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ORIGINAL ARTICLE

Synthesis and *in vitro* microbiological evaluation of novel diethyl 6,6'-(1,4-phenylene)*bis*(4-aryl-2-oxo-cyclohex-3-enecarboxylates)

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Abstract

Novel *bis* cyclohexenone ester derivatives 14–19 were synthesized and characterized by their spectral data. *In vitro* microbiological evaluations were carried out for all the novel compounds 14–19 against clinically isolated bacterial and fungal strains. Compounds 15, 16, 18 against *Staphylococcus aureus*, 14, 15 against β-Haemolytic streptococcus, 15, 19 against *Micrococcus luteus*, 17, 18 against *Salmonella typhii*, 14, 17 against *Shigella flexneri*, 15 against *Escherichia coli*, 16 against *Pseudomonas aeruginosa*, 15, 18, 19 against *Klebsiella pneumonia* exhibited potent antibacterial activity at an minimum inhibitory concentration (MIC) value of 6.25 µg/ml, whereas compound 16 against *Aspergillus flavus*, 17 against *A. niger*, 16, 18 against *Mucor indicus*, 15, 17–19 against *Microsporum gypseum* revealed excellent antifungal activity at an MIC value of 6.25 µg/ml.

Keywords: bis cyclohexenone esters, Michael addition, cyclocondensation, antibacterial activity, antifungal activity

Introduction

Literature survey reveals the value of chalcones as potent biologically active compounds. An important feature of chalcones from the chemical point of view is the ability to act as activated unsaturated systems in conjugated addition reactions of carbanions in the presence of basic catalysts[1,2]. This type of reaction may be exploited for the preparation of 3,5-diaryl-6-carbethoxycyclohexenones via Michael addition of ethyl acetoacetate. The mentioned cyclohexenones are effective synthons in some projected synthesis of structurally diverse heterocycles[3–5]. The motive for the preparation of cyclic chalcones is because they are excellent carriers of different types of biological activity[6,7]. Cyclohexenone carboxylates have known to possess effective biological activity, such as anti-HIV[8,9], anticancer[10], antifungal[11,12], antitumor[13], anticonvulsant[14,15], and antitubercular[16] activity. Novel cyclohexenoic long chain fatty alcohols are used in the treatment of neurological disorders[17]. Ambuic acid, a highly functionalized cyclohexenone exhibits antifungal activity[18].

The synthesis of 6-acetyl-5-aryl-2-cyclohexenones, 5-aryl-6-carbethoxy-2-cyclohexenones substituted in position 4 with a 2-thienyl moiety, 5-aryl-6-carbethoxy-3-(2-thienyl)-2-cyclohexenones[19,20] is already described. In recent years, there has been a great deal of interest in exploiting more than one proximal functional group for designing novel structures capable of performing a variety of functions. In view of the above and as part of the ongoing research on antimicrobials[3,21–23], we planned to synthesize highly functionalized *bis* cyclohexenone ester derivatives, whose various chemical functions commend them as valuable intermediates in subsequent reactions and to study their biopotentiality against clinically isolated bacterial and fungal strains.

Experimental

Chemistry

The progress of the reaction is monitored by thin layer chromatography (TLC) analysis. All the reported melting

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points are taken in open capillaries and are uncorrected. IR spectra are recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer and important absorption values (cm^{-1}) alone are listed. ^1H and ^{13}C NMR spectra are recorded at 400 and 100 MHz, respectively, on Bruker Avance II 400 NMR spectrometer using DMSO-d_6 as solvent. Two-dimensional HOMOCOR and HSQC spectra are recorded at Bruker DRX 500 NMR spectrometer. The ESI +ve MS spectra are recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis data are obtained on Carlo Erba 1106 CHN analyzer.

By adopting the literature precedent[24], *bis* chalcone derivatives **8–13** are prepared.

General procedure for the synthesis of *bis* cyclohexenone ester derivatives 14–19

To a solution of sodium ethoxide (0.001 mol) in 30 ml of absolute ethanol, freshly distilled ethyl acetoacetate (0.01 mol) and respective *bis* chalcones 8–13 (0.01 mol) in absolute ethanol (40 ml) was mixed. This mixture was refluxed in a water bath for 3–6 h by maintaining the temperature around (70–80°C). The reaction mixture was allowed to cool and filtered. Then the crude product was recrystallized from absolute ethanol to afford *bis* cyclohexenone ester derivatives **14–19**.

