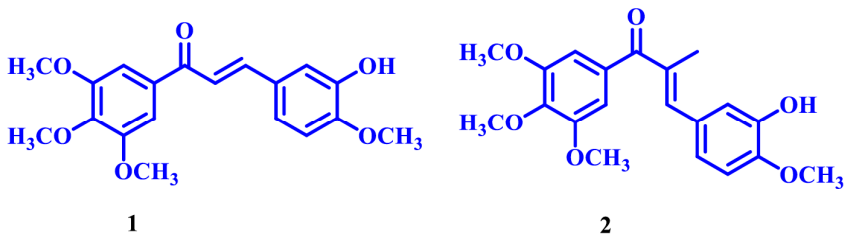


Fig. (1). Chemical structures of Tubulin Inhibitors containing Chalcone scaffolds.

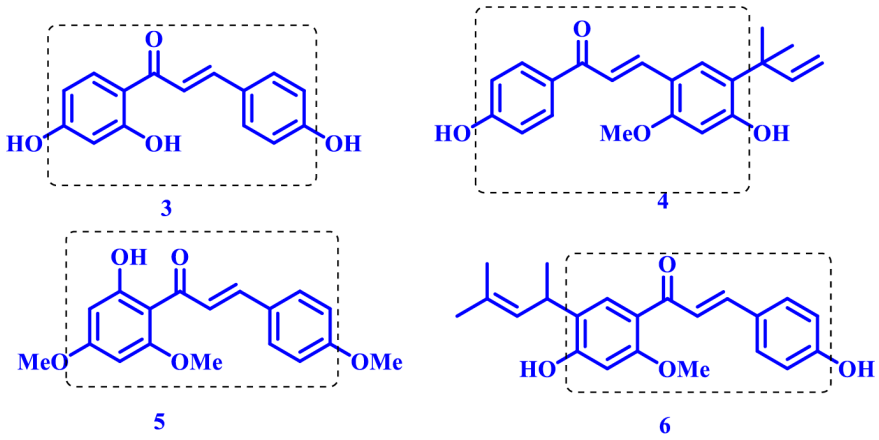


Fig. (2). Chalcone mimics of anticancer agents and potent inhibitors of skin carcinogenesis.

Exhibition of antitumor properties by chalcones and quinazolines in literature encouraged the authors towards synthesis of quinazoline-chalcone derivatives and evaluation of their cytotoxic studies.

2. BIOLOGICAL ACTIVITY

2.1. Cytotoxicity

The synthesized quinazoline linked chalcones (**13a-r)** were evaluated for their anticancer activity against 60 cancer cell lines derived from nine different types of human cancer (lung, leukemia, colon, melanoma, ovarian, renal, prostate and breast cancer). Results are expressed as percentage of growth inhibition (GI_{50}) determined relative to that of untreated control cells (Table **1**). Among the eighteen chalcones synthesized, three were active in the primary screen and these were further evaluated against a panel of 60 cell lines at five concentrations, and the results are given in Table **1**. These three compounds **13f, 13g** and **13h** exhibited a wide spectrum of activity against different cancer cell lines with mean GI_{50} values of 10.8, 13.4, and 0.93 μM, respectively. Specifically, compound **13h** exhibited excellent anticancer activity against sixty cancer cell line with GI_{50} values ranging from 0.23-2.38 μM, whereas compound **13g** also showed promising anticancer activity against different cancer cell lines, particularly against leukemia cell line with GI_{50} value of 0.26μM. Moreover, the other compound **13f** showed significant anticancer activity in the micro molar range against certain cell lines tested.

3. SAR STUDIES

In order to understand the structure activity relationship (SAR), we explored the modification on the 6, 7-positions of quinazoline ring as well as phenyl ring of the chalcones with electron donating and electron withdrawing substitutions. In case of compounds **13a-r**, quinazoline ring is unsubstituted, and the phenyl ring of chalcone is substituted with electron donating (**13f, 13g** and **13h**) groups. These compounds exhibited prominent cytotoxicity against leukemia and melanoma cancer cell lines. Compound **13h** (with 2, 4, 6 tri methoxy substitution) is most active among the series. Similarly dimethoxy substituted compounds are showing good anticancer activity. In case of compounds **13a-e** and **13i**, electron withdrawing substitution on chalcone showed moderate cytotoxicity. Multidrug resistance (MDR) is linked with the over expression of ATP-binding cassette (ABC) transporters. One of those is P-glycoprotein which is familiar as ATP-binding cassette, subfamily G, member 2 (ABCG2). ABCG2 is also known as breast cancer resistance protein. ABCG2 is inhibited by chalcones with a distinct polyspecificity by the A-ring moiety. In the present case, moderate to prominent anti-proliferation was observed with three compounds **13f, 13g** and **13h**. Though it is difficult to establish a lucid relationship between the substituting patterns on the ring A of chalcones and their cytotoxicity, an effort is made to explain role of position and number of methoxy groups in inhibition. Synthesized compounds having methoxy group on phenyl ring **(13f, 13g, and 13h)** are more active than those having methoxy group on Quinazoline as shown in Table **1**. It can be understood from the fact that chalcones are functionally asymmetric *i.e.*, higher potency can be observed by shifting the aromatic unit to the A-ring and methoxy substituents to the phenyl B-ring.

