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DABCO-catalyzed one-pot three component synthesis of dihydropyrano[3,2-c]chromene substituted quinazolines and their evaluation towards anticancer activity

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A facile DABCO promoted one-pot three component synthesis of a new series of C-C linked bis-heterocycle containing dihydropyrano[c] chromene as highly fused *oxa*-heteryl group at C-2 position of quinazoline was developed. Quinazoline-2-carbaldehyde, substituted 4-hydroxycoumarin and ethyl cyanoacetate were used as key components in the Knoevenagel-Michael addition reaction to get the titled compounds. These compounds were screened for anti-cancer activity against the breast cancer cell lines of MDA-MB 231, and MDA-MB 453.

Keywords: Multicomponent reaction, Knoevenagel-Michael reaction, Quinazoline, 4-hydroxycoumarin, DABCO, anti-cancer.

Quinazoline scaffold is consistently regarded as privileged structural moiety owing to its medicinal and pharmacological importance in various therapeutic agent[1-5], especially in anti-cancer agents[6]. Further, the *oxa*-heteryl quinazolines, wherein, the oxygen heterocyclic moiety linked at various position of quinazoline are well known for efficient medicinal properties, some of them even marketed as drugs. A class of 6-oxa-heteryl quinazolines, such as Lapatinib (**1a**) [7], Selatinib (**1b**) [8] and Icotinib (**1c**) [8] are marketed as anticancer drugs (Figure 1). Especially, 2-tetrahydrofuranyl / furanyl / benzofuranyl / morpholinyl / benzoxazinyl quinazolines exhibit pharmacological activities like anti-cancer by inhibiting tyrosine kinase[9], reactivation of mutant *p53* protein[10], anticholinergic activity by inhibiting cyclic guanosine 3', 5'-mono-phosphate phosphodiesterase[11], used for control of *m*RNA splicing by inhibiting clk4[12, 13]. Quinazoline derivative also acts as DPP-4 inhibitor for treating type-II diabetes[14]. Additionally, quinazoline bis-heterocyclic derivatives are used to treat schizophrenia, depression, anxiety, migraine, hallucinations, and insomnia by antagonizing the 5-HT_{2A} receptor[15]. A literature survey on 2-substituted *oxa*-heteryl

quinazoline derivatives revealed that a very few reports were found. Till date, particularly at 2-position of quinazoline synthesis and their activity, only benzofuran or benzoxazine as fused *oxa*-heterocycles were constructed.

