

An Efficient Synthesis of Aryloxyphenyl Cyclopropyl Methanone: A New Class of Antimycobacterial Agents[#]

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Abstract: An efficient, high yield and one pot synthesis of phenyl cyclopropyl methanones on reaction of different aryl alcohols with 4'-fluoro-4-chloro-butyrophenone in THF/DMF in presence of NaH /TBAB is reported. All the compounds were evaluated for their antitubercular activities against *M. tuberculosis* H37 Rv in vitro displaying MICs ranging from 25-3.125 mcg/ml. The most active compounds showed activity against MDR strains and one of them showed marginal enhancement MST in mice.

Key Words: Tuberculosis, Tetrabutylammonium bromide, Phenyl cyclopropyl methanones, Wolf- Kishner reduction

An increase in the global burden of tuberculosis with the worldwide mortality rate at 23% is major cause of concern in the socioeconomic and health sectors.^{1,2} Tuberculosis in HIV infected and immuno-compromised individuals and the increasing trend of MDR TB poses a threatening challenge particularly in the developing world.³ Although a number of lead molecules are in the pipeline of new drugs to combat the above challenge yet no new chemical entity has emerged in clinic for the last more than 40 years.^{4,5} Hence there is an emergent need to develop new drugs, acting through a novel mechanism, in cost effective manner. The incredible thickness mycobacterial cell wall being absent in human hosts owes a lot for the required long treatment of the disease and development of resistance against the known first line anti-TB drugs.⁶⁻⁸ Therefore, it is a selective target and many crucial enzymes required in the biosynthesis of cell wall macromolecules and their inhibitors are being looked as future hope in the treatment of this disease. One of such enzyme system is FAS-II required in the initial steps of mycolic acid biosynthesis.⁹ Many inhibitors of FAS-II are known and among them phenethyl alcohol¹⁰ and Triclosan¹⁰ are important for lead optimization. Further, during our work on this series of molecules antimycobacterial activity has been reported in simple acetophenones.¹¹ We also have identified a glycosylated phenyl cyclopropyl methanone (**1**) as very good antitubercular against MDR strains and in vivo too.¹²

Based on the above facts we thought to synthesize aryl cyclopropyl methanones and evaluate them for antimycobacterial activity. Our thought process for this class of molecules gained momentum because cyclopropyl ring is a common structural element of the mycobacterial cell wall¹³ and cyclopropyl ring is most common in a number of chemotherapeutics.¹⁴ Apart from the above aryl cyclopropyl ketones play prominent role as intermediates in the synthesis of many other biologically active compounds.¹⁵

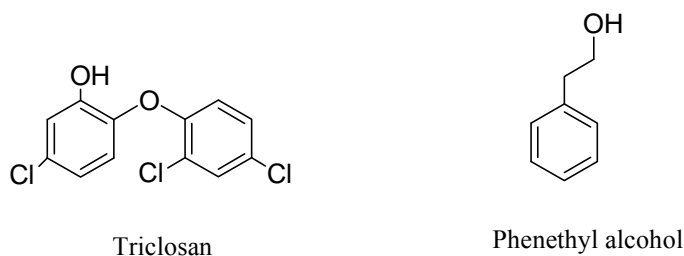
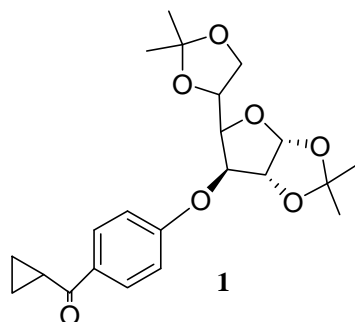
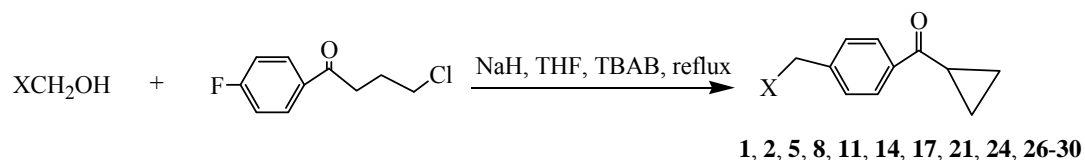