Position and number of methoxy substituents on the B-ring of chalcones play a vital role in their cytotoxic studies. Best inhibition was observed with two methoxy substituents

Table 1. The GI_{50} (the concentration required to reduce the growth of treated cells to half that of untreated cells) values for compounds 13f, 13g and 13h in sixty cancer cell lines.

Cancer Panel/cell line	Growth Inhibition GI_{50} (µM)		
	NSC: 760014 (13f)	NSC: 760016 (13g)	NSC: 760015 (13h)
Leukemia			
CCRF-CEM	2.55	4.04	0.62
HL-60(TB)	20.7	3.74	0.40
K-562	-	-	-
MOLT-4	5.68	2.40	0.89
SR	1.07	0.55	0.24
RPMI-8226	3.65	0.26	0.48
Non-small lung			
A549 / ATCC	3.66	3.20	0.68
EKVX	13.9	5.19	1.32
HOP-62	9.87	7.13	1.08
HOP-92	2.05	1.32	1.03
NCI-H226	3.53	2.45	1.03
NCI-H23	4.96	5.08	0.54
NCI-H322M	3.03	4.53	1.78
NCI-H460	13.0	14.2	0.49
NCI-H522	7.45	6.69	0.23
Colon			
COLO 205	14.8	9.58	0.86
HCC-2998	3.22	1.79	1.70
HCT-116	0.54	0.34	0.42
HCT-15	8.63	7.63	0.88
HT29	3.21	2.07	0.34
KM12	3.45	1.77	0.51
SW-620	3.51	2.38	0.52
CNS			
SF-268	6.55	3.06	1.12
SF-295	6.25	4.31	0.89
SF-539	7.73	4.97	1.19
SNB-19	11.5	9.99	1.31
SNB-75	5.19	24.1	0.28
U251	3.66	2.23	0.81
Ovarian			
IGROVI	20.7	13.5	1.55
OVCAR-3	5.10	4.00	1.12
OVCAR-4	3.26	4.28	1.29
OVCAR-5	39.6	20.3	1.78
OVCAR-8	7.03	3.67	1.38
NCI/ADR- RES	3.82	652	0.37
SK-OV-3	4.23	3.60	1.26

(Table 1) contd….

Cancer Panel/cell line	Growth Inhibition GI_{50} (μM)		
	NSC: 760014 (13f)	NSC: 760016 (13g)	NSC: 760015 (13h)
Renal			
786-0	7.96	4.20	1.44
A498	8.97	5.86	0.97
ACHN	13.6	1.39	1.67
CAKI-1	16.8	1.93	1.12
SN12C	1.09	7.30	0.38
TK-10	8.29	8.40	1.81
UO-31	4.57	1.05	1.44
RXF 393	2.20	2.93	1.60
Prostate			
PC-3	4.22	3.36	0.91
DU-145	8.16	6.71	1.47
Breast			
MCF7	2.17	1.84	0.46
MDA-B-231/ATCC	19.8	13.5	1.47
HS578T	15.3	8.21	0.76
BT-549	3.22	3.34	0.93
T-47D	3.61	6.20	1.64
MDA-MB-468	2.17	2.20	0.22
Melanoma			
LOX IMVI	2.81	1.37	0.87
MALME-3M	13.8	1.50	1.31
M14	6.24	1.54	0.59
MDA-MB-435	2.93	1.67	0.93
SK-MEL-2	13.9	1.40	0.41
SK-MEL-28	13.7	1.53	1.02
UACC-257	20.3	1.35	1.21

(**13f** and **13g**) compared to single substituents **(13d)**. Significant positive influence of polymethoxylation on the A-ring of chalcones in cytotoxic studies against tumor cell lines was explained many researchers [12]. Mahapatra *et al.* [13] reported that the best inhibitory effects were exhibited by chalcones connected to heteroatomic moiety in which at least two methoxy groups are present on B-ring [14]. Either di- or tri-methoxylation on aromatic ring of chalcones was highly beneficial to cell cycle arrest at G2/M [15]. Lower cytotoxic activity was reported in 2′-hydroxy-4′,6′-dimethoxychalcones and 2′,4′-diallyloxy-6′-methoxychalcones as the planarity is affected by the substitutions on the *ortho*position of the ring A [16]. However, in the present case, **13h** having two methoxy substituents at ortho positions is more active which can be explained on the basis that size of methoxy groups is lower to affect the planarity.