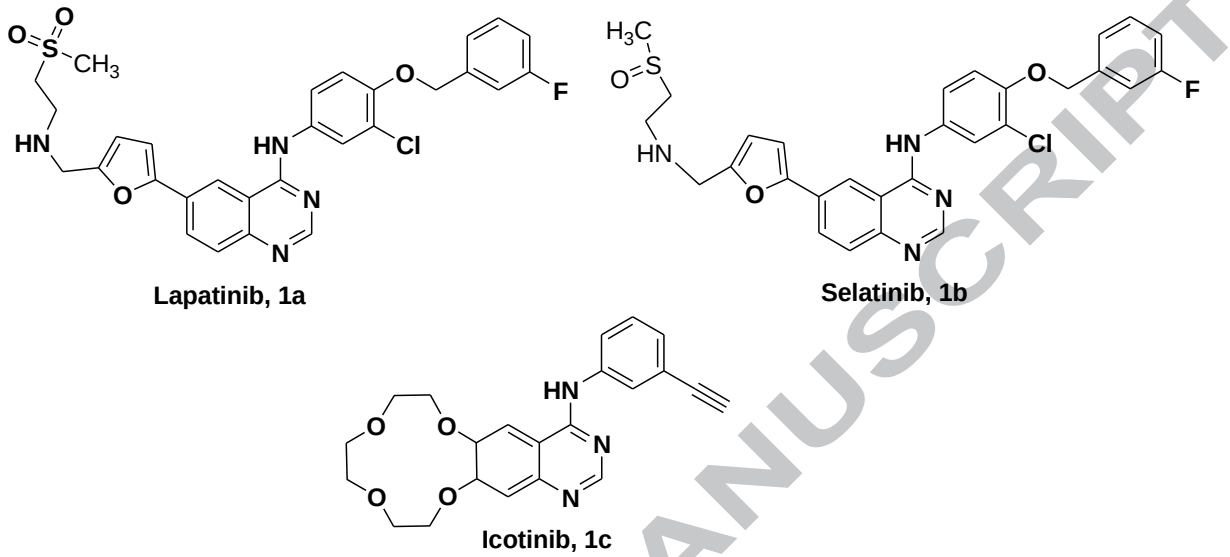
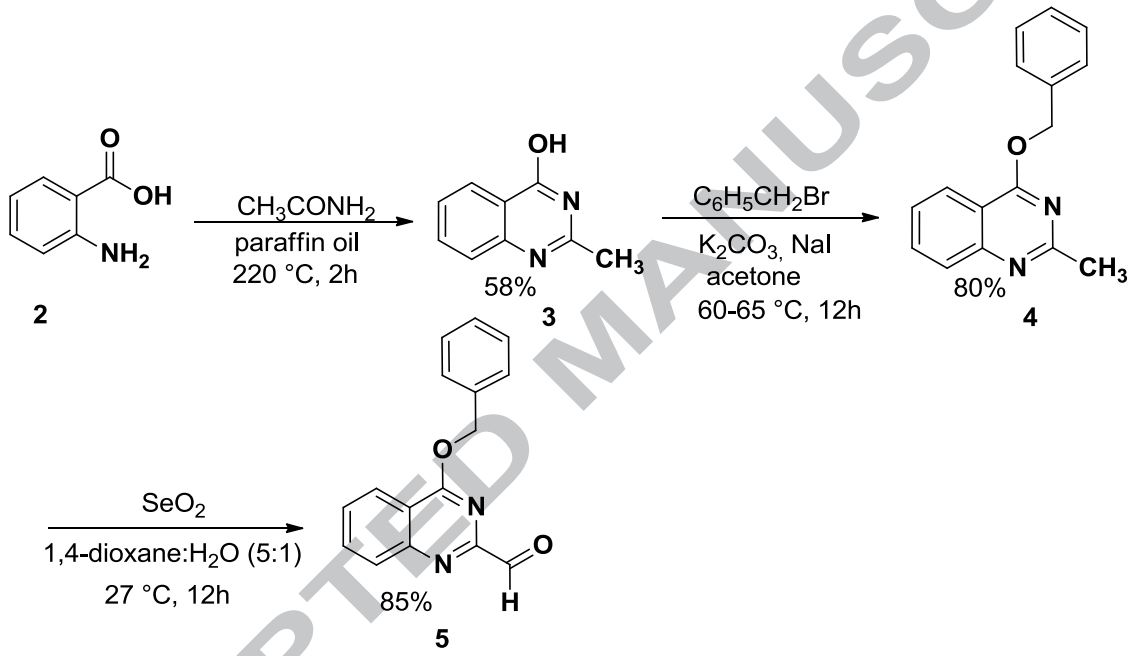


Figure 1. Marketed anticancer drugs of quinazoline derivatives

We have initiated a program for the synthesis of designed hybrid molecules that contains dihydropyrano[c]chromene motif as highly fused *oxa*-heterocyclic unit at C-2 position of quinazoline ring. Also, we are aware that the dihydropyrano[c]chromene compounds have a privileged medicinal scaffold owing to rigid heterocyclic ring system that has a possibility for inserting different functional groups to improve their efficacies as a result of binding to specific sites in the target. Furthermore, these compounds attracted interest over the years due to their varied biological properties, such as anti-cancer, spasmolytic, anti-coagulant, diuretic, anti-anaphylactic activities and also potassium channel activating properties[16-18]. 1,4-Diazobicyclo [2.2.2] octane (DABCO) is an inexpensive, commercially available and most common catalyst in organic reactions[19]. DABCO was also considered as one of the best catalysts in the multi-component synthesis of dihydropyrano[c]chromene derivatives[20].