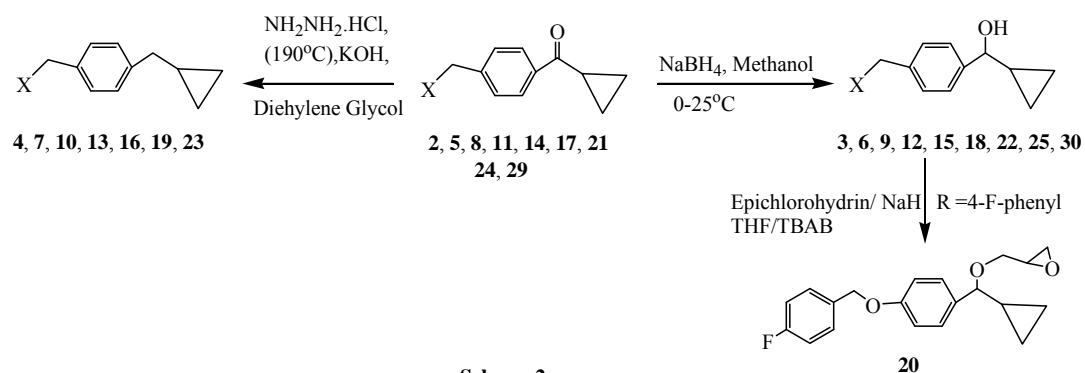
Fig.1 FAS-II Inhibitors



A number of methods for the synthesis of cyclopropyl ring exist in the literature¹⁶ and very recently we have reported rapid one pot procedure for the synthesis of combinatorial library of phenyl cyclopropyl methanones on solid phase.¹⁷ Since for biological evaluation a larger quantity of material is required and this led us to develop new method for their synthesis by conventional approach.



Scheme 1



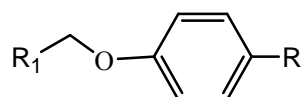
Scheme 2

Thus reaction of 4-chloro-4'-fluoro butyrophenone with 1,2:5,6-di-*O*-isopropylidene- α -D-glucofuranose alcohol in THF: DMF (1:1) in presence of NaH and tetrabutylammonium bromide (TBAB) as phase transfer catalyst at varying temperatures gave 4-*O*-benzyl- phenyl cyclopropyl methanones **1** in very good yields. Similar reaction with furylmethyl-, phenylmethyl-, 4-methoxy phenylmethyl-, 3,4-dimethoxy phenylmethyl-, 2-chloro phenylmethyl-, 4- fluoro phenylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, 4-chlorophenylmethyl-, 3,4-dichloro phenylmethyl-, cyclohexyl and cyclopentyl alcohols led to the formation





















of respective cyclopropyl phenylmethanones **2**, **5**, **8**, **11**, **14**, **17**, **21**, **24**, **26**, **27**, **28** and **29** respectively. The structures of all the compounds were determined on the basis of their spectroscopic data and microanalysis.¹⁸

To get an insight into the role of ketonic group on antitubercular activity profiles it has been partially and completely reduced to alcohol and methylene groups with sodium borohydride and Wolf Kishner reduction respectively in most of the active compounds. Thus reduction of cyclopropyl phenyl ketones **2**, **5**, **8**, **11**, **14**, **17**, **21**, **24** and **29** with NaBH₄ in ethanol gave compounds **3**, **6**, **9**, **12**, **15**, **18**, **22**, **25** and **30** respectively in good yields. However, reduction of compounds **2**, **5**, **8**, **11**, **14**, **17** and **21** with hydrazine hydrate followed by heating in presence of NaOH led to the respective cyclopropyl methanes **4**, **7**, **10**, **13**, **16**, **19** and **23** in moderate to good yields.