In the present case, hybrid molecules having halogen substituents (Cl, F, CF_3) on chalcones exhibited moderate antitumour activity whereas methoxy substituents exhibited higher activity. Similarly, relatively lower cytotoxicity was observed in biaryl-based chalcones having electron withdrawing groups (-F, -Cl or –Br) compared to those with electron donating groups (-OH or -OCH_3) on aromatic ring [17]. Moderate activity of halogen substituted compounds in this case is probably due to relatively low lipophilicity of these compounds. Literature survey shows that anthraquinone based chalcones containing electron-withdrawing substituents (–Cl and –CF_3) resulted in a considerable increase of cytotoxic activity in inhibition of HeLa cells [18]. Bulky substituents (Br or OMe group) in meta position of benzylidene affect anti-proliferative effects of chalcone analogues due to interaction with biological targets [19]. Less contribution of substituents (Cl, Br, NO_2, OH, CN, or CF_3) to antitumour activity was reported with chalcones containing quinazolines [20] and quinoxaline [21].

4. EXPERIMENTAL

4.1. Reagents and Media

The media for cell culture (MEM and DMEM) were purchased from Sigma-Aldrich. MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and

fetal bovine serum (FBS) was purchased from Himedia. Gentamycin sulphate was procured from Kasturba Hospital, Manipal. Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. NMR (^{1}H 300 MHz; ^{13}C 75 MHz) were recorded at room temperature in $CDCl_3$ as solvent and TMS as an internal standard (δ = 0 ppm), and the values were reported in the following order: chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of quartet), coupling constants (J in Hz), and integration. Mass spectra were recorded on a VG micromass70-70H instrument. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel GF-254 (100-200 mesh); spots were visualized under UV light at 254 nm.

I. Typicalexperimental procedure for the synthesis of title compound quinazoline-chalcones (13a-r)

A mixture of l-(4-(quinazolin-4-ylamino) phenyl)ethanone (263 mg, 1 mmol) and 4-fluorobenzaldehyde **12a** (124 mg, 1 mmol) was dissolved in 10 mL ethanol. To this mixture, sodium hydroxide (100 mg, 2.5 mmol) dissolved in 1.0 mL of water was added at 0-5^{o}C. The reaction mixture was stirred at room temperature for 45 min. Then, this reaction mixture was poured over crushed ice and acidified with dilute HCl. The light yellow solid thus obtained was filtered, washed with water and dried. The residue was purified on column chromatography (silica gel with 30% ethyl acetate in hexane) affording compound **13a** as a yellow solid, Yield: 81%; MR; 132-134^{o}C; DIPMS: m/z=400.1 (M+H),Elemental analysis: analysis calculated for $C_{23}H_{18}FN_3O_2$: C-72.17, H-4.54, and N-10.52; found C-72.23, H-4.41, and N-10.73;^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.14 (s, NH), δ8.80 (s, 1H), 8.12- 8.00 (m, 4H), 7.95 (d, 1H, *J*= 6.0 Hz), 7.88 (d, 1H, *J*= 6.0 Hz), 7.64 (d, 1H, *J*= 4.6 Hz), 7.55-7.60 (m, 4H), 7.56-7.59 (m, 2H), 7.48 (d, 1H, *J*= 14.5 Hz), 7.46-7.40 (m, 1H), 7.09 (m, 1H); ^{13}C NMR (CDC13, 75 MHz): δ175.1, 160.6, 159.0, 158.8, 154.4, 153.8, 153.7, 152.3, 147.1, 146.2, 145.7, 142.9, 139.3, 137.2, 136.1, 135.8, 133.6, 132.7, 130.6, 130.4, 128.9, 126.5, 124.2.

Following the same procedure as depicted for **13a**, the other quinazoline-chalcone derivatives**13b–r** were prepared by the Claisen-Schmidt condensation of corresponding benzaldehydes with substituted l-(4-(3,4-dihydroquinazolin-4-ylamino)phenyl)ethanones.

(E)-3-(2-Fluoro-4-(trifluoromethyl)phenyl)-l-(4-(quinazolin-4-ylamino) phenyl) prop-2-en-l-one (13b)

Yellow solid, Yield: 80%; MR; 134-146°C; DIPMS: m/z=437.62 (M+H); Elemental analysis: analysis calculated for $C_{24}H_{15}F_4N_3O$: C-65.90, H-3.46, and N-9.61; found C-65.96, H-3.49, and N-9.68;^{1}H NMR ($CDCl_3$, 300 MHz):δ8.97 (s, NH),δ8.83 (s, 1H),8.19- 8.09 (m, 4H), 8.04 (d, 1H, *J*= 7.5 Hz), 7.92 (d, 1H, *J*= 7.5 Hz), 7.89 (d, 1H, *J*= 15.0 Hz), 7.85-7.78 (m, 2H), 7.76-7.70 (m, 1H), 7.58 (d, 1H, *J*= 15.0 Hz), 7.50-7.46 (m, 1H), 7.23 (d, 1H, *J*= 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75MHz):δ 179.2, 166.8, 161.1, 160.5, 159.5, 158.7, 157.3,153.8,149.6,148.7,143.8,138.1,137.5,136.4,134.7, 133.9, 132.4, 129.1, 128.9,127.7, 125.8,124.0,122.2,120.5.

