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A facile synthesis of α, α′-*(EE)-bis(*benzylidene)-cycloalkanones and their antitubercular evaluations

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Abstract

An economical and facile synthesis of α, α′-*(EE)-bis(*benzylidene)-cycloalkanones was achieved by reaction of cycloalkanones with different aromatic aldehydes using ethanolic KOH in good yields. Few of the selected compounds were reduced with $NaBH_4$ to the respective α, α′-*(EE)-bis(*benzylidene)-cycloalkanols. All these compounds and our earlier synthesized cyclohexyl phenyl methanols were evaluated for their antitubercular, antifungal and antibacterial activities. Several compounds displayed moderate antitubercular activity with MIC 12.5-1.56 μg/mL. However, none of the compounds displayed any significant antifungal activity.

Keywords: α, α′-*(EE)-bis(*benzylidene)-cycloalkanones, Antifungal, Antitubercular

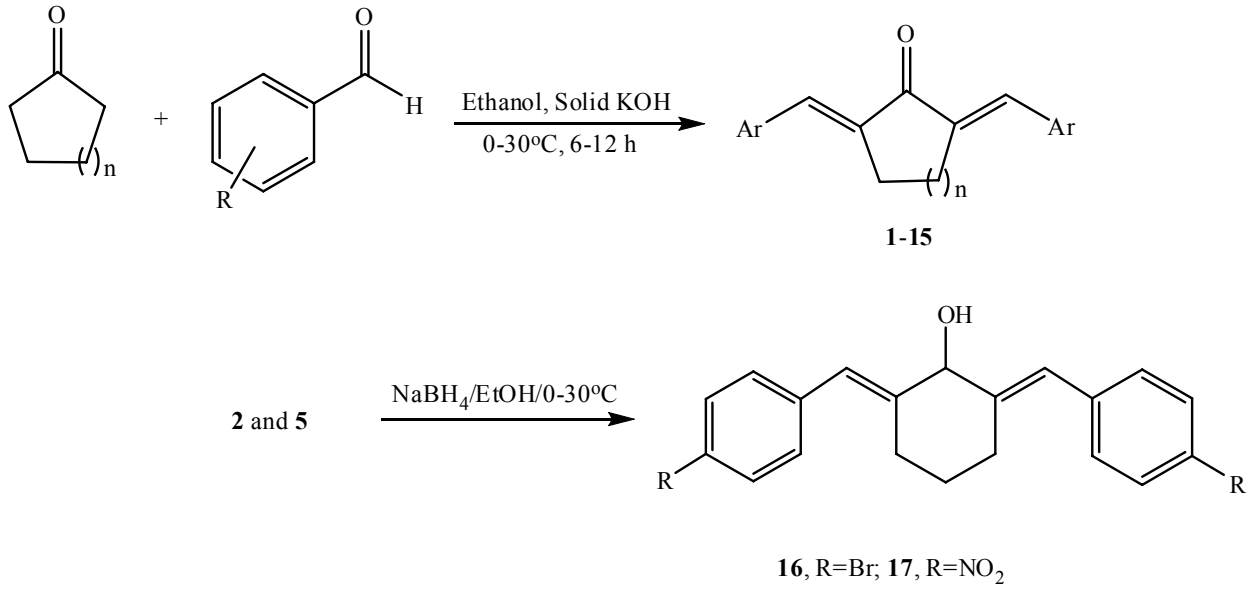
1. Introduction

Tuberculosis has re-emerged as a growing public health threat, killing young and middle aged people faster than any other diseases. Infection of Mycobacterium tuberculosis remains a leading cause of death [1]. It is estimated that nearly $9x10^6$ new cases of active TB disease occur every year [2]. A vast majority of the world's burden of tuberculosis (TB) is in developing countries, and it is estimated that only 23% of the prevalent active cases receive an appropriate antitubercular treatment [3]. An appropriate treatment of tuberculosis has led to the development of several bottlenecks in chemotherapy of tuberculosis. Emergence of MDR (multi drug resistance) and XDR (extremely drug resistance) tuberculosis and its synergy with HIV have further aggravated the problem of chemotherapy in tuberculosis. Although, a number of new

