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# C-dimethylated Chalcones*:* Towards Possible Dual Acting Agents- Against Tuberculosis and cancer (A549) and an SAR study

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**Abstract:**

A series of seventeen *C*-dimethyl chalcones on ring 'A' were synthesized by using a known condensation method. The chalcones include the utilization of heterocyclic aldehydes such as *N*,*N*-dimethyl benzaldehyde, furfural-2-aldehyde, and *N*-methyl indole aldehyde. All the compounds were found to possess moderate to potent anticancer activity on human lung adenocarcinoma cell line "A549" in the MTT cell proliferation method. The dimethyl chalcone **9f** with furfural ring was found to possess potent anti-cancer activity followed by **9m** with *o*-chloro substitution on ring 'B' with $IC_{50}$ values of 1.0 and 1.2 µg/mL respectively. Similarly compounds **9n**, **9j**, **9g, 9k, 9p**, **9d**, **9b,** and **9e** also showed good anti-cancer activity ($IC_{50}$ values from 6.5 to 9.8µg/mL). Further, the compounds were also compared with the known anti-TB activities in order to ascertain their properties towards dual acting agents against tuberculosis and cancer for more beneficiaries.

**Keywords:** Dimethyl chalcones, Anti-cancer, MTT–assay, A549, chalcones, Human lung adenocarcinoma.

## Introduction:

The previous literature revealed that many flavonoids are beneficial for human health [1]. Alkylated flavonoids isolated from plants have been reported as antimalarial [2], antioxidant [3], anticancer [4–6], anti-inflammatory [7] and antimicrobial agents [8] as well unsubstituted flavonoids. Anti-tubercular flavanones with methyl substitution on ring 'A' were isolated from the *Pisonia aculeate* of the *Nyctaginaceae* family [9] and their analogs were also synthesized for possible anti-tubercular profile [10] along with their respective chalcones for inhibition on HIV integrase [11].

In 2015, different alkyl substituted chalcones on ring 'B' of the $C_{15}$ skeleton were synthesized and to synthesize these chalcones from phloroacetophenone key intermediate [12,13,14] 2-hydroxy-4,6-dimethoxy-acetophenone was prepared. To synthesize some new *C*-alkylated chalcones [14] (having methyl substitution on ring B**)** by preparing required methyl substituted aromatic aldehydes.

The inflammation and fibrosis linked with TB could stimulate malignancy and triggered to blocking of lymphatics by pulmonary scarring and fibrosis initiating a delay in clearance of activated leucocytes. Subsequently, metastatic cell deposition was raised within fibrotic areas [15]. Instead, malnutrition and immune suppression affected by cancer were described as likely primary causes of contraction or recurrence of TB infection. Wu *et al.* [16] have described cancer as one of the major risk factors for TB. The possibility of co-infection with TB was reported in patients with malignancy, especially those with lung cancer [17]. In the present conditions of the drug resistance problem, dual acting agents against tuberculosis and cancer would be more beneficial. So, in our continuous efforts for bioactive flavonoids [10,11,14,18] here we attempted to develop the anticancer activity profile of the *C*-dimethylated chalcones (on ring 'A') whose anti-TB activity was already reported by us recently on *Mycobacterium tuberculosis* strain [19]. These synthesized compounds were screened for possible anti-cancer activity on the "A549" cell line (lung adenocarcinoma cell line).