Herein, we wish to report the DABCO catalyzed three-component reaction leading to a new series of C-C linked bis-heterocycles containing dihydropyrano [c] chromene, quinazoline scaffolds using 4-(benzyloxy)quinazoline-2-carbaldehyde, various substituted coumarins and ethyl cyanoacetate. Subsequently, the anticancer activities of the synthesized compounds were evaluated against MDA-MB 231 (Estrogen independent) and MDA-MB 453 (Breast adeno carcinoma) cell lines.

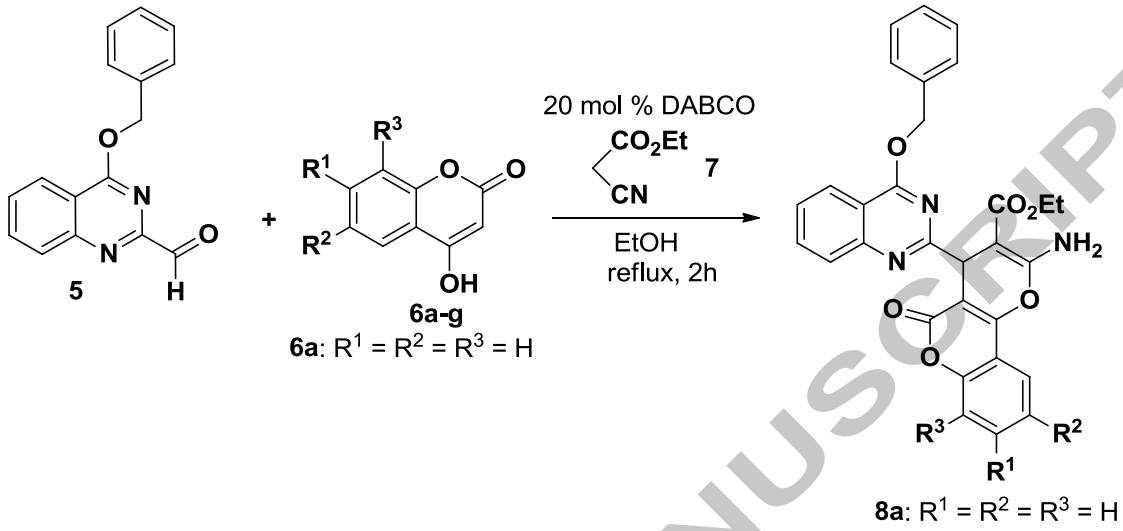
Initially, the required key precursor 4-(benzyloxy)quinazoline-2-carbaldehyde **5** was synthesized in four steps (over all yield 40%) starting from anthranilic acid **2** and acetamide as shown in **Scheme 1**. The cyclo condensation of anthranilic acid **2** with acetamide in paraffin oil at 220 °C for 2h led to quinazoline **3**[21]. The hydroxy compound **3** was treated with benzyl bromide in the presence of K_2CO_3 in dry acetone to give O-benzyl derivative **4**, which upon oxidation with SeO_2 afforded the carbaldehyde derivative **5** (Scheme 1). The compound **5** was characterized by ^1H, ^{13}C NMR and Mass spectra. In ^1H NMR spectrum, the compound **5** exhibited the characteristic aldehyde proton at δ 9.65 as singlet.