chemical entities have been discovered recently as potent antitubercular, yet no new drug has been entered in clinics since 1965 [4-6]. Therefore, there is an urgent need to develop new drugs, acting through a novel mode of action for the chemotherapy of TB. Recently, we have designed and developed novel aryloxy cyclopropyl phenyl methanones [7] as possible inhibitors of FAS-II enzyme required in chain elongation of mycolic acids keeping in mind the structure of triclosan [8] and the compounds displayed potent antitubercular activity both in vitro and in vivo. Moreover, several acetophenones [9], chalcones [10] and Mannich bases of benzylidene-cycloalkanones [11] have also been disclosed to possess moderate antitubercular activity and their mode of action is also postulated to be the inhibition of initial steps in fatty acid biosynthesis. *Bis*-benzylidene cyclolkanones have been reported to possess drug resistance reversal [12], cytotoxicity [13] and histone acetyl transferases (HAT) [14] inhibitory activities. N-acetyl transferase (NAT), a subtype of HAT plays very important role in initial stages of mycolic acid biosynthesis in mycobacterium and it is known that human NAT inactivates [15] the antitubercular drug INH (isoniazid) in humans. The above facts prompted us to synthesize benzylidene cycloalkanone analogs in an efficient and cost effective manner; and evaluated them for their antitubercular and antifungal activities.

2. Chemistry

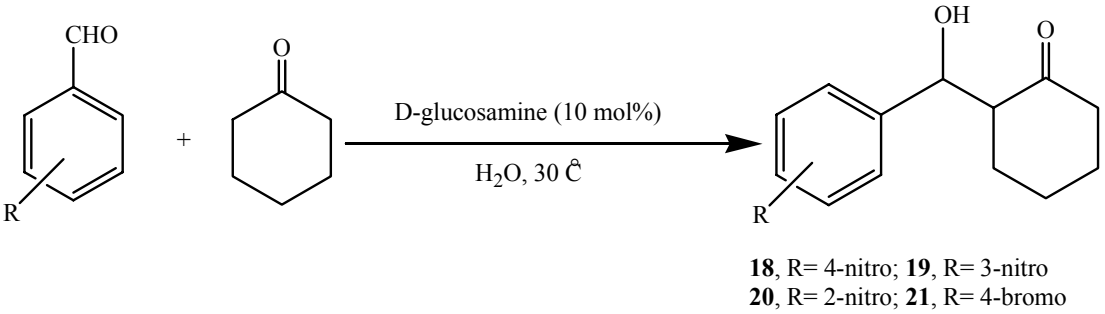
α, α′-*(EE)-bis(*benzylidene)-cycloalkanones (**1-15**) were prepared by reacting two equivalents of aromatic aldehydes with one equivalent of cycloalkanones in presence of solid KOH (5 mol%) in ethanol (Scheme1). The selected aldehydes comprise of furfuraldehyde, benzaldehyde, 4-bromobenzaldehyde, 4-chlorobenzaldehyde, 4-fluorobenzaldehyde, 4-nitrobenzaldehyde, 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde. The selected cycloalkanones were cyclopentanone, cyclohexanone and cycloheptanone.



Scheme 1. Synthesis of *bis*-benzylidene cycloalkanones and cycloalkanols

Two of the most active compounds **2** and **5** were reduced with $NaBH_4$ in ethanol to give the respective α, α′-*bis*-(benzylidene)-cycloalkanols **16** and **17** in good yields.

Cycohexylphenyl methanols **18**, **19**, **20** and **21** were prepared by D-glucosamine catalyzed aldol reaction of cyclohexanone with 4-nitro-, 3-nitro-, 2-nitro- and 4-bromobenzaldehydes as reported by us [16].