## 3. Materials and Methods:

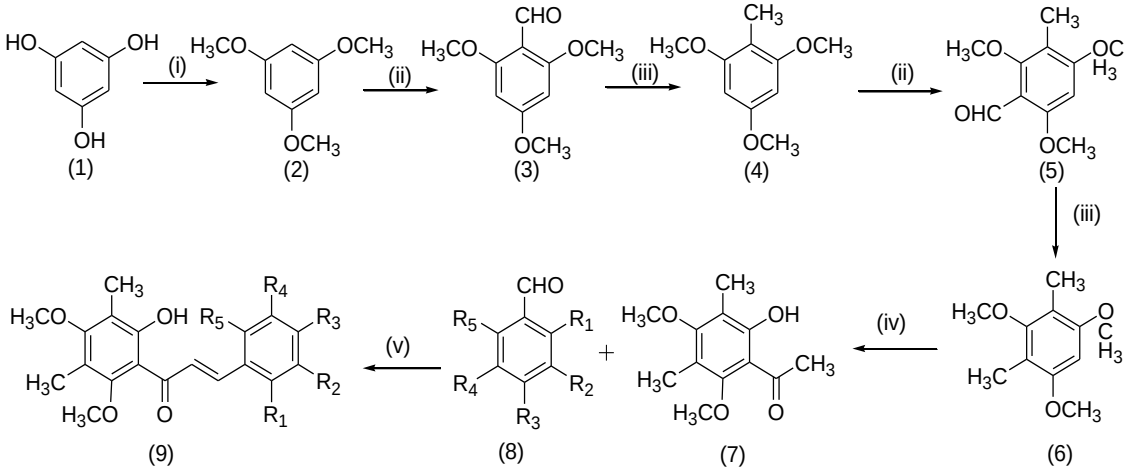
### Chemicals and reagents:

The starting material and all other chemicals purchased from Merck manufactures, Mumbai of AR grade.

### Instrumentation:

The melting ranges were determined by using the Meltemp apparatus. Proton NMR spectra were recorded on Bruker instrument at 400 MHz and Carbon NMR spectra were recorded at 100 MHz. Mass spectra were recorded on Agilent LC-1100 series LC-MS.

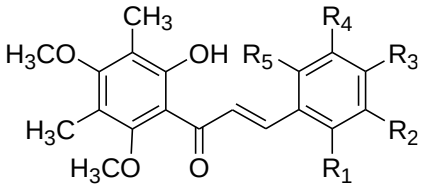
The chalcones (**9a-q**) of the present study (**Table 1**) were synthesized by following our reported **Scheme 1**, and all the compounds were confirmed by their melting points and spectral data [19].




**Reagents and conditions:** (i) DMS, Acetone, $K_2CO_3$,70°C, 8h. (ii) DMF, $POCl_3$, 0°C, 1h. (iii) $NH_2$-$NH_2.H_2O$, KOH, Ethyleneglycol, 80-90°C for 2h, 130-140°C for 2h. (iv) $CH_3COCl$, $AlCl_3$, Diethylether, 0°C, 6h (v) ArCHO, Aq. KOH/EtOH, 24h.

**Scheme 1:** Synthesis of new *C*-dimethylated Chalcones (**9a-q**)[19]

**Table1: Synthesized *C*-dimethyl chalcones (9a-q) with anticancer activity results**




| Compd. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | A549 $IC_{50}$ ($\mu$g/mL) | Anti-TB Activity [19] $MIC_{90}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|---|
| **9a** | H | H | $OCH_3$ | H | H | 18.9 ±1.80 | 1.6 |
| **9b** | H | $OCH_3$ | $OCH_3$ | H | H | 09.3± 1.10 | 6.25 |
| **9c** | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | H | 16.6± 0.64 | 1.6 |
| **9d** | H | H | Cl | H | H | 09.1± 2.20 | 12.5 |
| **9e** | H | H | F | H | H | 09.8± 1.50 | 12.5 |
| **9f** | Furfural aldehyde chalcone (heterocyclic) | | | | | 01.0± 0.30 | 6.25 |
| **9g** | *N*-methyl indole aldehyde chalcone (heterocyclic) | | | | | 08.5± 2.10 | 6.25 |
| **9h** | H | H | $N-(CH_3)_2$ | H | H | 18.3± 1.10 | 1.6 |
| **9i** | H | H | Br | H | H | 10.9± 0.90 | 6.25 |
| **9j** | H | $O-C_6H_5$ | H | H | H | 07.1± 2.70 | 3.12 |
| **9k** | $OCH_3$ | $CH_3$ | $OCH_3$ | H | $OCH_3$ | 08.5± 1.90 | 1.6 |
| **9l** | $OCH_3$ | H | $OCH_3$ | H | $OCH_3$ | 16.7± 1.10 | 6.25 |
| **9m** | Cl | H | H | H | H | 01.2± 0.45 | 6.25 |
| **9n** | F | H | H | H | H | 06.5± 1.13 | 6.25 |
| **9o** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | 10.3± 2.70 | - |
| **9p** | H | $O-C_2H_5$ | $OCH_3$ | H | H | 09.1± 0.66 | 1.6 |
| **9q** | H | $-O-CH_2-O-$ | | H | H | 13.2± 0.45 | 6.25 |