Bis cyclohexenone ester **14:** IR (KBr) ν (cm^{-1}): 3052, 2980, 2924, 2854, 1663, 1738, 1607, 757, 694; ^1H NMR (δ ppm), (*J* Hz): 0.93 (6H, t, CH_2CH_3 at C-1, J=5.2), 2.99–2.95 (2H, H_{5a}, m), 3.14–3.00 (2H, H_{5a}, m), 3.68–3.61 (1H, H_6, m), 3.95–3.87 (4H, m, CH_2CH_3 at C-1), 4.11 (1H, H_1, d, J=13.6), 6.54 (1H, d, H_3, J=2.0), 7.72–7.37 (14H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.28 C-5, 43.46 C-6, 59.89 CH_2CH_3 at C-1, 58.67 C-1, 122.89 C-3, 159.29 C-4, 169.31 C=O at C-1, 194.27 C-2, 130.10-124.16 -$C_{arom.}$, 140.28, 139.86, 138.00, 137.32 *ipso*-Cs.

Bis cyclohexenone ester **15:** IR (KBr) ν (cm^{-1}): 3063, 2986, 2925, 1664, 1738, 1600, 832, 756; ^1H NMR (δ ppm), (*J* Hz): 0.92 (6H, t, CH_2CH_3 at C-1, J=7.2), 2.99–2.94 (2H, H_{5a}, m), 3.12–3.05 (2H, H_{5a}, m), 3.67–3.59 (1H, H_6, m), 3.95–3.85 (4H, m, CH_2CH_3 at C-1), 4.09 (1H, H_1, d, J=14.3), 6.52 (1H, s, H_3), 7.81–7.18 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.27 C-5, 43.39 C-6, 59.89 CH_2CH_3 at C-1, 58.59 C-1, 122.84 C-3, 158.07 C-4, 169.15 C=O at C-1, 194.19 C-2, 128.91-115.64 -$C_{arom.}$, 162.11, 140.25, 133.80, 129.00 *ipso*-C's.

Bis cyclohexenone ester **16:** IR (KBr) ν (cm^{-1}): 3030, 2977, 2926, 2854, 1658, 1738, 1601, 811, 756; ^1H NMR (δ ppm), (*J* Hz): 0.92 (6H, m, CH_2CH_3 at C-1, J=7.0), 2.33 (6H, s, CH_3 at phenyl rings), 2.99–2.95 (2H, H_{5a}, m), 3.13–3.04 (2H, H_{5a}, m), 3.68–3.57 (1H, H_6, m), 3.92–3.90 (4H, m, CH_2CH_3 at C-1), 4.09 (1H, H_1, d, J=14.3), 6.52 (1H, s, H_3), 7.63–7.24 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.30 CH_2CH_3 at C-1, 21.32, 21.34 CH_3 at phenyl rings, 35.66, 35.56 C-5, 43.96, 44.08 C-6, 60.40, 60.37 CH_2CH_3 at C-1, 59.17 C-1, 123.88, 122.55 C-3, 159.63, 158.93 C-4, 169.85, 169.73 C=O at C-1, 194.71 C-2, 129.93-126.78 -$C_{arom.}$, 143.02, 141.00, 140.83, 140.53, 135.54, 134.87 *ipso*-C's.

Bis cyclohexenone ester **17:** IR (KBr) ν (cm^{-1}): 3052, 2980, 2927, 1665, 1738, 1609, 825, 679; ^1H NMR (δ ppm), (*J* Hz): 0.92 (6H, t, CH_2CH_3 at C-1, J=7.0), 2.97-2.93 (2H, H_{5a}, m), 3.07–3.00 (2H, H_{5a}, m), 3.66–3.63 (1H, H_6, m), 3.93–3.87 (4H, m, CH_2CH_3 at C-1), 4.11 (1H, H_1, d, J=15.6), 6.56 (1H, d, H_3, J=2.0), 7.76–7.38 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.20 C-5, 43.35 C-6, 59.91 CH_2CH_3 at C-1, 58.60 C-1, 123.28 C-3, 157.89 C-4, 169.09 C=O at C-1, 194.21 C-2, 129.78–127.61 -$C_{arom.}$, 140.22, 136.17, 135.14 *ipso*-C's.

Bis cyclohexenone ester **18:** IR (KBr) ν (cm^{-1}): 3063, 2974, 2923, 2849, 1664, 1737, 1607, 825, 756; ^1H NMR (δ ppm), (*J* Hz): 0.85–0.97 (6H, m, CH_2CH_3 at C-1), 2.99–2.92 (2H, H_{5a}, m), 3.08–3.00 (2H, H_{5a}, m), 3.68–3.56 (1H, H_6, m), 3.94–3.86 (4H, m, CH_2CH_3 at C-1), 4.03 (1H, H_1, d, J=14.5), 6.58 (1H, d, H_3, J=3.0), 7.67–7.28 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.31 CH_2CH_3 at C-1, 35.44 C-5, 43.90 C-6, 60.51 CH_2CH_3 at C-1, 59.22 C-1, 123.78 C-3, 158.41 C-4, 169.82 C=O at C-1, 194.59 C-2, 128.23–124.46 -$C_{arom.}$, 137.04, 132.22, 129.07 *ipso*-C's.

