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Syntheses of lipophilic chalcones and their conformationally restricted analogues as antitubercular agents

Imran Ahmad [a], Jay Prakash Thakur [b], Debabrata Chanda [b], Dharmendra Saikia [b], Feroz Khan [c], Shivani Dixit [d], Amit Kumar [d], Rituraj Konwar [d], Arvind Singh Negi [a], Atul Gupta [a,*]

[a] Chemical Sciences Division, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow 226 015, India
[b] Molecular Bio-prospection Department, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow 226 015, India
[c] Molecular and Structural Biology Department, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow 226 015, India
[d] Endocrinology Division, CSIR-Central Drug Research Institute, Jankipuram Extension, Sector-10, Sitapur Road, Lucknow 226021, India

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ABSTRACT

Lipophilic chalcones and their conformationally restricted analogues were synthesized and evaluated for their antitubercular efficacy against *Mycobacterium tuberculosis* H37Rv strain. Compounds **16**, **24**, **25a** and **25c** were found to be active MIC at 60, 30, 3.5 and 7.5 μg-mL^{-1}. In vitro cytotoxicity of compounds **16**, **24**, **25**a, **25c** and **26** in non-cancerous human epithelial kidney cell line (HEK-293) showed that most active compound **25a** was approximately 2.85 times selective towards tubercular versus healthy cells whereas compound **24** was found to be 16 times selective.

Tuberculosis (TB) is a global health priority not only due to its morbidity and mortality but also due to its high contagious nature.[1–4] In 2010, 8.8 million people fell ill with TB and 1.4 million were died of it.[5] Thus, it is second largest killer worldwide by a single infectious disease. TB becomes fiercer in combination with HIV or cancer. Even our combination therapy of PRISE is not sufficient to tackle the situation.[6–8] Poor management of chemotherapy has emerged drug resistance. The highly lipophilic cell wall of Mycobacterium is responsible for its virulence to some extent.[9] Streptomycin (**1**), rifampicin (**2**), rifapentine (**3**), isoniazide (**4**), ethionamide (**5**) and ethambutol (**6**) are some potential drugs which are being used for tuberculosis treatment (Fig. 1). However, drug resistance is a major drawback of these agents.

Therefore, there is an urgent need to explore new antitubercular agents. Ironically, the low number of potential new chemical entities is a worrying situation at present. Considering the severity of the problem, WHO has prepared a strategic plan in Berlin declaration 2007 to stop TB globally.

In the recent past, several coumarin derivatives have been reported to exhibit antimycobacterial activity. Isoimperatorin (**7**), Osthol (**8a**) and suberosin (**8b**) were isolated from *Arracacia tolucensis* exihibiting antitubercular activity MIC at 64.0 μg-mL^{-1}, 32.0 μg-mL^{-1} and 16 μg-mL^{-1} respectively (Fig. 1).[10] Other coumarin derivatives such as ferulenol (**9**) have also been reported to exhibit potent antitubercular activity (MIC = 2.0 μg-mL^{-1}) against other species of *Mycobacterium*.[11,12] Similarly, chalcones such as licochalcone A (**10**), present in *Glycyrrhiza inflata* exhibited potent antitubercular activity (MIC = 7.1 μg-mL^{-1}).[13] Furthermore, pyranones such as **11** and **12** present in *Piper sanctum* have been reported to have potent antitubercular activity in *Mycobacterium tuberculosis* (MIC = 32 μg-mL^{-1} and 4.0 μg-mL^{-1}, respectively) (Fig. 1).[14] It is known that a moderate to high level of lipophilicity of the compounds often attributes better antitubercular activity.

In the present study, taking structural learning from natural coumarins, chalcones and pyranones, we synthesized some lipophilic chalcones of prototype **I** and their conformationally restricted analogues (styrenylchromanone) based on prototype **II** which may be considered as hybrid of coumarin and chalcone nucleus. Further, for activity modulation, we planned to have a nitrogen moiety as it is essentially present in all the frontline antitubercular drugs (Fig. 2).