Scheme 2. Synthesis of hydroxy-(phenyl)-methyl cycloalkanones

Table-1: Synthesis of bis benzylidene cycloalkanones and cycloalkanols (**1**-**17**)

Compds.	**n**	**Ar/R**	**m.p. (°C)**	**known m.p (°C) [Ref]**	**% yield**
1	2	Furyl	131-133	140-142 [17]	69
2	2	4-bromophenyl	163-164	165-168 [23a,b]	86
3	2	3,4-dimethoxyphenyl	141-143	138-140 [20]	92
4	2	4-fluorophenyl	154-156	156-158 [23c]	85
5	2	4-nitrophenyl	154-157	161-162 [33]	87
6	3	4-bromophenyl	129-131	137 [21]	83
7	2	4-benzyloxyphenyl	199-201	190-191 [22]	86
8	2	3,4,5-Trimethoxyphenyl	218-219	208 [20]	88
9	2	Phenyl	117-119	117-118 [33]	91
10	2	4-chlorophenyl	142-145	147-148 [33]	85
11	3	4-chlorophenyl	118-120	Not reported	85
12	3	4-methoxyphenyl	95-97	126-127 [30]	87
13	3	3,4-dimethoxyphenyl	155-157	164-165 [33]	90
14	1	3,4-dimethoxyphenyl	190-192	186-188 [20]	84
15	2	2-naphthyl	199-202	212-213 [24]	89
16	2	4-bromophenyl	131-132	Not reported	75
17	2	4-nitrophenyl	143-145	Not reported	65

Table-2: In vitro Antitubercular activity of *bis*-benzylidene cyclohexanones (**1- 15**), *bis*-benzylidene cyclohexanols (**16** and **17**) and cyclohexyl phenyl methanols (**18-21**)

Compd no.	**n**	**cLog P***	**MIC H37 Ra**	**MIC H37 Rv**
1	2	3.68	>12.5	>12.5
2	2	6.46	1.56	12.5
3	2	4.64	>12.5	>12.5
4	2	5.18	12.5	>12.5
5	2	7.29	3.12	12.5
6	3	6.78	>12.5	>12.5
7	2	7.59	>12.5	>12.5
8	2	4.43	>12.5	>12.5
9	2	4.64	>12.5	6.25
10	2	5.76	>12.5	>12.5
11	3	6.17	>12.5	>12.5
12	3	4.8	6.25	>12.5
13	3	4.55	>12.5	>12.5
14	1	3.72	>12.5	>12.5
15	2	7.67	>12.5	>12.5
16	2	6.13	>12.5	12.5

17	2	3.32	>12.5	>12.5
18	2	3.43	>12.5	12.5
19	2	3.53	>12.5	12.5
20	2	3.55	>12.5	>12.5
21	2	3.07	>12.5	12.5
Isoniazid	-	-0.668	-	0.75
Ethambutol	-	0.1188	-	3.25

*cLogP was determined by OSIRIS Property Explorer Programme available at http://www.organic-chemistry.org/prog/peo/

Table-3: In vitro antifungal and antibacterial activity of synthesized compounds

Entry	**Minimum inhibitory conc. (MIC) in µg /mL against**									
	BACTERIA				**FUNGI**					
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**
1	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
2	>50	>50	>50	>50	>50	50	25	12.5	>50	>50
3	>50	>50	>50	>50	>50	>50	50	50	>50	>50
5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
8	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
9	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
10	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
12	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
14	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
15	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
16	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
18	>50	>50	>50	>50	50	>50	>50	>50	>50	>50
19	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

a. *E.Coli* (ATCC 9637), **b**. *Pseudomonas aurigonosa* (ACTT BAA-427), **c**. *Staphylococcus aureus* (ACTT-25923), **d**. *Klebsiella pneumoniae* (ACTT-27736), **e**. *Candida albicans*. **f**. *Cryptococcus neoformans*. **g**. *Sporothrix schenckii.* **h**. *Trichophyton mentagrophytes.* **i**. *Aspergillus fumigatus* **j**. *Candida parapsilosis* (ATCC-22019)