Scheme 1

Primarily, the evaluation of DABCO catalyst loading was studied using quinazoline-2-carbaldehyde **5** (1.0 equiv) substituted coumarin **6a** (1.1 equiv) and ethyl cyanoacetate **7** (1.1 equiv) refluxing in EtOH. Employing 5, 10 and 15 mol% DABCO resulted in the required compound **8a**[22] with inferior yields. The optimum catalyst loading for this knoevenagel-Michael reaction found to be 20 mol% of DABCO to afford the titled compound in high yields (82%). Loading 30 mol% of catalyst has no effect on the yields of the required compound **8a**. In the absence of catalyst, the reaction did not proceed at all and only starting materials were recovered (Table 1).

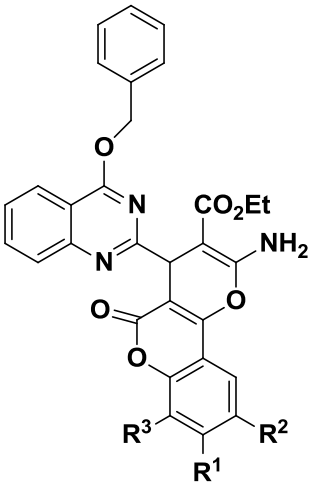
Table 1. Optimized conditions for **8a** compound with different mol % of DABCO in EtOH.



Entry	Catalyst (mol %)	Time	(%)Yield
1	DABCO (5 mol %)	2h	15
2	DABCO (10 mol %)	2h	25
3	DABCO (15 mol %)	2h	35
4	DABCO (20 mol %)	2h	82
5	DABCO (30 mol %)	2h	82
6	No catalyst	2h	No reaction

After optimization of the reaction conditions, the three component reactions of coumarins **6b-g** were carried out under standard protocol and the corresponding products **8b-g** were obtained in good to high yields (Table 2). The structures of compounds **8a-g** were deduced from their ^{1}H NMR, ^{13}C NMR, and IR spectral data. The molecular formulas for the compounds 8a-g were determined by HRMS from the corresponding $[M+H]^{+}$ ion peaks.

Table 2. DABCO catalyzed synthesis of dihydropyrano[c]chromenes **8a-g** via multicomponent reaction.[b, c]



8b-g

Entry	Compound	Substituent	% Yield[a]	m.p °C
1	**8b**	R^1 = Me, R^2 = Me, R^3 = H	80	258-260
2	**8c**	R^1= OMe, R^2 = H, R^3 = H	77	218-220
3	**8d**	R^1 = H, R^2 = F, R^3 = H	74	229-231
4	**8e**	R^1 = OMe, R^2 = Cl, R^3= H	79	242-245
5	**8f**	R^1 = H, R^2 = Cl, R^3 = Cl	83	262-264
6	**8g**	R^1 = H, R^2 = Cl, R^3 = Br	72	245-247

[a] Isolated yields but not optimized.
[b] All compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass and HRMS
[c] All the reactions are carried out using 0.75 mmol of **5.**

Anti-cancer activity against Breast cancer cell lines

The target compounds **8a-g** were evaluated for their cytotoxicity activity against two breast cancer cell lines, namely: MDA-MB 231 (Estrogen independent) and MDA-MB 453 (Breast adeno carcinoma cell line). Anti-cancer activities of the compounds were determined by a well-known colorimetric MTT assay based on mitochondrial reduction of yellow MTT tetrazolium salt dye to a highly colored violet-blue formazan product. Briefly, 1 x 10^4 cells (counted by Trypan blue exclusion dye method) were seeded in 96 well plates using DMEM (Dulbecco's Modified Eagle's Medium) with 10% FBS (Fetal Bovine Serum) medium and incubated for 24h before the treatment. After 24h, cells were treated with series of concentrations of each compound and incubated for 48h at 37°C in DMEM with 10% FBS medium. After 48h of incubation, media was replaced with 90 µL of fresh serum free media and 10 µL of MTT reagent (prepared in 5mg/ml in 1X PBS) and plates were incubated at 37°C for 4h. Then, media was removed carefully without disturbing the formazan crystals formed at the bottom of the plate and added with 200 µL DMSO and incubated at 37°C for

10-15 min to dissolve the formazan crystals. The absorbance at 570 nm was measured with a background deduction at 690 nm on a spectrophotometer (spectra max, Molecular Devices, USA). IC_{50} values were determined from plot with 50% cell viability (compared with control cells which received only DMSO+DMEM) versus concentration.

