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A Novel and Different Approach for the Synthesis of Quinoline Derivatives Starting Directly from Nitroarenes and Their Evaluation as Anti-Cancer Agents

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Abstract

A series of new quinoline derivatives (6-phenyl-6H-chromeno, [4,3-b] quinoline) have been prepared by using 4-chloro-2-phenyl-2H-chromene-3-carbaldehyde and various substituted nitroarenes as starting materials in the presence of Tin (II) chloride dihydrate and ethanol. The conversion in this synthesis involves the following steps (i) reduction of nitroarenes to anilines, (ii) Coupling of the anilines, chromene aldehydes (iii) Cyclization of resulting species and (iv) dehydration of cyclic intermediates. Several new quinolones have been prepared. We screened eight compounds of this novel series (6a-r) in three different cancer cell lines (B16F10, MCF7 and A549). The screened compounds showed moderate anticancer activity on two of the studied cell lines with best IC_{50} values of compound 6i (6.10±1.23 µM) and 6m (8.21±2.31 µM) on MCF7 cells. The selected compounds 6i and 6m led to morphological changes after treatment on MCF7 cell line. Interestingly, detailed studies suggested that the compounds 6i and 6m induced apoptosis in MCF7 cells in an oxidative stress independent manner without causing necrosis. In addition, we found destabilization of mitochondrial membrane potential behind the observed anticancer activity. Our results clearly indicate the promising anticancer potential of this novel series. This method is operationally simple and works with a diverse range of substrates.

Keywords: Quinolines, Chromeno-3-carbaldehyde, apoptosis, Anti-cancer agents

1. Introduction

The use of privileged structures in drug discovery has proven to be an effective strategy, allowing the generation of innovative hits/leads and successful optimization processes, (Musiol et al, 2017) Chromene and Quinolines are recognized as a privileged structures and useful templates for the design of novel compounds with potential pharmacological interest (Fig. 1), (Fernanda et al, 2017, Fernanda et al, 2014) Undoubtedly, quinoline-based compounds have a remarkable impact on anticancer drugs. The quinoline moiety offers a simply accessible, well-understood scaffold for designing new drugs. It is also a very potent molecule with the druggability for structure optimization through established synthetic pathways. For these reasons, quinoline-core anticancer drugs have a strong position in modern medicinal chemistry. (Musiol et al, 2017)

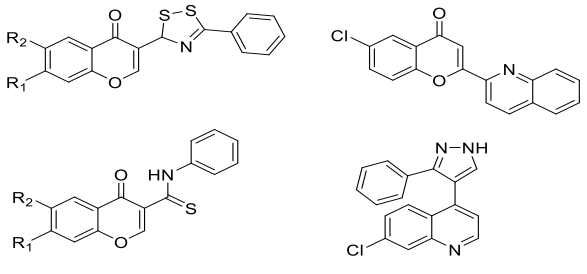


Figure 1. Chromene and Quinoline scaffolds emerged from the biological screening on cancer cell lines

Particularly in the field of neurodegenerative, infectious and inflammatory as well as diabetes and cancer. (Peczynska-Czoch, 1999) Moreover, as drug repurposing is becoming an attractive drug discovery approach, recent studies of chromene based drugs are also reported. (Musiol et al, 2017) Quinolines and Chromenes are frequently found structural units in biologically potent natural products, (Michael 2005) in particular, alkaloids.

In addition, various quinoline derivatives exhibit a broad range of pharmacological properties such as anti-cancer, (Peczynska-Czoch, 1999), (Connie, 1995) anti-tuberculosis, (Singh, 2004) anti-hypertensive, (Leonard, 2004) anti-HIV, (Nevinsky, 1999) and anti-alzheimer activities. (Luque, 2001) Substituted quinolones function as antagonists for $5HT_3$, NK-3, endothelin and leukotriene receptors. (Pinto et al, 1995) They are also utilized as intermediates for the design of bio-potent molecules. (Cuny et al, 2000)