4. Biology

All the synthesized compounds were evaluated for their antitubercular activity against *M. tuberculosis* H37Ra by MABA (microplate alamar blue assay) method [18], while Agar Micro dilution method [19] was used against *M. tuberculosis* H37Rv. INH and ethambutol were used as standard drugs. All of the above compounds were also screened against different strains of bacteria and fungi viz. E.*Coli* (ATCC 9637), P*seudomonas aurigonosa* (ACTT BAA-427), *Staphylococcus aureus* (ACTT-25923), *Klebsiella pneumoniae* (ACTT-27736), *Candida albicans* (ATCC-1405B), *Cryptococcus neoformans*, *Sporothrix schenckii, Trichophyton mentagrophytes., Aspergillus fumigatus and Candida parapsilosis* (ATCC-22019). Fungi were tested by NCCLS (National Committee for Clinical Laboratory Standards) method in RPMI 1640 medium and bacteria in Mueller Hinton Broth [28].

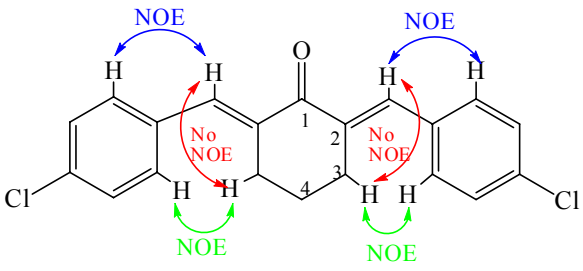
5. Results and discussion

5.1. Chemistry

Several reports exist for the synthesis [29-34] of benzylidene cycloalkanones involving use of organic and inorganic bases, metal catalyst, different type of Friedel Craft catalysts and TCT (Trichloro-1,3,5-triazine). We have used aqueous KOH /NaOH, TCT and many other catalysts for the condensation of cycloalkanones and aldehydes to get the desired α, α′-*(EE)-bis(*benzylidene)-cycloalkanones. In our hands, most of the catalysts resulted in the poor yields of the products as several byproducts were formed and it was difficult to isolate the desired compounds in pure form from the reaction mixture. Application of solid KOH (5 mol%) as a catalyst for condensation of different aldehydes with cycloalkanones in minimum amount of ethanol resulted in the desired α, α′-*(EE)-bis(*benzylidene)-cycloalkanones (**1-15**) in good yields (Scheme 1 and Table 1) and proved to most convenient method. This method is economical and eco-friendly as neither any byproduct was formed nor any toxic material was used during synthesis and reactions were carried out at ambient temperature. Work up of the reaction mixture was very simple involving just filtration of the product and washing with cold water followed by drying and crystallization. The structures of the *bis*-benzylidene cycloalkanones (**1-15**) were in accordance with their spectroscopic data and microanalyses. The IR spectrum of the compounds in general exhibited the absorption band at around 1731-1593 cm^{-1} indicating that the carbonyl

group and olefinic bonds of α, α′-*(EE)-bis(*benzylidene)-cycloalkanones are in conjugation. The ESMS (mass spectra) of the compounds showed their respective $[M+H]^+$ peaks. In the ^{1}H-NMR spectrum of the vinylic protons of the compounds occur either as singlet at around δ 7.50-7.30 ppm or merged with the multiplet of aromatic protons ranging from δ 8.25-6.65 ppm, while the methylene protons of C-3 was appeared as multiplet at around δ 2.96-2.36 ppm and the methylene protons of C-4 was observed as multiplet at around δ 1.97-1.67 ppm.