Compounds **8a-f** did not show toxicity even with 100µM concentrations in both MDA-MB 453 and MDA-MB 231 cell lines. However, the compound **8g** showed inhibition of breast cancer cell growth as reflected by its IC_{50} values. The compound **8g** showed an IC_{50} values at 45.7µM and 28.3µM concentrations in MDA-MB 453 and MDA-MB 231 cell lines respectively (Table 3). Also, we have performed a dose-dependent toxicity study in both the cell lines for compound **8g** and results are plotted in the form of graph (Figure 2). We found that compound 8**g** showed ~80% cell growth inhibitory effects at 50 µM and further at 100 µM concentration inhibit more than 95% cell growth (Figure 2) in MDA-MB 231 cells. Furthermore, **8g** showed its effective inhibition ~65% at 100µM concentration in MDA-MB 453 cells. This data suggests that **8g** derivative appears to have anti-cancer activity in breast cancer cells, MDA-MB 231. Although, the anticancer activity of **8g** is not comparable as standard activity, but functional group modifications at quinazoline-2-carbaldehyde **5** and substituted coumarins can guide to develop the drug leads and that will be future investigations from this laboratory.

Table 3. In vitro anticancer activity (IC_{50}) of compounds **8a-g** against breast cancer cell lines (MDA-MB 453, MDA-MB 231)

Entry	MDA-MB 453 (µM)[a]	MDA-MB 231 (µM)[a]
8a	>100	>100
8b	>100	>100
8c	>100	>100
8d	>100	>100
8e	>100	>100
8f	>100	>100
8g	**45.7 ± 2.47**	**28.3 ± 1.33**

[a] represents the concentration of the compound that causes cell growth inhibition.

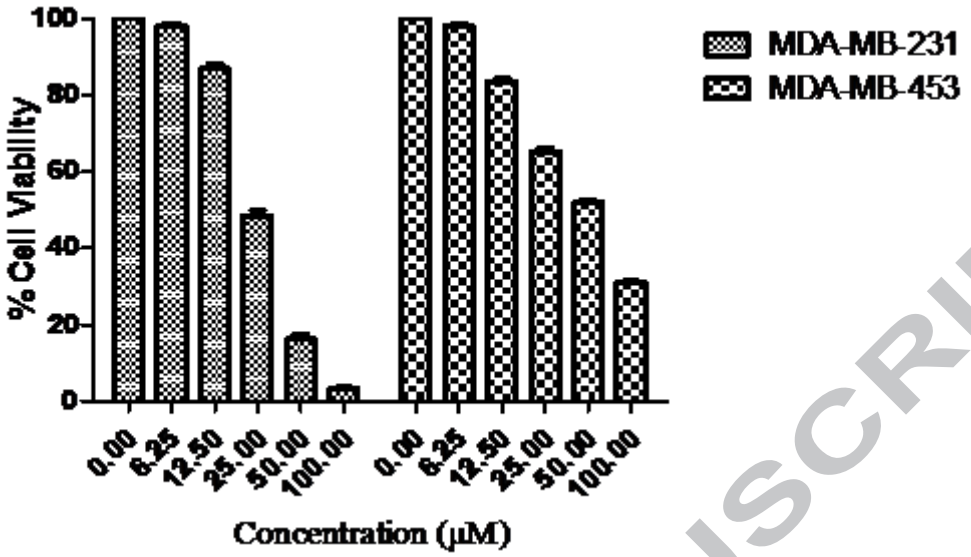


Figure 2. Concentration dependent anti-cancer activity of compound **8g** in breast cancer cells (MDA-MB 231and MDA-MB 453).