Bis cyclohexenone ester **19:** IR (KBr) ν (cm^{-1}): 3030, 2958, 2924, 2850, 1653, 1737, 1601, 831, 756; ^1H NMR (δ ppm), (*J* Hz): 0.94 (6H, m, CH_2CH_3 at C-1, J=7.2), 3.02–2.94 (2H, H_{5a}, m), 3.09–3.03 (2H, H_{5a}, m), 3.64–3.60 (1H, H_6, m), 3.81 (6H, s, OCH_3 at phenyl rings), 3.92–3.90 (4H, m, CH_2CH_3 at C-1), 4.06 (1H, H_1, d, J=14.5), 6.51 (1H, d, H_3, J=1.5), 7.71–6.99 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.30 CH_2CH_3 at C-1, 35.40 C-5, 43.95 C-6, 60.34 CH_2CH_3 at C-1, 59.17 C-1, 55.85 OCH_3 at phenyl rings, 121.53 C-3, 159.11 C-4, 169.78 C=O at C-1, 194.50 C-2, 128.70-114.68 -$C_{arom.}$, 161.74, 140.88, 129.74 *ipso*-C's.

Microbiology

Materials

All the clinically isolated bacterial strains namely *Staphylococcus aureus*, β-Haemolytic streptococcus, *Micrococcus luteus*, *Bacillus subtilis*, *Salmonella typhii*, *Shigella felxneri*, *Vibreo cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and fungal strains namely *Aspergillus flavus*, *Aspergillus niger*, *Mucor indicus*, *Rhizopus arrhizus* and *Microsporum gypseum* are obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity by disc diffusion method

The *in vitro* activities of the compounds are tested in Sabourauds dextrose broth (SDB) (Hi-media, Mumbai) for fungi and nutrient broth (NB) (Hi-media, Mumbai) for bacteria by the disc diffusion method following the reported method[25]. The respective hydrochlorides of the test compounds **14–19** are dissolved in water to obtain 1 mg ml^{-1} stock solution and the different concentrations (100, 200, 500 ppm) were prepared from the stock solution. Seeded broth (broth containing microbial spores) is prepared in NB from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal

spores from 1- to 7-day-old Sabourauds agar (Hi-media, Mumbai) slant cultures are suspended in SDB. Sterile paper disc of 5 mm diameter is saturated with the three different concentrations and such discs are placed in each seeded agar plates. The petri plates are incubated in BOD incubator at 37°C for bacteria and at 28°C for fungi. The zone of inhibition is recorded by visual observations after 24 h of inhibition for bacteria and after 72–96 h of inhibition for fungi. Moreover, the zone of inhibition is measured by excluding the diameter of the paper disc. Ciprofloxacin is used as standards for bacteria and fluconazole as standard for fungi under analogous conditions.

In vitro antibacterial and antifungal activity by two-fold serial dilution method

Minimum inhibitory concentration (MIC) in μg/ml values is carried out by two-fold serial dilution method[26]. The respective test compounds (**14–19**) are dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg ml^{-1} stock solution. Seeded broth (broth containing microbial spores) is prepared in NB from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C, while fungal spores from 1- to 7-day-old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. The colony-forming units (cfu) of the seeded broth are determined by plating technique and adjusted in the range of 10^4–10^5 cfu/ml. The final inoculum size was 10^5cfu/ml for antibacterial assay and 1.1–1.5 × 10^2 cfu/ml for antifungal assay. Testing is performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH of 5.6 for fungi (SDB). Exactly 0.4 ml of the solution of test compound was added to 1.6 ml of seeded broth to form the first dilution. One millilitre of this is diluted with a further 1 ml of seeded broth to give the second dilution and so on till six such dilutions are obtained. A set of assay tubes containing only seeded broth is kept as control. The tubes are incubated in BOD incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The MICs are recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin is used as standard for bacterial studies and fluconazole is used as standard for fungal studies.