The *EE* geometry of the double bonds in the above compounds (**1-15**) was based on earlier literature reports [25, 26, 27]. It is reported that the methine protons are in close proximity of the carbonyl group which exerted an anisotropic effect, resulting in the downfield shifting and overlapping of the vinylic protons with the aromatic protons and appearance of vinylic protons in the region of δ 7.15-7.95 is an indicative of such compounds with *E* configuration and in the region of δ 6.8 indicates *Z* configuration [25, 26], as for example, the olefinic protons of *Z*-2-phenyl methylenecylohexanones and Z-2-phenyl methylene-6,6-diphenyl cyclohexanones are generally observed at δ 6.27 and 6.22 [27]. In ^{1}H NMR spectra of compounds **1-15** the vinylic protons appear either as singlet at around δ 7.50-7.30 ppm or observed along with the multiplets of aromatic protons ranging from δ 8.25-6.65 ppm. The *EE* geometry of the two double bonds was further substantiated by NOE experiment on a prototype compound (**10**). The vinylic proton at δ 7.7 ppm showed NOE with the the aromatic *ortho* protons and at the same time it did not show any NOE with the methylene protons at C-3 (δ 2.8 ppm) (Fig. 1). The latter, clearly shows the *EE* geometry of the olefinic bond.



EE geometry

Fig. 1. NOE in *EE* geometry

The IR spectrum of the *bis*-benzylidene cycloalkanols (**16** and **17**) exhibited the absorption band at around 3300 and 1480 cm^{-1} indicating the presence of hydroxyl group and olefinic bonds. In the ^{1}H-NMR spectrum of the compound the vinylic protons are merged with aromatic protons and appeared in the range of δ 8.21-6.54 ppm and hydroxyl proton observed around δ 4.50 ppm while the proton of C-1 occurred at δ 2.79 ppm and the methylene protons of C-3 and C-4 appeared as such.

5.2. Biology

Out of all the screened compounds **2**, **4, 5**, **9, 12**, **16**, **18**, **19** and **21** displayed antitubercular activity with MIC ranging from 12.5 to 1.56 μg/mL against either the avirulent strain *M. tuberculosis* H37Ra or the virulent strain *M. tuberculosis* H37Rv. As evident from Table 2, compounds **2**, **4**, **5** and **12** display antitubercular activity against the avirulent strain *M. tuberculosis* H37Ra, a surrogate of *M. tuberculosis* H37Rv. On the other hand compounds **2, 5, 9**, **16**, **18**, **19** and **21** show activity against *M. tuberculosis* H37Rv. Therefore, two of the above compounds **2** and **5** are active against both the avirulent and virulent strains of *M. tuberculosis*.

Among all the compounds screened against different strains of bacteria and fungi only one compound (compound **2)** show activity against the two fungi, *Sporothrix schenckii* and *Trichophyton mentagrophytes* with MIC 25 and 12.5μg/mL respectively. Other compounds displayed MIC ≥50μg/mL against all the fungi which were tested for these compounds.

A closer look into the SAR (structure activity relationship) of these compounds reveals that among all the benzylidene cycloalkanones, compounds **1**, **3**, **6**, **7**, **8**, **10**, **11**, **13**, **14**, **15, 17**and **20** do not exhibit antitubercular activity against either of the strains *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv as they have MIC >12.5 μg/mL. Further, it is also evident that reduction of carbonyl group in the most potent compounds (**2** and **5**) of the series to their respective *bis*-benzylidene cycloalkanols (**16** and **17**), results in the loss of antitubercular activity indicating that the carbonyl group is essential for their antitubercular activity. It is also interesting to note that among the *bis*-benzylidene cycloalkanones, only *bis*-benzylidene cyclohexanones display antitubercular activity while their counter parts with cyclopentanone and cycloheptanone moieties are inactive as their MIC values were >12.5 μg/mL. It is also clear that the substitution on aromatic ring at the 4th position generally leads to compounds having better activity in