(E)-3-(3-Chloro-4-fluorophenyl)-1-(4-(quinazolin-4-ylaamino) phenyl) prop-2-en-1-one (13c)

Yellow solid, Yield: 79%; MR; 128-130°C; DIPMS: m/z=453.41 (M+H); Elemental analysis: analysis calculated for $C_{24}H_{15}ClF_3NO$: C-65.90, H-3.46, and N-9.61; found C-65.97, H-3.51, and N-9.72;^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.81 (s, 1H), 8.07- 8.00 (m, 2H), 7.89 (s, 1H), 7.69-7.75 (m, 3H), 7.72 (d, 1H, *J* = 15.8 Hz),7.70-7.65 (m, 1H), 7.52 (d, 1H, *J*= 15.8 Hz), 7.51-7.47 (m, 2H), 7.29-7.24 (m, 1H), 6.81-6.71 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ178.3, 165.5, 161.3,160.9, 159.7, 159.0,158.6, 157.4,140.7, 139.6, 138.4, 137.1, 136.2,134.9,133.1,130.7,129.2,128.4,127.5,125.1, 123.8, 121.6,120.3; HRMS (ESI m/z) for $C_{23}H_{16}ClFN_3O$, calculated 404.09604, found 404.09488 $[M+H]^+$.

(E)-3-(3-Fluoro-4-methoxyphenyl)-1-(4-(quinazolin-4-ylamino) phenyl) prop-2-en-l-one (13d)

Yellow solid, Yield: 75%; MR; 118-120°C; DIPMS: m/z=399.94 (M+H); Elemental analysis: analysis calculated for $C_{24}H_{18}FN_3O_2$: C-72.17, H-4.54, and N-10.52; found C-72.23, H-4.61, and N-10.68;^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.91 (s, 1H), 8.05- 8.12 (m, 2H), 8.05 (s, 1H), 7.89-7.85 (m, 3H), 7.72 (d, 1H, *J*= 15.8 Hz),7.65-7.60 (m, 1H), 7.49 (d, 1H, *J*= 15.8 Hz), 7.31-7.36 (m, 1H), 6.95-6.99 (m, 1H), 6.54-6.49 (m, 2H), 3.60 (s, 3H).

(E)-3-(2,5-Dimethoxyphenyl)-1-(4-(quinazolin-4-ylamino) phenyl)prop-2-en-l-one (13e)

Yellow solid, Yield: 80%; MR; 122-125°C; DIPMS: m/z=411.96 (M+H); Elemental analysis: analysis calculated for $C_{25}H_{21}N_3O_3$: C-72.98, H-5.14, and N-10.21; found C-73.14, H-5.19, and N-10.33;^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.84 (s, 1H), 8.11- 8.08 (m, 2H), 8.04 (s, 1H), 7.99-7.95 (m, 3H), 7.92 (d, *J*= 15.8 Hz, 1H),7.85-7.80 (m, 1H), 7.62 (d, *J*= 15.8 Hz, 1H), 7.61-7.57 (m, 1H), 7.19-7.14 (m, 1H), 6.94-6.84 (m, 2H), 3.87 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ189.5, 157.0, 154.5, 153.4, 153.3, 149.9, 142.4, 139.9,133.8,133.2,134.4,130.0,129.9,128.9,127.0,124.4,122.8, 120.3,120.2,117.1, 113.7, 112.4, 115.6, 115.2.

(E)-3-(3,4-Dimethoxyphenyl)-1-(4-(quinazolin-4-ylamino)phenyl)prop-2-en-l-one (13f)

Yellow solid, Yield: 82%; MR; 160-163°C; DIPMS: m/z=411.91 (M+H); Elemental analysis: analysis calculated for $C_{25}H_{21}N_3O_3$: C-72.98, H-5.14, and N-10.21; found C-73.14, H-5.23, and N-10.36;^{1}H NMR (CDC1$_3$, 300 MHz): δ 9.70 (s, 1H, NH), 8.70 (s, 1H), 8.54 (d, *J*= 8.0 Hz, 1H), 8.18 (d, 2H, *J*= 9.0 Hz), 8.09 (d, 2H, *J*= 8.0 Hz), 7.84 (d, 1H, *J*= 8.0 Hz), 7.79 (d, 1H, *J*= 7.0 Hz), 7.69 (d, 1H, *J*= 15.0 Hz), 7.65 (s, 1H), 7.53 (d, 1H, *J*= 15.0 Hz), 7.28-7.23 (m, 2H), 6.91 (d, 1H, *J*= 8.0 Hz), 3.95 (s, 3H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ182.4, 162.2, 154.6, 151.4, 150.2,144.7, 142.4, 139.4,133.2, 129.9, 129.2, 127.9, 127.0, 123.1, 120.3, 120.1, 119.8,115.2, 111.1, 110.1, 102.8, 101.7, 101.5, 55.9.