**Anti-Cancer activity**

**MTT cell proliferation assay method:** The compounds were tested on A549 (Human lung carcinoma cell line) cells using MTT cell proliferation assay [20]. A549 cell line as obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10 % fetal bovine serum (FBS). The cells (2000 cells per well) were seeded in a 96-well microplate containing 100 $\mu$L of DMEM + 10 % FBS medium per well and incubated at 37 ºC with 5 % $CO_2$. The cells were treated different concentration of compounds up to72 hours for every 24 hours interval. Controls were maintained with 0.5 % DMSO. After 72 hours treatment, 5$\mu$L of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (R & D Systems, USA) along with 45 $\mu$L of phenol red-free DMEM (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37 ºC with 5 % $CO_2$ for 4 hours. Thereafter, 50 $\mu$L of solubilization buffer (R & D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 hours the optical density was measured at 550 nm using a micro plate reader (Bio-Rad, USA).

**Results and Discussion:**

Chalcones are versatile molecules and important intermediates for the synthesis of various flavonoids and different heterocyclic compounds [21]. Chalcones can be synthesized in different ways [22,23]. The present chalcones **(9a-q)** were synthesized by Claisen-Schmidt reaction.

Chalcones (**9a-q**) are synthesized, the condensation between acetophenone (2-hydroxy-3,5-dimethyl-4,6- dimethoxyacetophenone) and heterocyclclic or aromatic substituted aldehydes in the presence of basic media in alcoholic solvent at room temperature for 24h. The acetophenone was prepared from phloroglucinol as a starting material by methylation, formylation and wolf-kishner reductions as reported previously [19]. All the compounds were well confirmed by their physical constants and spectral data comparison [19].

The anti-cancer activity was performed by following the MTT cell proliferation method on lung adenocarcinoma cell line i.e. A549 and results were summarized in **Table 1**.

**SAR Study**

A series of seventeen new *C*-dimethylchalcones were synthesized as reported previously [19] by changing different substituents on aromatic aldehyde and tested their anticancer activity on lung adenocarcinoma (A549) cell lines. All the synthesized compounds showed promising anticancer activity on A549 cell lines. Among these tested dimethyl chalcones on A549 cell line, **9f** which was synthesized using furfural-2-aldehyde showed more potent anti-cancer activity with $IC_{50}$ of 1.0$\mu$g/mL when compared to other synthesized compounds. Surprisingly, the compound **9m** which was obtained by using *o*-chloro substituted benzaldehyde also showed potent activity with $IC_{50}$ value 1.2 $\mu$g/mL, it is almost nearer to the activity of the compound **9f** (1.0 $\mu$g/mL). The compound **9n** (*o*-fluoro) showed very good activity with $IC_{50}$ value of 6.5 $\mu$g/mL. The compound **9j** having 3-phenoxy substitution on *meta*-position also showed good activity with $IC_{50}$ value 7.1$\mu$g/mL. The compounds **9g** (*N*-methyl indole), **9k** (3-methyl-2,4,6-trimethoxy), **9p** (3-ethoxy-4-methoxy), **9b (**3,4-dimethoxy), **9o** (3,4,5-trimethoxy) also showed good activity with $IC_{50}$values of 8.5, 8.5, 9.1, 9.3 and 10.3 $\mu$g/mL respectively. The halo substitution containing on *para*- position compounds **9d** (*p*-chloro), **9e** (*p*-fluoro), and **9i** (*p*-bromo) showed better activity with $IC_{50}$ values of 9.1, 9.8, and 10.9$\mu$g/mL respectively**.** The remaining compounds **9a** (4-methoxy), **9c** (2,3,4-trimethoxy), **9h** (4-N,Ndimethyl), **9l** (2,4,6-trimethoxy) and **9q** (3,4-methylenedioxy) showed moderate anticancer activity with $IC_{50}$ values of 18.9, 16.6, 18.3, 16.7 and 13.2 $\mu$g/mL respectively. Basing on the above activity results tetrahydrofuran ring containing compound showed more superior activity followed by halogen substitution on *ortho*- or 2$^{nd}$ position also showed superior activity. When compared to the halo substituted compounds 2-substituted compounds are more active than 3-substituted compounds and 4-substituted compounds, moreover the chloro- substituted compounds showed superior activity than *fluoro*- substituted compounds and bromo- substituted compounds. When compared the fluoro- and bromo- compounds, *fluoro*- compounds showed more activity than bromo- compounds. Dramatically 3,4-alkoxy compounds showed more activity than mono alkoxy substituted compounds and tri-alkoxy substituted compounds.