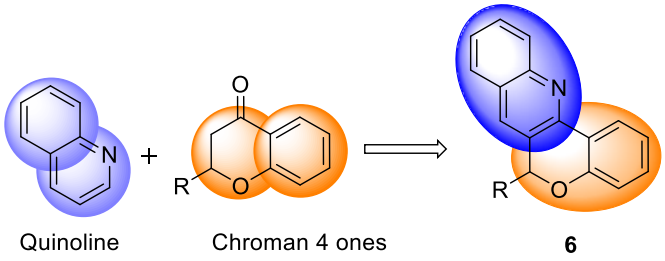
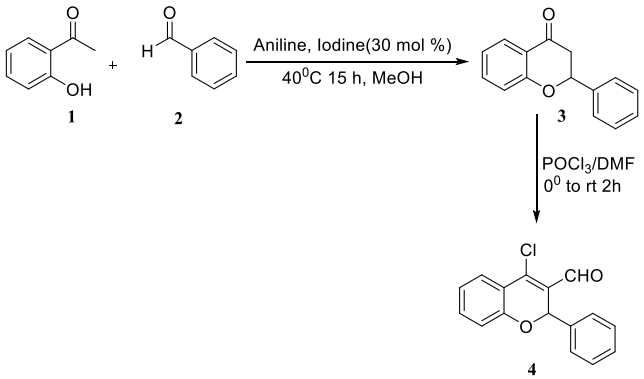


Figure 2. Combination of Chromene & Quinolines hybrids as potential anticancer agents

2. Results and Discussion

The ability to create carbon-carbon carbon-nitrogen bonds is at the heart of organic synthesis, heterogeneous catalytic processes are particularly apt at creating such bonds, especially in reductive amination or relay sequences where more one bond is formed allowing for rapid assembly of complex structures. The present method has proved to be a particularly powerful tool for the intramolecular creation of carbon-carbon and carbon-nitrogen bonds by using $SnCl_2 \cdot H_2O$ and EtOH. We herein report a protocol for the construction of chromeno quinoline hybrid comprising Tin (II) Chloride dihydrate catalyzed reductive cyclization of nitroarenes and chromenes, nitroarenes reduction is useful in pharmaceutical industry because nitro-substituted product can be reduced by $SnCl_2 \cdot H_2O$ to yield aryl amine. It involves reduction of the nitro arenes to anilines (Liu et al, 2002) which in the proximal presence of Chromeno 3-aldehyde (Z) would undergo cyclization, and dehydration of the intermediates to furnish the desired product, in straightforward manner (Fig. 2).

In continuation of our work, we report the method by using starting material 4-Chloro-2-aryl-2H-3-chromene carbaldehyde **4** prepared from 2-phenyl chroman-4-one **3**, through Vilsmeier-Haack reaction, (Subbarao et al, 1973) and compound **3** prepared by simple Mannich approach(Subbarao et al, 1973), (Baker et al, 1933) **Scheme 1**.



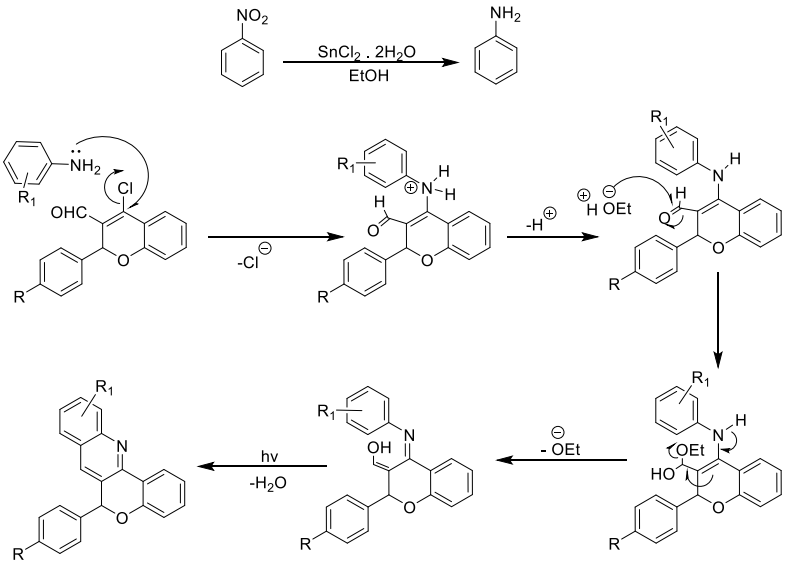
Scheme 1. Synthesis of starting material Chromene3-carbaldehyde using Mannich approach