(E)-3-(3,5-Dimethoxyphenyl)-l-(4-(quinazolin-4-ylaamino)phenyl)prop-2-en-l-one (13g)

Yellow solid, Yield: 85%; MR; 158-160°C; DIPMS: m/z=411.76 (M+H); Elemental analysis: analysis calculated for $C_{25}H_{21}N_3O_3$: C-72.98, H-5.14, and N-10.21; found C-

73.11, H-5.19, and N-10.38; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.91 (s, 1H, NH), 8.73 (s, 1H), 8.60 (d, 1H, *J*= 8.3 Hz), 8.18 (d, 1H, *J*= 16.9 Hz), 8.15 (d, 1H, *J*= 16.9 Hz), 8.21-8.12 (m, 2H), 7.89-7.81 (m, 2H), 7.69 (s, 2H), 7.63-7.58 (m, 1H), 6.87 (m, 2H), 6.52 (s, 1H), 3.85 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ190.5, 185.6, 183.2, 183.1, 180.1, 176.9, 170.5, 168.1, 161.0, 154.5, 150.4, 148.6, 144.5, 133.2, 129.9, 129.1, 127.0, 122.3, 120.4, 120.1, 106.3, 102.7, 95.7, 94.7, 55.4.

(E)-1-(4-(Quinazolin-4-ylamino) phenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-l-one (13h)

Yellow solid, Yield: 83%; MR; 148-150°C; DIPMS: m/z=441.64 (M+H); Elemental analysis: analysis calculated for $C_{26}H_{23}N_3O_4$: C-70.74, H-5.25, and N-9.52; found C-70.91, H-5.37, and N-9.73;^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.70 (s, 1H, NH), 8.64 (s, 1H), 8.54-8.49 (m, 1H), 8.13 (m, 1H), 8.09 (d, 1H, *J*= 15.4 Hz), 8.05-8.0 (m, 2H), 7.87 (d, 1H, *J*= 15.4 Hz), 7.83-7.74 (m, 2H), 7.70 (m, 1H), 7.56-7.52 (m, 1H), 6.16 (s, 2H), 3.96 (s, 6H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ 190.8, 163.1, 161.1, 153.4, 149.6, 142.0, 135.9, 134.7, 133.2, 130.0, 129.7,128.5, 126.9, 121.5, 120.7, 120.5, 119.9,115.2, 106.5, 90.4, 90.1.

(E)-1-(4-(Quinazolin-4-ylamino) phenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-l-one (13i)

Yellow solid, Yield: 81%; MR; 135-137°C; DIPMS: m/z=441.73 (M+H); Elemental analysis: analysis calculated for $C_{26}H_{23}N_3O_4$: C-70.74, H-5.25, and N-9.52; found C-70.91, H-5.33, and N-9.73;^{1}H NMR ($CDC1_3$, 300 MHz): δ8.85 (s, 1H), 8.09 (d, 1H, *J*= 7.6 Hz), 8.03-7.91 (m, 4H),7.87-7.82 (m, 2H), 7.71 (d, 1H, *J*= 16.2 Hz), 7.61-7.58 (m, 1H), 7.41 (d, 1H, *J*= 16.2 Hz), 6.85 (s, 2H), 3.93 (s, 6H), 3.90 (s, 3H).

(E)-1-(4-(6,7-Dimethoxyquinazolin-4-ylamino)phenyl)-3-(4-fluorophenyl)prop-2-en-l-one (13j)

Yellow solid, Yield: 75%; MR; 165-166°C; DIPMS: m/z=429.58 (M+H); Elemental analysis: analysis calculated for $C_{25}H_{20}N_3O_3$: C-69.92, H-4.69, and N-9.78; found C-70.18, H-4.83, and N-9.91; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.00 (s, 1H, NH), 8.66 (s, 1H), 8.09-8.00 (m, 4H), 7.77-7.71 (m, 4H), 7.50 (s, 1H), 7.35 (d, 1H, *J*= 15.0 Hz), 7.24 (d, 1H, *J*= 15.0 Hz), 7.19 (s, 1H), 4.01 (s, 3H,), 3.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ188.6, 173.5, 169.6, 168.6, 166.9, 164.5, 159.8, 158.6, 155.7, 149.4, 146.5, 146.1, 145.9, 144.5, 143.7, 139.6, 133.5, 130.6, 129.5, 96.8, 54.4, 53.2, 45.7, 41.3.

(E)-1-(4-(6,7-Dimethoxyquinazolin-4-ylamino)phenyl)-3-(2-fluoro-4-(trifluoromethyl) phenyl) prop-2-en-l-one (13k)

Yellow solid, Yield: 86%; MR; 138-139°C; DIPMS: m/z=479.88 (M+H); Elemental analysis: analysis calculated for $C_{26}H_{20}$ $F_3N_3O_3$: C-65.13, H-4.20, and N-8.76; found C-65.19, H-4.32, and N-8.92; ^{1}H NMR ($CDC1_3$, 300 MHz): δ 9.25 (s, 1H, NH), 8.62 (s, 1H), 8.14-8.07 (m, 4H), 7.88-7.79 (m, 4H),7.62 (s, 1H), 7.50 (d, 1H, *J*= 15.4 Hz), 7.45 (d, 1H, *J*= 15.4 Hz), 7.21 (s, 1H), 4.06 (s, 3H), 4.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ 182.8, 162.3, 161.5, 160.2, 159.1, 158.4, 156.9, 155.8, 154.2, 150.6, 140.8, 138.5, 129.6, 129.0, 127.2, 125.0, 118.6, 95.3, 59.5, 46.4, 48.5.