Results and discussion

Chemistry

The conventional approach for the synthesis of target molecules diethyl 6,6'-(1,4-phenylene)*bis*(4-aryl-2-oxocyclohex-3-enecarboxylates) **14–19** are as follows: *Bis* chalcones **8–13** are synthesized by the Claisen-Schmidt condensation of terephthaldehyde **1** and respective substituted acetophenones **2–7** in the presence of alcoholic sodium hydroxide. Treatment of *bis* chalcones **8–13** with ethyl acetoacetate in the presence of sodium ethoxide in refluxing ethanol (Scheme 1 and Table 1) afford target molecules, *bis* cyclohexanone ester derivatives **14–19**. The structures of all the synthesized *bis* cyclohexanone ester derivatives **14–19** are confirmed by FT-IR, MS, ^{1}H NMR, and ^{13}C NMR spectral studies and elemental analysis. Moreover, compound **16** is also characterized by two-dimensional ^{1}H-^{1}H HOMOCOR and ^{1}H-^{13}C HSQC spectral studies. The reaction mechanism involves the formation of Michael addition product by ethyl acetoacetate with *bis* chalcones **8–13** in the presence of base, sodium ethoxide. Later, the addition product undergoes intramolecular aldol reaction in the presence of sodium ethoxide base to give the title compounds **14–19**.

To discuss the spectral data of the synthesized compounds, methyl substituted compound **16** is chosen as the representative compound.

Analysis of FT-IR spectrum of diethyl 6,6'-(1,4-phenylene)*bis*(2-oxo-4-p-tolylcyclohex-3-enecarboxylate) 16

FT-IR spectrum of diethyl 6,6'-(1,4-phenylene)*bis*(2-oxo-4-*p*-tolylcyclohex-3-enecarboxylate) **16** shows two strong characteristic absorptions at 1738 and 1658 cm^{-1} are due to ester carbonyl and ketone functional groups, respectively. The band at 1601 cm^{-1} is due to the presence of C=C stretching frequency. The absorption frequency at 3030 and 2977 cm^{-1} is assigned to aromatic C-H stretching vibration and the absorption frequencies at 2926 and 2854 cm^{-1} is assigned to aliphatic C-H stretching vibration. The observed ester carbonyl, ketone, and C=C stretching vibrational bands are supporting evidence for the formation of synthesized compound **16**.

Analysis of ^{1}H NMR spectrum of *bis* cyclohexenone ester 16

In the ^{1}H NMR spectrum of **16**, a triplet observed at 0.92 ppm (J=7.0 Hz) corresponding to six protons and this signal is due to ester methyl protons at C-1. A multiplet observed at 3.92–3.89 ppm corresponding to four protons, and this signal is due to ester methylene protons at C-1. Three multiplets are obtained in the range 2.99–2.95, 3.13–3.04, and 3.68–3.57 ppm and they are due to H-5a, H-5e, and H-6 protons. The doublet at 4.09 ppm (J=14.3 Hz) has been assigned to H-1 proton. The singlet observed in downfield region at 6.52 ppm is due to H-3 proton. The aromatic protons appeared as a multiplet in the range 7.63–7.24 ppm.

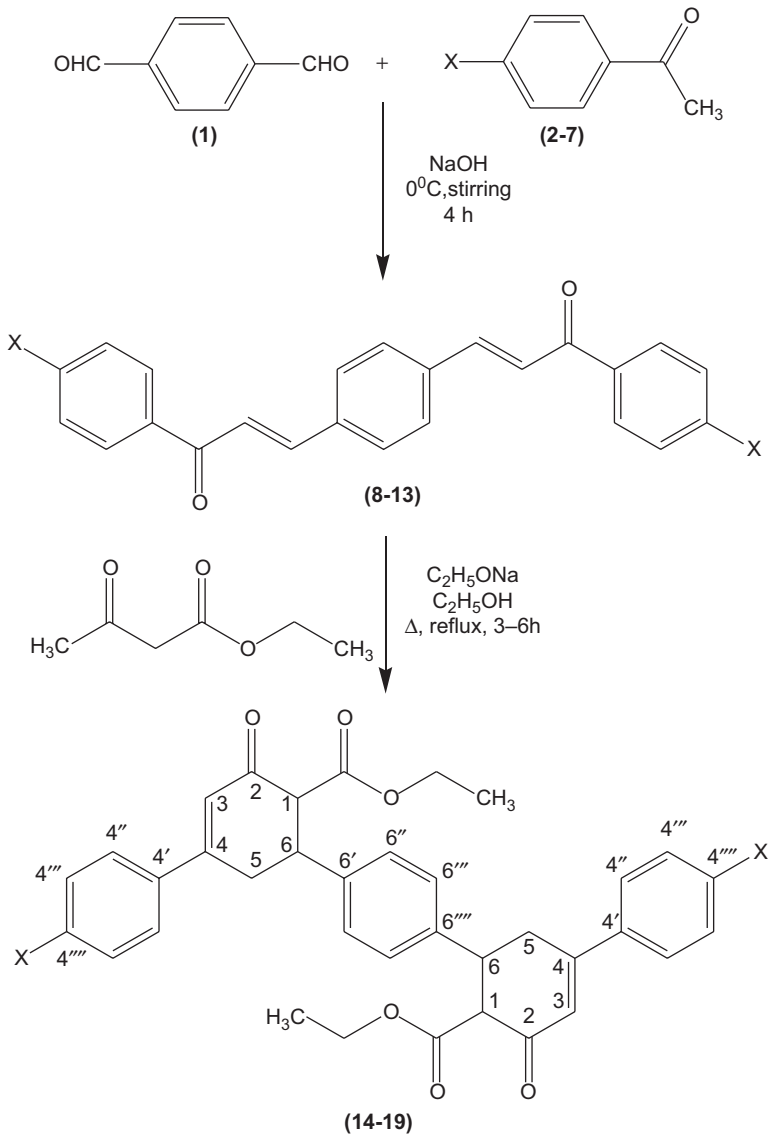
Analysis of ^{13}C NMR spectrum of *bis* cyclohexenone ester 16