Molecular Docking studies:

In breast cancer cells Estrogens Receptor alpha (ERα) protein is highly expressed. In order to know the level of anticancer activity of **8g** against breast cancer lines (MDA-MB 231, MDA-MB 453), the molecular docking studies have been performed by using Glide (**Schrödinger Release 2015-3**: Maestro, version 10.3, Schrödinger, LLC, New York, NY, 2015) software. Crystal structure of ERα protein has downloaded from protein data bank [PDB deposition number :3UUD, 1.60A][23].

The affinity of **8g** at the receptor site of ERα protein and the best ligand binding poses were identified using the lowest binding energy, high docking score and the number of H-bonding, residue π- π interactions, hydrophobic interactions at receptor site (Figure 3 – 6). The **Table 4** represents the docking score and binding energies of **8a-g**. From the **Table 4**, it is evident that **8g** possess low binding energy (-44.69) and considerable high docking score (-3.521). As shown in **Figure 3**, the compound **8g** was found to be best in fitting into the active site of receptor. This may be due to possible hydrogen bonding interaction that formed with Leu-409 and π-π interaction with Tyr-331 of receptor. The hydrogen bonding was formed between backbone -NH of Leu-409 and oxygen of C=O of ester group in **8g** and it is observed as 1.80Å (Figure 4). The π-π interaction is indicated between Tyr-331 of receptor and olefinic bonds of pyran ring in **8g** as shown in **Figure 6**.

Table 4. Showing docking score and Binding energy of 8a-g

Structure	Docking score	Binding energy(Kcal/mol)
8a	-2.683	-37.694
8b	-2.642	-35.392
8c	-1.998	-38.355
8d	-2.582	-33.841
8e	-3.207	-35.251
8f	-2.854	-35.612
8g	**-3.521**	**-44.685**

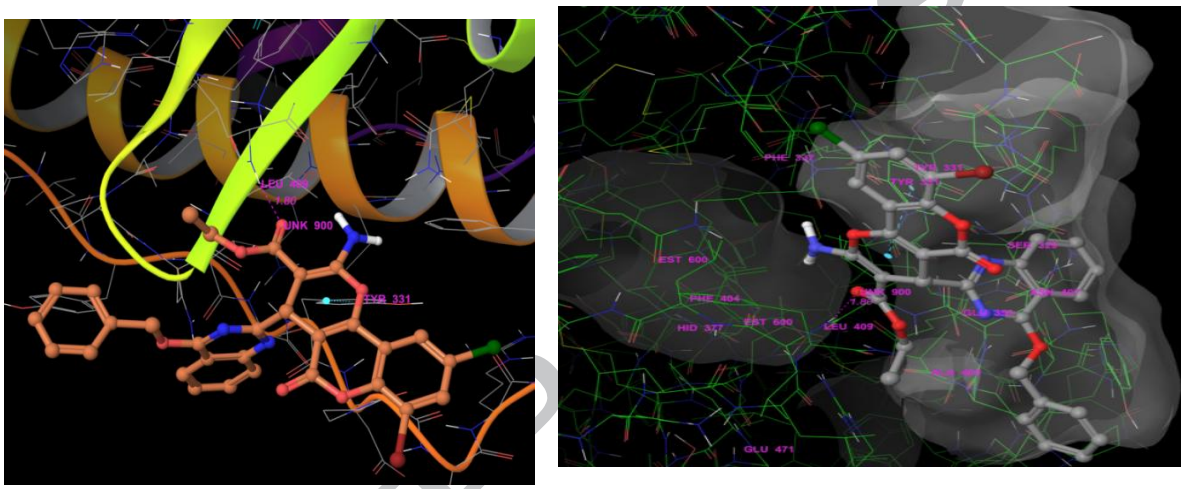


Figure 3. Illustrates compound **8g** (In stick) with ERα protien (3 UUD) and showing how it fits into the pocket. The protein is presented as cartoon and coloured spectrum. **8g** in active site (H bonding was shown by dotted pink line and pi-pi interactions are in sky blue dotted line)