comparison to other compounds either with unsubstituted aromatic ring or having substitution on other aromatic positions. Compounds **16**, **18**, **19** and **21** are moderate antitubercular agents as they have MICs values < 12.5 μg/mL. However, compounds **2, 5, 9** and **12** are good antituberculars as they have MICs in the range of 1.56–6.25 μg/mL. Among the hydroxy-(phenyl)-methyl cycloalkanones **18**-**21**, except compound **20** with nitro substituent at 2-position of the aromatic ring**,** other compounds of the series **18**, **19** and **21** with substituents (-NO_2 or -Br) either at 3- or 4-positions on the aromatic rings display moderate antitubercular activity against *M. tuberculosis* H37Rv with MIC 12.5 μg/mL while they were inactive against the avirulent strains (MIC >12.5 μg/mL). Further as the compounds did not display any significant activity against fungi or other bacteria, they may be specific to mycobacterium.

6. Conclusion

In conclusion, we have developed a simple, economical and efficient method for the synthesis of *bis*-benzylidene cycloalkanones and evaluated them for their antitubercular activity. Three of the compounds displayed MIC in the range of 6.25- 1.56 μg/mL, a criterion for further optimization of the series for new antitubercular agents.

7. Experimental

7.1. Chemistry

Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light and/or spraying a 20% $KMnO_4$ aq. soln. Column chromatography was performed on silica gel (60–120 mesh, E. Merck). IR spectra were recorded as thin films or in chloroform soln. with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 in $CDCl_3$. Chemical shift values are reported in ppm relative to $SiMe_4$ as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m (multiplet); J in hertz. FAB mass spectra were performed using a mass Spectrometer Jeol SX-102 and ESI mass spectra with Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

7.1.2. General experimental procedure for the preparation of α, α′-(*EE*) *bis*-(substituted-benzylidene)-cycloalkanones:

To a stirring solution of the cycloalkanone (1 mmol) and aromatic aldehyde (2 mmol) in minimum amount of ethanol, solid KOH (5 mol %) was added. The reaction mixture was stirred at ambient temperature till the disappearance of the starting materials (TLC). The solid separated after complete reaction was filtered and washed with water and dried. The product, so obtained, was crystallized with ethanol to give the desired compounds in good yields.

2,6-(*EE*) *Bis*-furan-2-yl-methylene-cyclohexanone (1):. IR (KBr): ν_{max} cm^{-1} 3452, 1777, 1593; MS (FAB): 255 $[M+H]^+$. ^1H NMR (300MHz, $CDCl_3$): 7.56-7.53 (d, J=7.1 Hz, 4H, olefinic protons and ArH), 6.66 (d, J=3.18 Hz, 2H, ArH), 6.51 (dd, J=1.68 and 1.56 Hz 2H, ArH), 3.05-2.93 (m, 4H, CH_2), 1.93-1.88 (m, 2H, CH_2). ^{13}C NMR (50MHz, $CDCl_3$): δ 188.4, 153.2, 144.5, 133.2, 123.6, 116.1, 112.5, 31.0, 30.1, 28.4 and 22.1. Anal. Calc. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55 %. Found: C, 75.54; H, 5.58 %.

2,7-(*EE*) *Bis*-(4-bromobenzylidene)-cycloheptanone (6): IR (KBr): ν_{max} cm^{-1} 3449, 1673, 1606; MS (FAB) : 447 $[M+H]^+$. ^1H NMR (300MHz, $CDCl_3$): δ 7.61-7.11 (m, 10H, olefinic protons and ArH), 2.73-2.67 (m, 4H, CH_2), 1.98-1.81 (m, 4H, CH_2). ^{13}C NMR (50MHz, $CDCl_3$): δ 204.0, 142.0, 134.6, 134.3, 131.7, 131.6, 130.9, 43.3, 31.3, 29.9, 28.7, 28.0, 27.7 and 25.4. Anal. Calc.for $C_{21}H_{18}O_1Br_2$: C, 56.53; H, 4.07 %. Found: C, 56.54; H, 4.09 %.