In one of the most active compounds (**18**) the hydroxyl group was further derivatised to its *O*-(epoxy-*n*-propyl) derivative (**20**) by its reaction with epichlorohydrin in THF in presence of phase transfer catalyst following our earlier reported method.



Comps. no.	CH ₂ R ¹	R	MABA MIC (μg/ml) against <i>M. tuberculosis</i> H37Ra	Agar Microdilution MIC (μg/ml) against <i>M. tuberculosis</i> H37Rv
2	Furyl methyl		25	25
3	Furyl methyl		>25	>25
4	Furyl methyl		25	25
5	Phenyl methyl		25	6.25
6	Phenyl methyl		>50	25
7	Phenyl methyl		>50	12.5
8	4-methoxy phenyl methyl		>50	12.5
9	4-methoxy phenyl methyl		>50	3.12
10	4-methoxy phenyl methyl		>25	25
11	3,4-methoxy phenyl		25	12.5

	methyl			
12	3,4-methoxy phenyl methyl		3.12	>50
13	3,4-methoxy phenyl methyl		12.5	25
14	2-chloro phenyl methyl		>25	12.5
15	2-chloro phenyl methyl		>25	-
16	2-chloro phenyl methyl		>25	>50
17	4-fluoro phenyl methyl		>25	12.5
18	4-fluoro phenyl methyl		25	6.25
19	4-fluoro phenyl methyl		nd	12.5
20	4-fluoro phenyl methyl		nd	3.12
21	3-pyridyl methyl		>25	25
22	3-pyridyl methyl		>25	>25
23	3-pyridyl methyl		>25	-
24	4- pyridyl methyl		>25	12.5
25	4- pyridyl methyl		>25	>25
26	4-chloro phenyl			25
27	3,4-dichloro phenyl			25
28	cyclohexyl		>25	>25
29	cyclopentyl		>25	25
30	cyclopentyl		25	3.12

All the compounds synthesized were evaluated for their antitubercular activity in against *M. tuberculosis* H37Rv MABA method¹⁹ while Agar Microdilution method²⁰ was used against *M. tuberculosis* H37 Rv. One of the compounds was also screened against MDR strains and in vivo in mice model.²¹ As evident from Table-1 except compounds **3**, **12**, **16**, **22**, **25**, **26**, **27** and **28** all other compounds displayed activity with MIC ranging from 25µg /mL to 3.25 µg /mL.

A closure look into the structure activity relationship in these compounds with the compounds synthesized among the cyclopropyl phenyl methanones **21**, **26**, **27** and **28** are inactive as their MICs are >25µg/mL, however, compounds **5**, **8**, **11**, **14**, **17** and **24** have MICs in the range of 12.5- 6.25 µg/mL. It is interesting to note that the partially reduced alcohols **9** (3.12µg/mL), **18** (6.25µg/mL) and **30** (3.12µg/mL)

of the corresponding phenyl cyclopropyl methanones **8** (12.5µg/mL), **17** (12.5µg/mL) and **29** (25µg/mL) did offer better protection. Among the completely reduced cyclopropyl phenyl methanes none of the compounds offer better inhibition than the parent ketones as the MICs were either retained or enhanced. Further, replacement of aryloxy moiety in these compounds with heteroaryloxy group did not improve the antitubercular efficacy. Replacement of aryloxy group with cyclopentyloxy moiety in the alcohols did offer better result. However, in general replacing the aryloxy group with cycloalkyloxy group gave compounds with comparable activities. One of the cyclopropyl phenyl methanol with 4-fluorobenzyloxy substituent offer very good MIC and fluoro group is known to play very important role in biological activity profile of the molecules it was derivatised in to respective *O*-(*n*-epoxypropyl) derivative which has MIC of 3.12µg/mL. Among the most active compoundsand ...were chosen for evaluating against MDR strains and they have the MIC of.....µg/ml. These compounds were further evaluated in vivo in mouse model and offer protection to the extent of.....