The synthesized compounds were investigated for their antitubercular potential in *Mycobacterium tuberculosis* $H_{37}R_V$ strain radiometrically. Further, **16**, **24**, **25a**, **25c** and **26**, which showed significant antitubercular activity were evaluated for their toxicity

* Corresponding author. Tel.:+91 522 2718556.
E-mail address: atisky2001@yahoo.co.in (A. Gupta).

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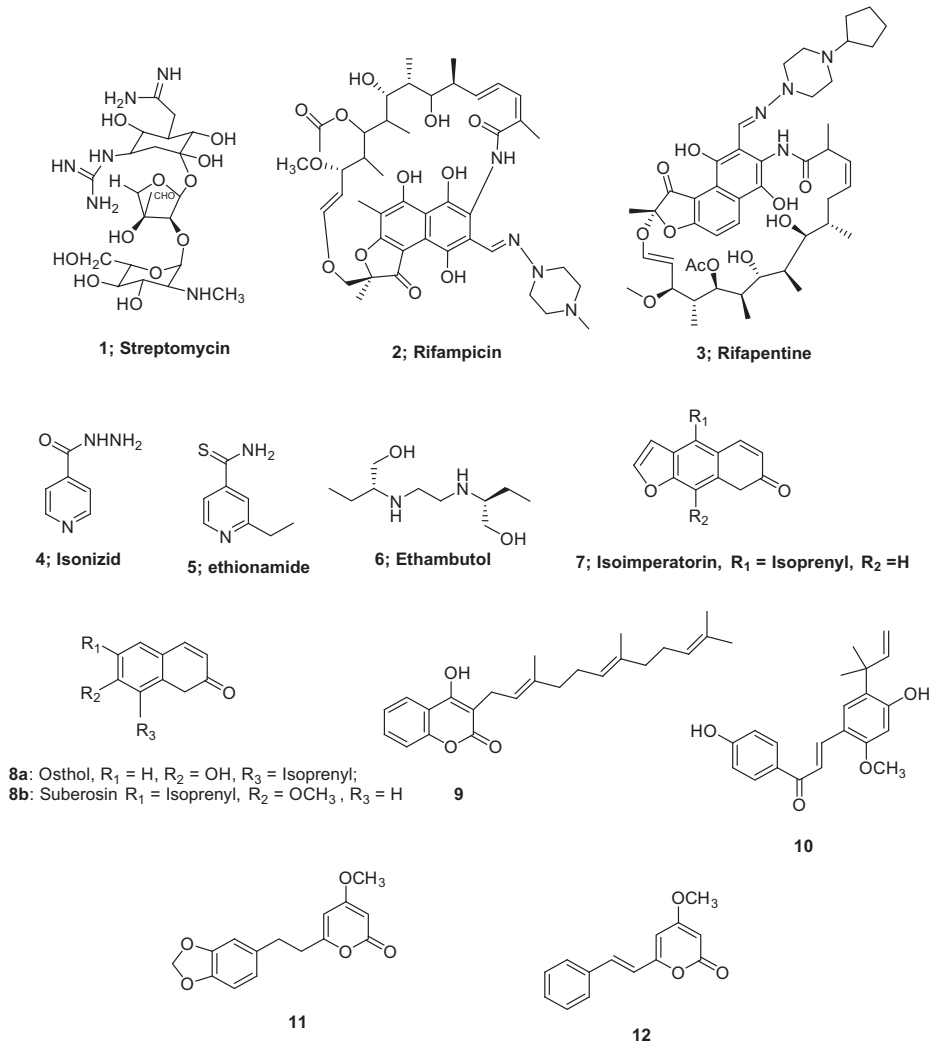


Figure 1. Some synthetic and natural anti-tubercular agents.