2,7-(*EE*) *Bis*-(4-chlorobenzylidene)-cycloheptanone (11): IR (KBr): ν_{max} cm^{-1} 3021, 1731, 1603; MS (FAB): 343$[M+H]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 7.40-7.19 (m, 10H, olefinic protons and ArH), 2.65 (m, 4H, CH_2), 1.97-1.95 (m, 4H, CH_2). ^{13}C NMR (50MHz, $CDCl_3$): δ 198.1, 142.1, 134.9, 134.6, 131.0, 129.1, 129.0, 43.6, 31.7, 29.1, 28.8 and 25.7. Anal. Calc.for $C_{21}H_{18}O_1Cl_2$: C, 70.60; H, 5.08 %. Found: C, 70.59; H, 5.10 %.

2,7-(*EE*) *Bis*-(4-methoxybenzylidene)-cycloheptanone (12): IR (KBr): ν_{max} cm^{-1} 3018, 1731, 1603; MS (FAB): 335$[M+H]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 7.45-7.26 (m, 6H, olefinic protons and ArH), 6.93-6.87 (m, 4H, ArH), 3.81 (s, 6H, OCH_3), 2.70 (m, 4H, CH_2), 1.97 (m, 4H, CH_2); ^{13}C NMR (50MHz, $CDCl_3$): δ 199.7, 160.0, 140.0, 135.8, 131.6, 129.0, 114.3, 55.5, 30.2, 28.8, 28.4 and 25.7. Anal. Calc.for $C_{23}H_{24}O_3$: C, 82.63; H, 7.18 %. Found: C, 82.68; H, 7.09 %.

2,7-(*EE*) *Bis*-(3,4-dimethoxybenzylidene)-cycloheptanone (13): IR (KBr): ν_{max} cm^{-1} 3010, 1695, 1596; MS (FAB) : 395$[M+H]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 7.29 (s, 2H olefinic protons), 7.05-6.83 (m, 6H, ArH), 3.89 (s, 12H, OCH_3), 2.71 (m, 4H, CH_2), 2.00 (m, 4H, CH_2). ^{13}C NMR (50MHz, $CDCl_3$): δ 198.1, 150.6, 149.2, 140.0, 135.9, 129.4, 122.9, 113.4, 111.4, 56.0, 29.1 and 28.6. Anal. Calc.for $C_{25}H_{28}O_5$: C, 73.51; H, 6.91 %. Found: C, 73.49; H, 6.93 %.

2,5-(*EE*) *Bis*-(3,4-dimethoxy-benzylidene)-cyclopentanone (14): IR (KBr): ν_{max} cm^{-1} 3020, 1730, 1595; MS (FAB) : 367$[M+H]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 7.50 (s, 2H, olefinic protons) 7.18-6.89 (m, 6H, ArH), 3.92 (s, 12H, OCH_3), 3.09 (m, 4H, CH_2). ^{13}C NMR (50MHz, $CDCl_3$): δ 196.0, 150.6, 149.3, 135.6, 134.0, 129.4, 124.9, 113.9, 111.5, 56.2, and 26.8 Anal. Calc.for $C_{25}H_{28}O_5$: C, 76.61; H, 6.36 %. Found: C, 76.61; H, 6.39 %.

7.1.3. General experimental procedure for the preparation of α, α′-*bis*(benzylidene)-cycloalkanols:

A solution of α,α'-(*EE*) *bis*(substituted-benzylidene)-cycloalkanones ((1mmol) and ethanol (…) was stirred at 0 °C for 10 minutes. $NaBH_4$ (1 equivalent) was slowly added and stirred at 30˚C till the disappearance of starting material (TLC). The reaction mixture was brought to 0°C and excess of $NaBH_4$ was quenched by saturated aq. solution of NH_4Cl and solid so obtained was filtetred. The solid cake was washed with ethanol, the combined filtrate was evaporated under reduced pressure to give a crude mass. The latter was dissolved in ethylacetate, organic layer was washed with water, dried (anhy. Na_2SO_4) and concentrated in vacuum to give a gummy mass. The latter was chromatographed over SiO_2 using a gradient of hexane: EtOAc as eluent to afford the pure products.