General procedure for the synthesis of compounds:

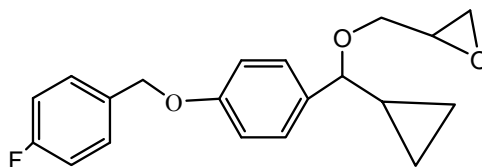
Physical data of the selected compounds:

17. Colourless oil, FAB MS $m/z = 271 [M+H]^+$, IR $\nu_{\max} \text{ cm}^{-1}$ 2945 (C-H stretching), 1654 (C=O), 1600 (C-O); ^1H NMR (200 MHz, CDCl_3) $\delta = 8.00$ and 6.90 (each d, $J = 8.80$ Hz, each 2H, Ar-H), 7.34 and 7.01 (each m, each 2H, ArH), 5.04 (s, 2H, OCH_2Ar), 3.80 (s, 3H, OCH_3) 2.60 (m, 1H, cyclopropyl CH), 1.21 and 0.98 (each m, each 2H, cyclopropyl CH_2 's); ^{13}C NMR (50 MHz, CDCl_3) $\delta = 199.2$ (C=O), 165.4, 162.6, 160.5, 132.5, 132.4, 131.8 (ArC), 130.6, 129.8, 129.6, 116.2, 115.7, 114.9 (Ar-CH), 69.8 (OCH_2Ar), 17.0 (cyclopropyl CH), 11.5 (cyclopropyl CH_2 's).

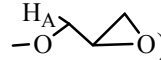
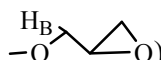
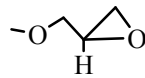
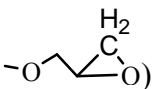
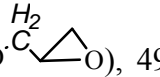
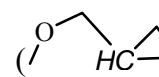
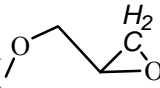
18. Colourless solid, FAB MS $m/z = 273 [M+H]^+$, IR $\nu_{\max} \text{ cm}^{-1}$ 3339.7 (OH stretching), 2933.4 (C-H stretching), 1609.7 (C-O); ^1H NMR (200 MHz, CDCl_3) $\delta = 8.07$ and 7.98 (each d, $J = 8.80$ Hz, each 2H, Ar-H), 7.41 and 7.47 (each m, each 2H, ArH), 7.25 and 7.07 (each m, each 4H, ArH), 5.08 (s, 2H, OCH_2Ar), 3.80 (s, 3H, OCH_3) 2.60 (m, 1H, cyclopropyl CH), 1.73 (s, 1H, OH exchangeable with D_2O) 1.20 and 0.98 (each m, each 2H, cyclopropyl CH_2 's); ^{13}C NMR (50 MHz, CDCl_3) δ 132.5, 132.4, 131.8 (ArC), 129.8, 128.6, 116.1, 115.6 (Ar-C), 78.5 (CHOH), 69.7 (OCH_2Ar), 19.5 (cyclopropyl CH), 3.1, 2.0 (cyclopropyl CH_2 's).

19. Colourless –solid FAB MS $m/z = 256 [M+H]^+$, IR $\nu_{\max} \text{ cm}^{-1}$ 2916.6 (C-H stretching), 1607.3 (C-O); ^1H NMR (200 MHz, CDCl_3) $\delta = 7.25$ and 7.21 (each d, each 2H, Ar-H), 7.15 and 7.05 (each m, each 2H, ArH), 6.95 and 6.92 (each m, each 2H, ArH), 6.80 and 6.72 (each m, each 2H, ArH), 4.80 (s, 2H, OCH_2Ar), 2.32 (d, 2H, $-\text{CH}_2$) 1.22 (m, 1H, cyclopropyl CH), 0.78 , $.02$ (each m, each 2H, cyclopropyl CH_2 's); ^{13}C NMR (50 MHz, CDCl_3) δ 157.3, 135.1, 116.0, 115.6 (ArC), 129.8, 128.6, 116.1, 115.6 (Ar-C), 69.8 (OCH_2Ar), 39.8 (CH_2), 12.5 (cyclopropyl CH), 5.03 (both cyclopropyl CH_2 's).