(E)-3-(3-Chloro-4-fluorophenyl)-l-(4-(6,7-dimethoxy-quinazolin-4-ylamino)phenyl)prop-2-en-l-one (13l)

Yellow solid, Yield: 86%; MR; 145-146°C; DIPMS: m/z=463.62 (M+H); Elemental analysis: analysis calculated for $C_{25}H_{19}$ $FClN_3O_3$: C-64.73, H-4.13, and N-9.06; found C-64.96, H-4.28, and N-9.32;^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.74 (s, 1H), 8.08-8.01 (m, 4H), 7.96 (d, 1H, *J*= 15.1 Hz), 7.70 (s, 1H), 7.66-7.69 (m, 2H), 7.49 (d, 1H, *J*= 15.1 Hz), 7.01-7.05 (m, 2H), 4.00 (s, 6H).

(E)-1-(4-(6,7-Dimethoxyquinazolin-4-ylamino)phenyl)-3-(3-fluoro-4-methoxyphenyl)prop-2-en-l-one (13m)

Yellow solid, Yield: 84%; MR; 128-129°C; DIPMS: m/z=459.64 (M+H); Elemental analysis: analysis calculated for $C_{26}H_{22}$ FN_3O_4: C-67.97, H-4.83, and N-9.15; found C-68.31, H-4.92, and N-9.37;^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.42 (broad, 1H, NH), 8.56 (s, 1H), 8.15-8.03 (m, 4H), 8.08 (d, 1H, *J*= 15.3 Hz), 7.79 (s, 2H), 7.73-7.68 (m, 3H), 7.60 (d, 1H, *J* = 15.3 Hz), 7.17-7.10 (m, 2H), 4.02 (s, 6H), 4.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ 188.0, 172.6, 156.0, 154.3, 152.3, 148.9, 146.7,143.8,142.1, 132.0,129.8, 129.7, 128.9,121.2,120.5, 115.5,115.4, 114.4,109.4, 106.5, 103.7, 101.3, 56.2, 55.6.

(E)-3-(2,5-Dimethoxyphenyl)-1-(4-(6,7-dimethoxyquinazolin-4-ylamino)phenyl)prop-2-en-l-one (13n)

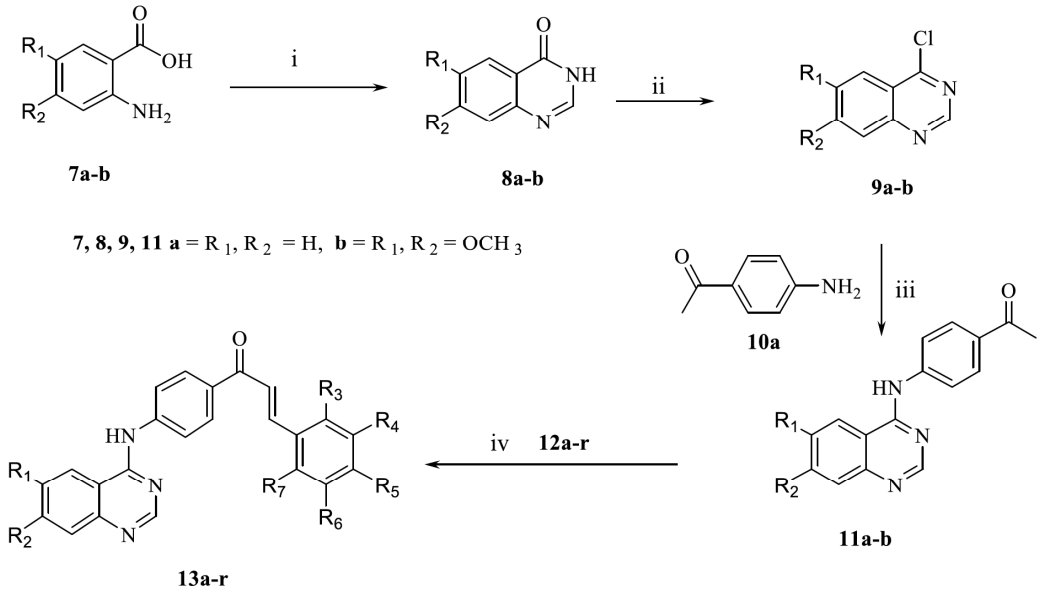
Yellow solid, Yield: 84%; MR; 136-137°C; DIPMS: m/z=471.86 (M+H); Elemental analysis: analysis calculated for $C_{27}H_{25}N_3O_5$: C-68.78, H-5.34, and N-8.91; found C-69.03, H-5.44, and N-9.11; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.62 (s, 1H), 8.05 (s, 2H), 7.70 (d, 1H, *J*= 14.8 Hz), 7.39 (d, 1H, *J*= 14.8 Hz), 7.15 (m, 2H), 7.00 (m, 2H), 6.49 (s, 2H), 6.35 (s, 1H), 4.00 (s, 6H), 3.85 (s, 3H), 3.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ189.2, 162.8, 161.3, 160.5, 158.3, 156.7, 155.8, 154.4, 149.8, 148.8, 147.6, 145.6, 144.7, 144.1, 140.5, 137.6, 135.8, 133.4, 130.7, 120.5, 116.4, 101.8, 56.2, 55.6, 45.9, 45.4.