The most common synthetic routes to prepare the flavanone **3** occurs via chalcone intermediate which involves the base catalyzed aldol condensation of 2-hydroxy acetophenones **1** with aromatic or conjugated aldehydes **2** in the presence of aniline and Iodine. The resulting chalcone **3** cyclized to produce flavanones via intramolecular SN^2 reaction. (Ching-Fa et al, 2012)

To explore the feasibility of this proposed strategy the reaction between known nitroarene (**5,** 1.2 eq) and Chromeno 3-carbaldehyde (**4,** 1 eq) with commercially available $SnCl_2 \cdot 2H_2O$ in EtOH at room temperature was performed (Table 1). Gratifyingly both starting materials were completely consumed in 8-12 h at room temperature and gave desired quinolone products (**6**) in 68-87% yield (entry 1-18)

The nitro arenes contained electron withdrawing and electron donating groups. All example mentioned in Table 1 were performed easily when compared to earlier reports. The table also shows that the yields of substituted anilines are not affected by the nature or the position of the aromatic substituents on the corresponding nitrobenzene.

A plausible mechanism of the conversion is shown in Scheme 2. Initially, the nitroarenes **1** are reduced to anilines by $SnCl_2 \cdot 2H_2O$ and these anilines react immediately with Chromene 3 aldehyde **3**, producing the corresponding imines. The imine intermediate cyclized in the presence of light produces the quinoline derivatives **6**. The conversion takes place spontaneously and no intermediate could be isolated.



Scheme 2. The plausible mechanism of the formation of the 6- phenyl-6*H*-chromeno [4,3-*b*] quinoline 6(a-r)

Table 1. Synthesis of the 6- phenyl-6*H*-chromeno [4,3-*b*] quinoline 6 (a-r) from Chromeno 3 carbaldehyde

Entry	R	R^1	Product 6	Time (h)	Yield[c](%)
1	H	H	**a**	8	85
2	H	3-OMe, 4-OMe, 5-OMe	**b**	2	68
3	H	3-Me	**c**	1	81
4	4-Br	H	**d**	9	78
5	OMe	3-F	**e**	10	84
6	H	2-Me, 3-Me	**f**	8	81
7	OMe	3-Me	**g**	12	77
8	H	2-Me, 3-Br, 5-Cl	**h**	12	81
9	3-Cl	4-OH	**i**	10	86
10	H	4-F	**j**	8	81
11	H	4-Cl	**k**	10	83
12	H	3-Br	**l**	12	85
13	H	3-NO_2	**m**	10	82
14	H	3-Me, 4-OMe	**n**	12	87
15	3-Br	4-OH	**o**	12	72
16	H	2-OMe, 3-OMe, 4-OMe	**p**	12	78
17	3-Cl	2-OMe, 3-OMe, 4-OMe	**q**	8	81
18	3-Cl	H	**r**	12	85

[a]Reagents and conditions: nitroarene **5** (1.2 mmol), aldehyde **4** (1.0 mmol), $SnCl_2.2H_2O$ (0.2 mmol), In EtOH (5 mL), reflux.

[b]All the products were fully characterized by the usual spectroscopic techniques.