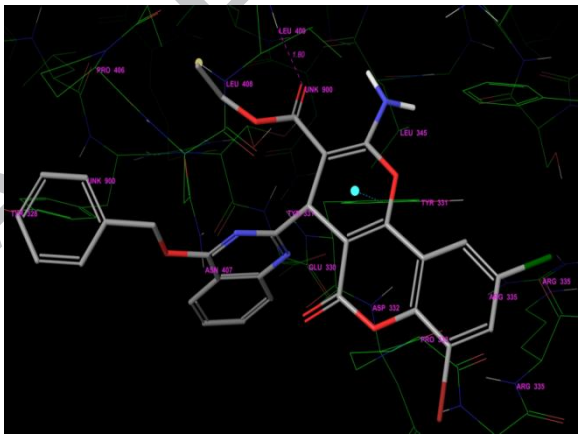


Figure 4. Showing H bonding distance(pink dotted line), (shown between backbone –NH of Leu 409 of receptor and –CO of ester in **8g**

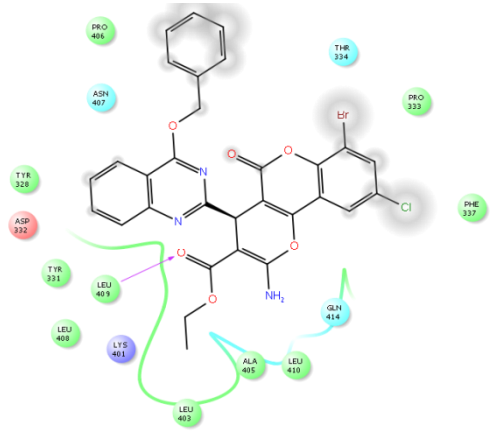


Figure 5. **8g** involved in hydrophobic interactions by fluorescent green colour) and polar interactions (shown shown in cyan)

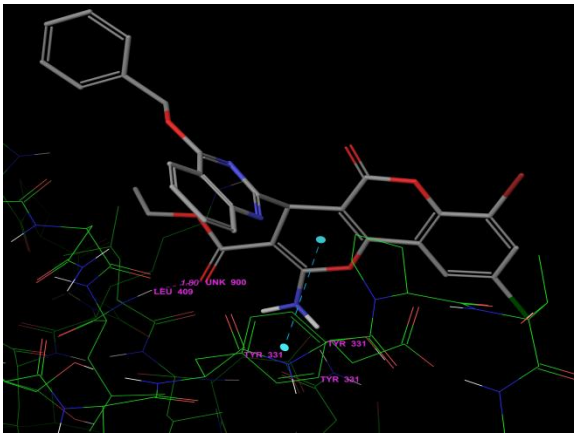


Figure 6. The π-π interaction is indicated between Tyr-331 of receptor and olefinic bonds of pyran ring in **8g**.

The derivative **8g**, along with H bonding and π-π interactions shows other type of interactions like hydrophobic interaction (takes place with pro-333, Phe-337, Pro-406 , Tyr-328 , Tyr-331, Leu-408, Leu-403, Ala-405 and Leu-410 amino acids), and polar interaction (with amino acids Thr-334, Gln-414 and Asn-407) (Figure 5). Bromo and chloro substituents in phenyl group oriented towards the hydrophobic pockets appear to be a positive influence in binding affinity[24] with protein as indicated by their high glide score and least binding energy. These docking studies revealed that the various possible interactions between **8g** and receptor site makes the compound easy to fit into the active site and it gives supporting evidence for the best anticancer activity of the compound.