(E)-3-(3,4-Dimethoxyphenyl)-1-(4-(6,7-dimethoxyquinazolin-4-ylamino) phenyl) prop-2-en-l-one (13o)

Yellow solid, Yield: 81%; MR; 139-140°C; DIPMS: m/z=471.81 (M+H); Elemental analysis: analysis calculated for $C_{27}H_{25}N_3O_5$: C-68.78, H-5.34, and N-8.91; found C-68.85, H-5.42, and N 9.12-;^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.60 (s, 1H), 8.08 (d, 2H, *J*= 7.0 Hz), 7.91 (d, 2H, *J*= 7.0 Hz), 7.69 (d, 1H, *J*= 15.5 Hz), 7.39 (d, 1H, *J*= 15.5 Hz), 7.20 (s, 1H), 7.10 (s, 1H), 6.74 (s, 2H), 6.50 (s, 1H), 4.01 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 3.62 (s, 3H).

(E)-3-(3,5-Dimethoxyphenyl)-1-(4-(6,7-dirnethoxyquinazolin-4-ylarnino)prop-2-en-l-one (13p)

Yellow solid, Yield: 84%; MR; 134-135°C; DIPMS: m/z=471.71 (M+H); Elemental analysis: analysis calculated for $C_{27}H_{25}N_3O_5$: C-68.78, H-5.34, and N-8.91; found C-69.12, H-5.43, and N-9.13; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.69 (s, 1H), 8.05 (d, 2H, *J*= 8.5 Hz), 7.92 (d, 2H, *J*= 8.5 Hz), 7.71 (d, 1H, *J*= 15.8 Hz), 7.46 (d, 1H, *J*= 15.8 Hz), 7.25 (s, 1H), 7.13(s,1H), 6.75(s,2H), 6.50(s,1H), 4.03(s,3H), 4.00 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H).



13a=R_1=R_2=H; R_5=F: R_3=R_4=R_6=R_7=H
13b=R_1=R_2=H; R_5=CF_3; R_3=F R_4=R_6=R_7=H
13c=R_1=R_2=H; R_5=F; R_4=Cl R_3=R_6=R_7=H
13d=R_1=R_2=H; R_5=OCH_3; R_4=F R_3=R_6=R_7=H
13e=R_1=R_2=H; R_3=R_5=OCH_3; R_4=R_6=R_7=H
13f=R_1=R_2=H; R_4=R_5=OCH_3;R_3=R_6=R_7=H
13g=R_1=R_2=H; R_4=R_6=OCH_3;R_3=R_5=R_7=H
13h=R_1=R_2=H; R_3=R_5=R_7=OCH_3;R_4=R_6=H
13i=R_1=R_2=H; R_4=R_5=R_6=OCH_3;R_3=R_7=H
13j=R_1=R_2=OCH_3; R_5=F: R_3=R_4=R_6=R_7=H
13k=R_1=R_2=OCH_3; R_5=CF_3; R_3=F R_4=R_6=R_7=H
13l=R_1=R_2=OCH_3; R_5=F; R_4=Cl R_3=R_6=R_7=H
13m=R_1=R_2=OCH_3; R_5=OCH_3; R_4=F R_3=R_6=R_7=H
13n=R_1=R_2=OCH_3; R_3=R_5=OCH_3; R_4=R_6=R_7=H
13o=R_1=R_2=OCH_3; R_4=R_5=OCH_3;R_3=R_6=R_7=H
13p=R_1=R_2=OCH_3; R_4=R_6=OCH_3;R_3=R_5=R_7=H
13q=R_1=R_2=OCH_3; R_3=R_5=OCH_3;R_4=R_6=H
13r=R_1=R_2=OCH_3; R_4=R_5=R_6=OCH_3;R_3=R_7=H

Reagents and Conditions: (i) formamide, 150°C, 3h **(ii)** $POCl_3$, 100°C, 4h **(iii)** isopropanol, reflux, 5h (iv) substituted benzaldehydes **(12a-r)** ethanol, 10% aq NaOH solution, rt, 6h.

Scheme 1. Synthesis of novel hybrid quinazoline chalcone derivatives.