2.1 In Vitro Cytotoxicity Assay

In the present study with series compounds **6**(**a-r**), three cancer cell lines (B16F10, A549 and MCF-7) were used. All the cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. B16F10, and MCF-7 cells were maintained in DMEM (Sigma Aldrich, USA) media supplemented with 10% fetal bovine serum (FBS, Gibco, USA), and Penicillin-Streptomycin (100 IU/mL each; Sigma Aldrich, USA), whereas A549 cells were maintained in RPMI-1640 (Sigma Aldrich, USA) media supplemented with FBS and antibiotic mix. The cytotoxicity assay was performed as per MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (Himedia, India) assay protocol as described previously.

Briefly, for cytotoxicity evaluation, 96 well plates (Eppendorf, Germany) were seeded with approximately 8000 cells/well and incubated overnight at 37 ºC and 5% CO_2 in a cell culture grade humidified incubator with HEPA filter for circulation of clean air (Eppendorf, Germany). The selected compounds **6**(**a-r**) were incubated with cells for 48 hours at six different concentrations in triplicates (3.12, 6.25, 12.5, 25, 50, and 100 µM) prepared in respective plain media. After incubation, drug solutions were discarded and the cells were incubated with 100 µL MTT solution/per well (0.5 mg/mL, prepared in plain media) for 4 hours. Thereafter, the formed formazan crystals were dissolved by adding 200 µL dimethyl sulfoxide/well, and kept on orbital shaker for 20 min. Thereafter, the absorbance was measured at 570 nm and results were quantified for the calculation of IC_{50} values.

2.2 Result Description

To assess the cytotoxicity profile, we screened eight compounds of this novel series **6(a-r)** in three different cancer cell lines (B16F10, MCF-7 and A549). The screened compounds showed moderate anticancer activity on two of the studied cell lines with IC_{50} values of compound **6m** 33.97±3.27 µM on B16F10 and **8.21±2.31** µM on MCF7 cells, whereas A549 human lung carcinoma cells showed higher resistance with IC_{50} value of 45.85±5.14 µM. In addition, compound **6b** and **6p** also showed decent anticancer activity on two cells lines. Compound **6i** produced anticancer cancer effect on MCF 7 cells with IC_{50} value of **6.10±1.23** µM. Another compound 6n exhibited promising anticancer activity on B16F10 cells with IC_{50} value of 14.66±1.43 µM. Overall, the A549 lung carcinoma cell lines exhibited higher resistance as evident from the IC_{50} values of individual compounds as shown in table. The representative comparative percentage viability graph is shown in Figure 3.

Table 2. Cytotoxicity evaluation: The IC_{50} values of different compound 6a-6r on three different cancer cell lines. Values are represented as mean±S.D. (n=3 of three independent experiments)

Compound	IC_{50} Value on B16F10 cells (µM)	IC_{50} Value on MCF-7 cells (µM)	IC_{50} Value on A549 Cells (µM)
6a	62.11±7.39	31.46±3.83	98.01±3.21
6b	94.45±8.32	17.10±2.84	>100
6c	14.66±1.43	31.15±1.83	99.09±4.21
6d	26.49±3.64	21.20±1.63	65.77±8.42
6e	45.85±6.14	35.09±5.23	88.87±5.21
6f	62.11±7.39	31.46±3.83	98.01±3.21
6g	82.23±5.57	70.95±8.37	54.45±2.87
6h	>100	54.43±6.98	89.12±9.32
6i	18.45±3.25	**6.10±1.23**	54.67±5.14
6j	26.49±3.64	21.20±1.63	65.77±8.42
6k	45.85±6.14	35.09±5.23	88.87±5.21
6l	62.11±7.39	31.46±3.83	98.01±3.21
6m	33.97±3.27	**8.21±2.31**	45.85±5.14
6n	14.66±1.43	31.15±1.83	99.09±4.21
6o	33.97±3.27	14.37±2.84	78.78±5.31
6p	33.97±3.27	14.37±2.84	78.78±7.31
6q	33.97±3.27	14.37±2.84	78.78±4.31
6r	14.66±1.43	31.15±1.83	99.09±4.21