2,6-*Bis*-(4-bromobenzylidene)-cyclohexanol (16): IR (KBr): ν_{max} cm^{-1} 3021, 1724, 1596; MS (FAB): 417 $[M-OH]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 7.74-6.54 (s, 2H, olefinic protons and m, 8H, ArH), 4.64 (s, 1H, CHOH), 2.79-2.73 (m, 1H, CH), 2.40-2.33 (m, 4H, CH_2), 1.61 (m, 2H, CH_2). Anal. Calc. for $C_{20}H_{18}O_1Br_2$: C, 55.33; H, 4.18 %. Found: C, 55.30; H, 4.20 %.

2,6-*Bis*-(4-nitro-benzylidene)-cyclohexanol (17): IR (KBr): ν_{max} cm^{-1} 3213, 1656 and 1513; MS (FAB): 366.9 $[M+H]^+$. ^1H NMR (200MHz, $CDCl_3$): δ 8.21-8.16 (m, 4H, ArH), 7.37-7.33 (m, 6H, olefinic protons and ArH), 4.25 (s, 1H, CHOH), 2.79-2.72 (m, 1H, CH), 2.07-1.99 (m, 4H, CH_2), 1.67-1.58 (m, 2H, CH_2). Anal. Calc.for $C_{20}H_{18}N_2O$: C, 65.57; H, 4.95%. Found: C, 65.53; H, 4.99%.

8. Biological Activity:

8.1. Activity against *M. tuberculosis* H_{37}Ra Strain

All the synthesized compounds were evaluated for their efficacy against *M. tuberculosis* H_{37}Ra at active concentration ranging from 50μgmL^{-1} to MIC using two fold dilutions in the initial

screen. Log phase culture of *M. tuberculosis*H_{37} Ra is diluted so as to give final $OD_{550\,nm}$ of 0.05 in Sauton's medium. In 96 well white plate 190μL of culture is dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed to 96 well plates so as to make final test concentration 25μg ml^{-1} (5μg test compound is dispensed in 10μL of DMSO). Then the plate is incubated at 37 °C/5% CO_2 for 5 days. On 5th day 15μL Alamar blue solution is added to the each well of plate. The plate is again incubated overnight at 37 °C/5% CO_2 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 to 1.56μg ml^{-1}

8.2. Activity against M. tuberculosis H_{37}Rv strain

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H_{37}Rv was performed by agar micro dilution method where twofold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H_{37}Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 μg ml^{-1} concentration of extracts/compounds was prepared in 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 h followed by streaking of *M. tuberculosis* H_{37}Rv (5×10^4 bacilli per 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 H_{37}Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

8.3. Antifungal and Antibacterial activity

Minimum inhibitory concentration of compounds was tested according to standard micro broth dilution technique as per NCCLS guidelines in flat bottom 96 well tissue culture plates (CELLSTAR_ Greiner bio-one GmbH, Germany) in RPMI 1640 medium buffered with MOPS (3-[N-morpholino]propanesulfonic acid) (Sigma Chem. Co., MO, USA) for fungal strains and in Muller Hinton broth (Titan Biotech Ltd, India) for bacterial strains. The concentration ranges for

the tested compounds were 50–0.36 and 32–0.0018 mg/mL for standard compounds. Plates were incubated at 35^{o}C in a moist chamber (24 h for all the bacterial strains, 48 h for *C. albicans* and *C. parapsilosis*, 72 h for *Aspergillus fumigatus*, *S. schenckii*, and *Cryptococcus neoformans*, and 96 h for *Trichophyton mentagrophytes)*. MICs were determined as 90% inhibition of growth with respect to the growth control spectrophotometrically.

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