The chalcones were also found to have anti-TB activity against Mycobacterium tuberculosis strain (H37Rv) in the Microplate Alamar Blue assay (MABA) method in our recent report [19] and were included in **Table 1**. Among these studied chalcones shown in **Table 1**, some of the compounds were

found to possess good dual potency, for example, the heterocyclic chalcone **9f (**furfural-2-aldehyde) with heteroaromatic ring furan was found to be potent anti-cancer compound 1.0 $\mu$g/mL with the anti-TB activity of 6.5 $\mu$g/mL; while in compound **9k** (3-methyl-2,4,6-trimethoxy) the anti-tubercular activity is 1.6 $\mu$g/mL with anticancer activity of 8.5 $\mu$g/mL. Similarly the chalcone **9c** (2,3,4-trimethoxy) compound showed potential anti-tubercular activity 1.6 $\mu$g/mL with anticancer activity of 16.6 $\mu$g/mL; while the chalcone **9m** (*o*-chloro) compound with halogen substitution at *ortho* position was found to be potent anti-cancer activity 1.2 $\mu$g/mL with the anti-TB activity of 6.25 $\mu$g/mL. so, some of these dimethyl chalcones found to be showing good anticancer and anti-TB activities, so the development of these chalcone skeletons may further enhance the suitability for curing TB along with malignancy [17]. Moreover, recently the authors also studied the *In Silico* docking studies of the present synthesized ligands for potential pharmacological properties [24].

**Conclusion:**

A series of seventeen *C*-dimethyl chalcones on ring 'A' were synthesized and studied for their anticancer activity on the A549 cell line. Among the tested dimethyl chalcones, **9f** (furfural-2-aldehyde) with a heteroaromatic ring was found to be potent anticancer molecule followed by **9m** with *o*-chloro substitution on ring 'B' with $IC_{50}$ values 1.0 and 1.2 µg/mL respectively. Similarly the dimethyl chalcones **9n** (*o*-fluoro), **9j** (3-phenoxy phenyl), **9g** (*N*-methyl indole), **9k** (3-methyl-2,4,6-trimethoxy), **9p** (3-ethoxy-4-methoxy), **9d** (*p*-chloro), **9b (**3,4-dimethoxy), **9e** (*p*-fluoro), **9o** (3,4,5-trimethoxy) and **9i** (*p*-bromo) substitution on ring 'B' showed moderate activity with $IC_{50}$ values 6.5, 7.1, 8.5, 8.5, 9.1, 9.1, 9.3, 9.8, 10.3 and 10.9 respectively**.** Moreover, the compounds **9f, 9k, 9m,** and **9c** were also found to show dual-acting potency for both anticancer and anti-TB activities.

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**Conflict of interest:**

The authors declare no conflict of interest.

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