We examined the anti-proliferative activity of compounds to understand the concentration dependant effect of the selected compounds on cellular morphology of MCF-7, cells were treated with desired concentration of **6i** and **6m** (7.5, 15 and 30 µM) and cisplatin (10 µM) as standard anticancer agent. (Kunal et al, 2014) Results of the morphological assessment based on phase contrast microscopy revealed profound changes in cellular morphology in a dose dependant manner (Fig 4). There was significant enhancement of chromatin condensation and apoptotic cells in a dose dependant manner as observed via EtBR/AO staining (Shivendra, 2011) (Fig 5). Interestingly, the death mechanism was found to be apoptosis as we did not observe any orange stained nucleus which is characteristic of cells dying via necrosis as EtBr can cross the nuclear membrane only if the membrane is leaky. In the present study, the cells were stained green mainly by acridine orange which is a vital dye and stains the nuclear material green.

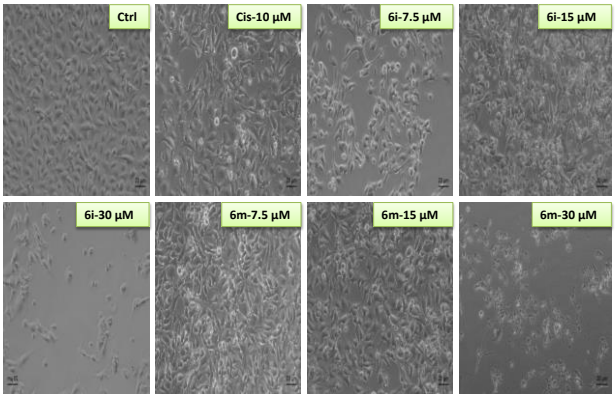


Figure 4. Effect of the selected compounds 6i and 6m on morphological changes after treatment at concentrations of 7.5, 15 and 30 µM for 24 h on MCF-7 cells as observed by phase-contrast microscopy, 10 µM of cisplatin was used as standard compound

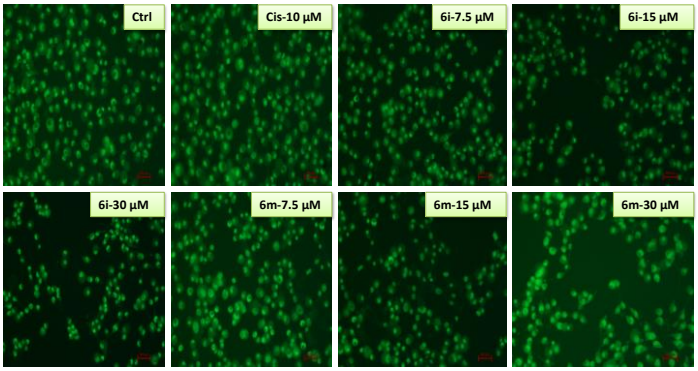


Figure 5. Effect of the selected compounds 6i and 6m on nuclear morphology after treatment at concentrations of 7.5, 15 and 30 µM for 24 h and staining with AO/EtBr on MCF-7 cells as observed by fluorescent microscopy

After the morphological assessment, we investigated the dose dependant effect of the selected compounds on reactive oxygen species levels in MCF-7 cells. The cells were treated with selected compounds at dose dependant concentrations. Interestingly, we did not observe significant increase in the intracellular oxidative stress indicating that the cell death induced by the synthesized series compound is independent of oxidative stress (Fig 6).