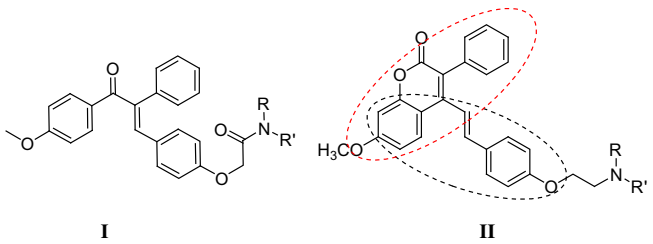


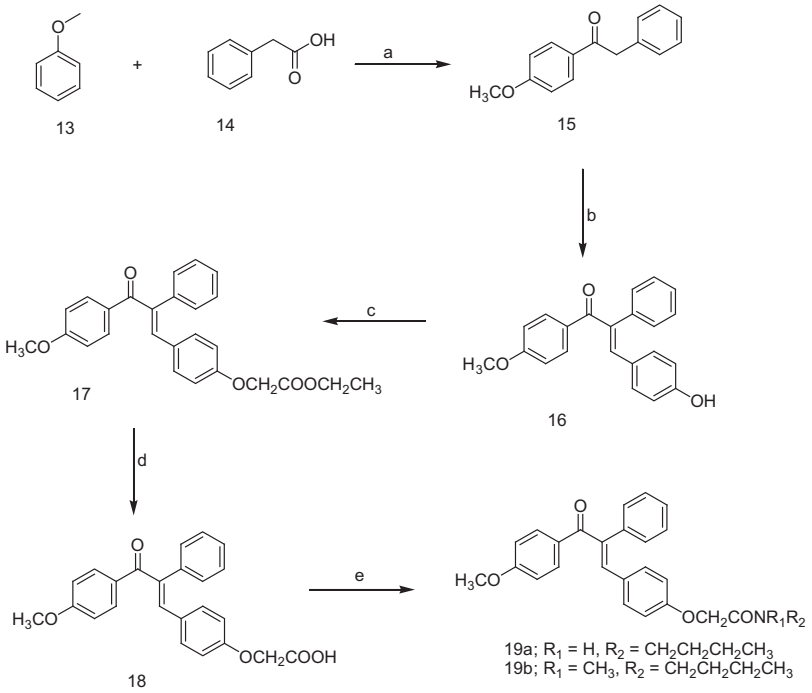
Figure 2. Prototype **I** and **II**.

in in vitro MTT assay using non-cancerous human epithelial kidney cell line (HEK-293) derived from human embryonic kidney cells.

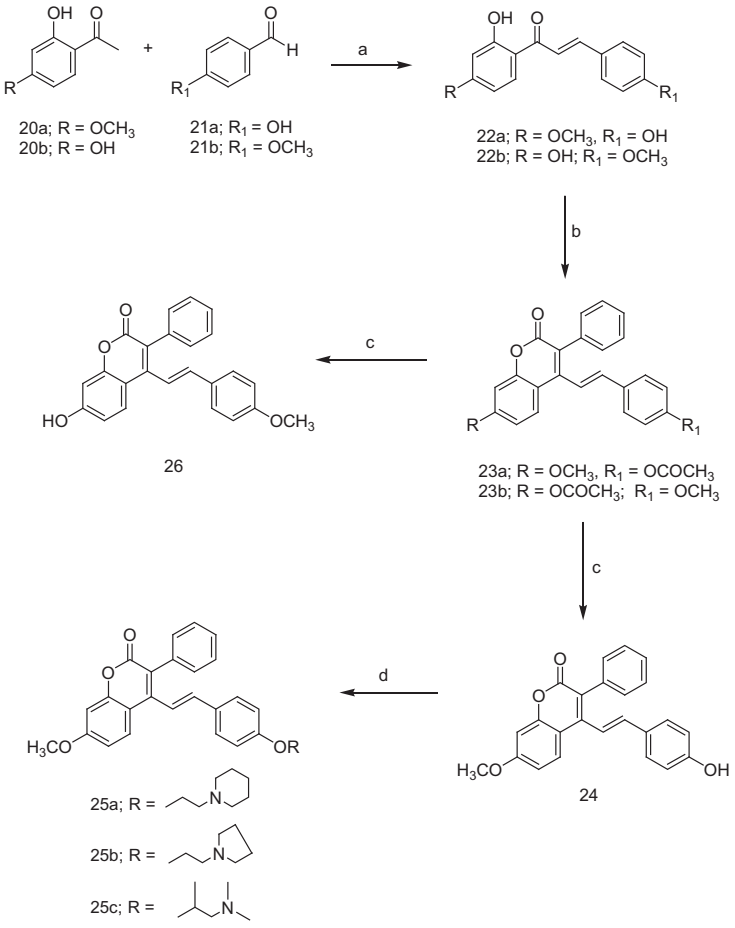
The synthesis of chalcone derivatives of type (**I**) was started from Friedel–Crafts acylation of anisol (**13**) with phenylacetic acid (**14**) using polyphosphoric acid (PPA) at 100 °C as reported earlier which yielded deoxybenzoin **15** in good yield as single product (scheme 1).[15] Compound **15** was reacted with 4-hydroxybenzaldehyde in presence of piperidine and dry benzene at reflux yielding compound **16** as single product with trans geometry confirmed by X-Ray analysis.[15] Compound **16** on condensation with ethyl bromoacetate gave corresponding ester derivative **17**. On hydrolysis under basic reaction condition compound **17** gave corresponding carboxylic acid derivative **18** in quantitative yield. The subsequent treatment of acid **18** with different amines in presence of 1-hydroxy benzotriazole (HOBt) and dicyclohexylcarbodiimde (DCC) in dichloromethane (DCM) under basic reaction conditions at reflux afforded amide derivative of chalcone **19a** and **19b** in 68 and 75% yields, respectively.