20.



Colourless solid, FAB MS $m/z = 328 [M+H]^+$, IR $\nu_{\max} \text{ cm}^{-1}$ 3338.7 (OH stretching), 2930.4 (C-H stretching), 1608.7 (C-O); ^1H NMR (200 MHz, CDCl_3) $\delta = 7.42$ and 7.38 (each d, $J = 8.60$ Hz, each 2H, Ar-H), 7.28 (m, 4H, ArH), 7.10 and 6.96

(each d, $J = 8.60$ each 2H, ArH), 5.01 (s, 2H, OCH₂Ar), 3.72 (m, 1H, )
 3.54 (m, 1H, ) , 3.39 (m, 1H, ) , 2.74 (m, 2H, ) ,
 1.25 (m, 1H, cyclopropyl-CH), 0.48, 0.44 and 0.42 (m, 4H, cyclopropyl CH₂'s);
¹³C NMR (50 MHz, CDCl₃) δ 165.3, 158.6, 134.6, 133.2 (ArC), 127.4, 127.3,
 126.2, 126.1, 113.7, 113.3, 112.7 (Ar-CH), 83.8 (CHOH), 67.4 (OCH₂Ar),
 66.9 () , 49.1 () , 42.7 () , 15.5 (cyclopropyl CH),
 2.3, 0.1 (cyclopropyl CH₂'s).

6.1.1. Activity against *M. tuberculosis* H₃₇Ra Strain

All the glycosyl amino alcohols synthesized were evaluated for their efficacy against *M. tuberculosis* H₃₇Ra at concentration ranging from 50 µg/mL to 1.56 µg/mL using two fold dilution in the initial screen. Log phase culture of *M. tuberculosis* H₃₇ Ra is diluted so as to give final OD_{550 nm} of 0.05 in Sauton's medium. In 96 well white plate 190 µL of culture is dispensed in each well. A DMSO solution of test compounds is dispensed to 96 well plates so as to make final test concentration 25 µg/mL (5 µg test compound is dispensed in 10 µL of DMSO). Then the plate is incubated at 37 °C / 5 % CO₂ for 5 days. On 5th day 15 µL Alamar blue solution is added to the each well of plate. The plate is again incubated overnight at 37 °C/ 5 % CO₂ incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found active (>90 % inhibition as compared with control) at this concentration are then tested at 6 serial dilutions starting from 50 µg/mL to 3.12 µg/mL [19].

6.1.2. Activity against *M. tuberculosis* H₃₇Rv strain

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H₃₇Rv was performed by agar microdilution method [20] where two fold dilutions of each test compound was added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H₃₇Rv growing on L-J medium was harvested in 0.85 % saline with 0.05 % Tween-80. A suspension of 1 µg/mL concentration of extracts/compounds was prepared in dimethyl sulfoxide (DMSO). This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentration of compound keeping the volume constant *i.e.* 0.1 mL. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 hours followed by streaking of *M. tuberculosis* H₃₇Rv (5 x 10⁴ bacilli/tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H₃₇Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

In vivo Screening:

In vivo activity:

The activity of compound **13** was evaluated *in vivo* in experimental tuberculosis in mice as described previously.²¹ Hence, the efficacy of the compound **13** against challenge of *M. tuberculosis* H₃₇Rv was tested at 100 mg/Kg. Mice were infected intravenously via lateral veins with 10⁷ CFU. Mice were divided into 2 groups of 10 mice each after 2 days of infection. One was of compound treated by intraperitoneal (i.p.) route, whereas the other group served as untreated control. At 25mg /Kg dose, the compound gives a marginal protection (*Fig.22*). The compound seems to protect mice at nontoxic concentration against *M. tuberculosis* infection. However, at higher doses it causes toxicity in mice. It will be interesting to prepare analogs of compound which will be nontoxic to eukaryotes but are strongly antitubercular.

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