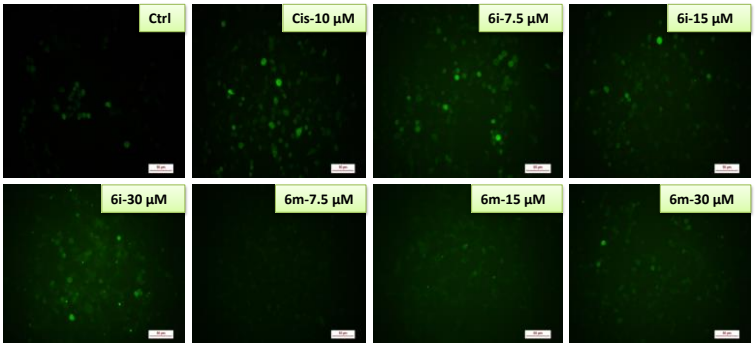


Figure 6. Effect of the selected compounds 6i and 6m on intracellular reactive oxygen species levels after treatment at concentrations of 7.5, 15 and 30 µM for 4 h and staining with DCFDA as observed by fluorescent microscopy

In addition, we estimated the integrity of mitochondrial membrane potential (MMP) via JC-1 staining. Strikingly, the compounds showed significant alteration of MMP in a dose dependant manner indicating the role of mitochondrial stress behind the observed anticancer effect (Kamal et al, 2015) (Fig. 7).

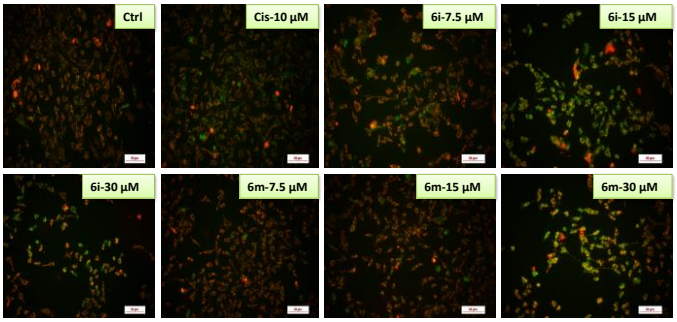


Figure 7. Effect on mitochondrial membrane potential of the selected compounds 6i and 6m after treatment at concentrations of 7.5, 15 and 30 µM for 24 h and staining with JC-1 as observed by fluorescent microscopy

3. Conclusions

In summary, a simple access for the synthesis of diverse novel quinolone derivatives (6-phenyl-6H-chromeno, [4, 3-b] quinolone) have been prepared by using 4-chloro-2-phenyl-2H-chromene-3-carbaldehyde and various substituted nitroarenes as starting materials in the presence of Tin chloride (II) dehydrate and ethanol. The conversion in this synthesis involves the following steps (i) reduction of nitroarenes to anilines, (ii) Coupling of the anilines, chromene aldehydes (iii) Cyclization of resulting species and (iv) dehydration of cyclic intermediates. Highly sterically demanding products, ambient reaction conditions, cost-effective catalytic system, good yields, operational simplicity, atom and step-economies are salient features of this strategy. A number of drug-like small molecules were prepared quickly and efficiently. We screened eighteen compounds of this novel series 6(a-r) in three different cancer cell lines (B16F10, MCF7 and A549). The selected compounds 6i and 6m led to morphological changes after treatment on MCF7. Further detailed studies such as acridine orange/ethidium Bromide (AO/EB), JC-1, DCFDA staining suggested that the compounds 6i and 6m induced apoptosis in MCF7 cells in an oxidative stress independent manner. Moreover, the selected compounds caused disruption of mitochondrial membrane potential indicating impact on mitochondrial stability thus inducing apoptosis. Our results clearly suggest that these hybrids have the potential to be developed as apoptosis inducers against breast carcinoma.

General reaction procedure for the synthesis of 6a–r

To as stirred solution of nitro arene (1.2 mmol) and appropriate chromene 3- carbaldehyde (1.0 mmol) in EtOH (5 mL), $SnCl_2 \cdot 2H_2O$ catalyst (0.2 mmol) were added and the reaction mixture was stirred at room temperature for 12 h. The progress of reaction was monitored by TLC. The resulting solution was washed with sat. aq $NaHCO_3$ (3 X 5 mL) and extracted with ethyl acetate (3 X 5mL). The organic layer was dried with sodium sulphate and concentrated under reduced pressure and further purified by column chromatography by using ethyl acetate/n-hexane to afford pure compounds 6a–r. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS.

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