The synthesis of target compounds of prototype (**II**) was started with 2-hydroxy-4-methoxy-acetophenone (**20a**) as reported earlier (scheme 2).[16] Briefly, compound **20a** was condensed with 4-hydroxybenzaldehyde (**21a)** in presence of piperidine and dry benzene under reflux to afford 1-(2-hydroxy-4-methoxy-phenyl)-3-(4-hydroxy-phenyl)-propenone (**22a**). Compound **22a** on reaction with phenyl acetic acid in acetic anhydride and triethyl amine at reflux gave 7-methoxy-3-phenyl-4{2-[4-acetoxy-phenyl]-vinyl}-benzopyran-2-one (**23a)** in 86% yield. Compound **23a** on hydrolysis in 2% methanolic NaOH gave 7-methoxy-3-phenyl-4{2-[4-hydroxy-phenyl]-vinyl}-benzopyran-2-one **24** in 80% yield. Similarly, compound **26** was synthesized by reaction of **20b** and **21b** using same sequence of reactions. Condensation of compound **24** with 2-chloroethyl alkylamine hydrochloride in acetone in presence of K_2CO_3 under reflux gave the desired products **25**(**a–c**) in 78–86% yields. The synthesized compounds were characterized by the use of different spectroscopy techniques.[17]

To evaluate the potential of basic pharmacophore of chalcone, **16** (hydroxyl derivative) was evaluated for its antitubercular efficacy in *Mycobacterium tuberculosis* $H_{37}R_V$ strain which showed



Scheme 1. Reagent and conditions (a) Polyphosphoric acid, heating at 100 °C; (b) 4-hydroxybenzaldehyde, piperidine, dry benzene, reflux; (c) ethyl bromoacetate, anhy. K_2CO_3, dry acetone, reflux; (d) NaOH, methanol, reflux; (e) alkylamines, DCC, HOBt, DMAP, DCM, reflux.



Scheme 2. Reagent and conditions (a) piperidine, dry benzene, reflux; (b) phenylacetic acid, Et_3N, acetic anhydride, reflux; (c) 2% methanolic NaOH, room temperature; (d) 2-chloroethyl alkyl amines hydrochlorides, anhy. K_2CO_3, dry acetone reflux.