In summary, we accomplished syntheses of a library of new series of quinazoline based C-C linked bis-heterocyles **8a-g** in which the C-2 position is occupied dihydropyrano[c]chromene moiety. The titled compounds were screened for their anticancer activity against breast cancer cell lines (MDA-MB 231, MDA-MB 453). Substitution at phenyl ring of *oxa*-heteryl moiety with bromine and chlorine atoms emerged as active compound **8g** in both breast cancer cell lines. Further, it was evidenced by the molecular docking studies. Hence, it is a strong impetus us to synthesize halo substituted derivative of quinazoline based C-C linked bis-heterocyles as good leads for further study. Study towards this direction is currently underway.

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Supplementary data

Supplementary data associated with detailed experimental procedures for the synthesis and spectral data of the synthesized compounds included as separate file

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22. *Procedure for the synthesis of compound 8a:* 4-Benzyloxy-quinazoline-2-carbaldehyde **5** (0.2g, 0.75 mmol), ethyl cyanoacetate **7** (0.087ml, 0.83 mmol), 4-hydroxycoumarin **6a** (0.83 mmol) and DABCO (20 mol %) were added to ethanol taken in R.B flask. The reaction mixture was refluxed for 35 min. Then resulted reaction mixture was cooled to 15 $^{\circ}$C, the solid separated out, filtered. The compound was recrystallised by EtOH as solvent, if necessary crude solid was passed through a silica gel column using EtOAc/hexane 3:7 mixture as eluent to yield the corresponding product **8a**. Yellow solid; IR (KBr, υ_{max}, cm^{-1}): 3377, 1706, 1670, 1649 ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (d, 1H, J = 8.5 Hz, ArH), 8.24 (dd, 1H, J = 6.2 Hz, J = 1.5 Hz, ArH), 7.87 (t, 1H, J = 8.7 Hz, J = 7.0 Hz, ArH), 7.59-7.49 (m, 3H, ArH), 7.29-7.22 (m, 2H, ArH), 6.90-6.88 (m, 3H, PhH), 6.81-6.77 (m, 2H, PhH), 6.57 (s, 2H, NH_2), 5.14 (AB-q, 2H), 3.86-3.81 (m, 2H,), 3.46 (s, 1H, 4(sp^3)-H), 0.71 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.6, 162.1, 158.1, 152.4, 143.4,137.1, 136.3, 134.0, 131.8, 128.7, 127.6, 126.1, 124.6, 124.4, 123.4, 123.3, 116.7, 116.0, 115.5, 95.3, 58.4 47.0, 13.3. ESI-HRMS: $[M+H]^+$ m/z calculated for $C_{30}H_{24}N_3O_6$: 521.1660; found at 521.1651.
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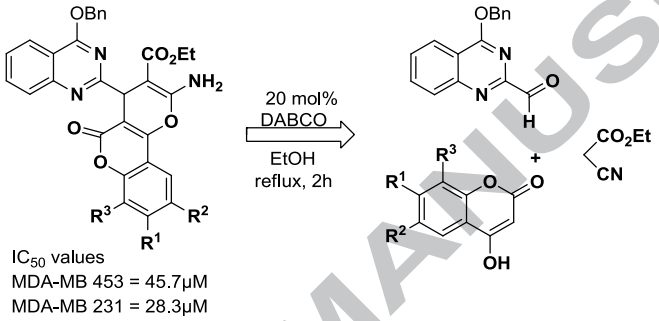
Graphical abstract

DABCO-catalyzed one-pot three component synthesis of dihydropyrano[3,2-c]chromene substituted quinazolines and their evaluation towards anticancer activity

Sumathi Vodnala, A. K. D. Bhavani*, Ramakrishna Kamutam, V. G. M.Naidu, Promila, Ch. Prabhakar



The novel carbon-carbon linked dihydropyrano[3,2-c]chromene substituted quinazolines were achieved via DABCO induced Knoevenagel-Michael addition reaction and resulting derivatives were evaluated towards anticancer activity.