(E)-l-(4-(6,7-Dimethoxyquinazolin-4-ylamino)phenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-l-one (13q)

Yellow solid, Yield: 83%; MR; 138-140°C; DIPMS: m/z=471.67 (M+H); Elemental analysis: analysis calculated for $C_{27}H_{25}N_3O_5$: C-68.78, H-5.34, and N-8.91; found C-68.94, H-5.47, and N-9.16; ^{1}H NMR ($CDC1_3$, 300 MHz): δ 8.65 (s, 1H), 8.20 (d, 1H, *J*= 15.4 Hz), 8.09-8.01 (m, 4H), 7.95 (d, 1H, *J*= 15.4 Hz), 7.80 (s, 1H), 7.51 (s, 1H), 7.23 (s, 1H), 6.18 (s, 1H), 4.80 (s, 3H), 4.03 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ 177.7, 176.2, 167.5, 165.8, 164.1, 162.3, 162.2, 161.1, 160.0, 150.3, 149.9, 137.2, 136.2, 135.9, 135.7, 121.6, 121.5, 121.4, 121.3, 121.0, 105.2, 68.3, 68.0, 66.0, 65.0, 58.7.

(E)-1-(4-(6,7-Dirnethoxyquinazolin-4-ylamino)phenyl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-l-one (13r)

Yellow solid, Yield: 81%; MR; 160-162°C; DIPMS: m/z=501.67 (M+H); Elemental analysis: analysis calculated for $C_{28}H_{27}N_3O_6$: C-67.06, H-5.43, and N-8.38; found C-67.38, H-5.64, and N-8.46; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (s, 1H), 8.08-8.05 (m, 1H), 8.0-7.85 (m, 3H),7.75 (d, 1H, *J*= 15.6 Hz), 7.40 (d, 1H, *J*= 15.6 Hz), 7.31-7.20 (m, 2H), 6.84 (s, 2H), 4.00 (s, 6H), 3.91 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ 189.1, 155.7, 154.9, 153.3, 153.1,149.7, 147.7,144.7,143.3,140.3,133.0,130.3,129.8,129.6,121.1,120.3, 120.1, 107.7, 105.5, 109.4, 99.2, 99.1, 60.9, 56.2, 56.1.

4.2. Cytotoxic Test

The inhibition of the cellular growth was estimated using MTT (3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [22]. The test is based on ability of viable cells to reduce a soluble yellow tetrazolium salt to blue farmazan crystal. The test is based on reduction of a soluble form of yellow tetrazolium salt in the presence of viable cells to crystal form of blue farmazan.

5. RESULTS AND DISCUSSION

Taking into consideration of antitumor activity of chalcones having methoxy and halo groups, fruitful cytotoxic studies were envisaged in the current study for chalcones having those substituents. Variation in hydrophobicity and hence improved incursion of these synthesized chalcones into the cancer cells can be expected due to substitution of a heterocyclic compound like quinazoline on chalcones.

5.1. Chemistry

Hybrid molecules of quinazoline and chalcones having the substituents at different positions were prepared. In general, chalcones with para -OCH_3 substitution on aromatic B ring have relatively higher activities compared to others. So, in single methoxy substituted compound **(13d)** preparation,

substituent position is para. Eighteen novel compounds **(13a-r)** were synthesized successfully in good yields *via* substituted benzaldehydes (**12a-r)** by employing the reaction sequences shown in Scheme **1**.

The quinazoline-chalcone derivatives (**13a-r**) have been prepared by the Claisen-Schmidt condensation of various substituted benzaldehydes (**12a-r**) with substituted l-(4-(3,4-dihydroquinazolin-4-ylamino)phenyl)ethanones **(11a-b)** in the presence of 10% aqueous NaOH. The substituted l-(4-(3,4-dihydroquinazolin-4-ylamino)phenyl)ethanones **(11a-b)** have been obtained by nucleophillic displacement reaction of 4-chloroquinazoline **(9a-b)** with 4-amino acetophenone**10a**. Substituted 4-chloroquinazolines (**9a-b**) has been prepared from substituted quinazolin-4(3H)-ones (**8a-b**) in $POCl_3$. The intermediates **8a-b** have been obtained by the reaction of substituted anthranilic acid (7) in DMF as shown in Scheme **1**. The compound **13a** was confirmed based on its spectral data.

CONCLUSION

In conclusion, a series of quinazoline linked chalcone conjugates were synthesized and evaluated for their *in vitro* cytotoxicity. Most of these quinazoline linked chalcone compounds exhibited significant cytotoxicity with IC_{50} values ranging from 0.93 to 30.54 μM. Three potential compounds **13f, 13g** and **13h** exhibited cytotoxicity against leukemia (GI_{50} value of 1.07, 0.26 and 0.24 μM), Non-small lung (GI_{50} values of 2.05,1.32 and 0.23 μM), colon (GI_{50} values of 0.54, 0.34 and 0.34 μM) and breast (GI_{50} values of 2.17, 1.84 and 0.22 μM) cell line, respectively. Based on these biological results, it is evident that compound **13h** has the potential to be considered for further detailed studies either alone or in combination with existing therapies as potential anticancer agents.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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