Table 1
Antimycobaterial and cytotoxic activities of compounds against *Mycobacterium tuberculosis* H_{37}Rv strain and human epithelial kidney cell line (HEK-293) respectively

Compound	MIC (μg-mL^{-1})	HEK-293 (IC_{50} in μg/mL)	Log*P*
16	60	50	4.887
18	250	—	4.460
19a	n.a	—	5.049
19b	n.a	—	5.296
22	n.a[a]	—	3.107
23	n.a	—	3.107
24	30	>250	4.933
25a	3.5	05	6.078
25b	n.a	—	6.078
25c	7.5	05	5.759
26	125	>250	4.933
Rifampicin	2.0	—	2.615
Streptomycin	2.0	—	−4.112

n.a. = not active at 250 μg-mL^{-1} concentration, (—) = Not determined, Log*P* = octanol/water partition coefficient, values were calculated using scigress explorer (Fujitsu) software.

activity at MIC 60 μg-mL^{-1} (Table 1).[18] Modification of compound **16** for activity modulation was made through transformation into alkylcarboxylic acid and corresponding amide derivatives (**18**, **19a** and **19b**). These compounds were found to be either far less active than **16** (hydroxyl derivative) or inactive. It is known in literature that molecular flexibility plays an important role in drug protein interactions. We therefore planned to synthesize conformationally restricted chalcones which may also be considered as hybrid of chalcone and coumarin derivatives (styrenylchromanone). This modification was made in such a way that the total lipophilic nature of compounds be almost similar (Log*P* values for compound **16** and **24** are 4.887 and 4.933, respectively, Table 1). The synthesis of these styrenylchromanones is briefly described in scheme 2. Compound **24**, the basic pharmacophore of prototype II, was showed antitubercular activity at MIC 30 μg-mL^{-1} (whereas compounds **16** showed activity at MIC 60 μg-mL^{-1}) (Table 1). Compound **24** possessing two fold higher activity than **16** was taken further for modification by incorporation of a nitrogen moiety present in many frontline antitubercular drugs. The modified compounds **25a–c** were synthesized by aminoalkylation of compound **24** as shown in scheme 2. The minimum inhibitory concentration (MIC) for compounds **25a** and **25c** were found to be 3.5 and 7.5 μg-mL^{-1}, respectively, which are close to the MIC values of rifampicin and streptomycin (MIC = 2.0 μg-mL^{-1} respectively) standard antitubercular drugs used as control. However, compound **25b** was found to be inactive in this assay.

In view of good antitubercular activity of compounds **16**, **24**, **25a, 25c** and **26**, their toxicity was evaluated in in vitro MTT assay using non-cancerous human epithelial kidney cell line (HEK-293) derived from human embryonic kidney cells as model of normal cell.[19] The IC_{50} values for studied compounds showed that compounds **16** (hydroxyl derivative of chalcone), **24** and **26** (hydroxy derivatives of conformationally restricted chalcones, styrenylchromanone) required relatively higher concentration for inhibition of cell population (50 and 250 μg/mL respectively) Table 1. Both compound **25a** and **25c** inhibited 50% cell population at 5 μg/mL concentration in this assay. Since, theoretically, MIC value represents IC_{100} and is approximately double of IC_{50} value, therefore comparing the in vitro antitubercular and cytotoxic activities of compounds (MIC vs IC_{50} values), the most active compounds **25a** could be considered to be approximately 2.85 times selective towards tubercular versus healthy mammalian cells whereas compound **24** was found to be 16 times safe with respect to its MIC values.

However, chalcone derivative (**16**) which had MIC value 60 μg/mL presented low selectivity towards tubercular versus healthy cells.

In conclusions, a series of lipophilic chalcones and their conformationally restricted analogues has been explored as antitubercular agents. Compounds **16**, **24**, **25a** and **25b** showed significant antitubercular activity. In vitro cytotoxic activity data showed that the most active compound **25a** exhibiting potent antitubercular activity (MIC = 3.5 μg-mL^{-1}) was found to be approximately 2.85 times selective towards tubercular versus healthy cells whereas, greater selectivity was observed for compound **24** as compared to conformationally flexible chalcone derivative (**16**).

Overall, the biological activity results of these molecules ravelled that conformationally restricted chalcones were superior over their open chain analogues possibly due molecular flexibility and may be a good lead in future for development of new antitubercular drugs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.096.

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