The ^{13}C resonances at 194.71 ppm are assigned to C-2 carbonyl carbon, whereas two resonances observed at 169.85/169.73 ppm are assigned to ester carbonyl carbons. The ^{13}C resonances at 35.66/35.56 and 43.96/44.08 ppm are due to the C-5 and C-6 carbons, respectively. The ^{13}C resonance observed at 60.40/60.37 and 14.30 ppm is assigned to ester methylene and methyl carbons at C-1, respectively. The signal observed at 59.17 ppm is assigned to C-1 carbon, whereas the carbon signal resonates at 123.88/122.55 ppm is assigned to C-3 carbon. The aromatic carbons are observed in the range of 129.93–126.78 ppm. C-4 carbon resonates at 157.89 ppm.

The remaining ^{13}C signals at 143.02, 141.00, 140.83, 140.53, 135.54, 134.87 ppm are due to *ipso* carbons.

Analysis of ^{1}H-^{1}H COSY spectrum of *bis* cyclohexenone ester 16

In the HOMOCOSY spectrum of **16**, the signal at 4.09 ppm shows correlation with the signal at 3.68–3.57 ppm. Similarly, the signal at 3.68–3.57 ppm shows correlation with the signal at 4.09 ppm as well as with the signal at 2.99–2.95 and 3.13–3.04 ppm. Likewise, the signal at 2.99–2.95 ppm shows correlation with the signals at 3.13–3.04 and 3.68–3.57 ppm. Moreover, the signal at 3.13–3.04 ppm shows correlation with the signals at 6.52 ppm, 2.99–2.95 and 3.68–3.57 ppm. Also, the signal at 6.52 ppm shows correlations with the signal at 3.13–3.04 ppm. From the observed correlation, it reveals that three multiplets observed in the range 2.99–2.95, 3.13–3.04, and 3.68–3.57 ppm due to H-5a, H-5e, and



Scheme 1. Synthesis of novel *bis* cyclohexenone ester derivatives.

Table 1. Physical and analytical data of compounds 14–19.

Compounds	X	Reflux timeΔ (h)	Yield (%)	m.p. (0C)	Elemental analysis (%) CFound (calculated)	HFound (calculated)	m/z (M)+. Molecular formula
14	H	5	82	62	76.71 (76.85)	5.99 (6.09)	562C36H34O6
15	F	3	86	90	72.14 (72.23)	5.31 (5.39)	598C36H32F2O6
16	CH3	6	90	222	77.12 (77.26)	6.38 (6.48)	590C38H38O6
17	Cl	4	80	72	68.31 (68.47)	4.98 (5.11)	630C36H32Cl2O6
18	Br	3	78	128	59.87 (60.02)	4.37 (4.48)	718C36H32Br2O6
19	OCH3	6	85	186	73.11 (73.29)	6.03 (6.15)	622C38H38O8

H-6 protons, whereas the doublet at 4.09 ppm and the singlet at 6.52 ppm are assigned to H-1 and H-3 proton. One multiplet at 3.92–3.89 shows correlation with the triplet at 0.92 ppm and vice versa. These mutual correlations reveal that the triplet observed at 0.92 ppm is due to ester methyl protons at C-1 and multiplet observed at 3.92–3.89 ppm is due to ester methylene protons at C-1. The methyl protons at aromatic ring observed at 2.33 ppm show correlation with the aromatic protons observed in the range 7.63–7.24 ppm. All the ^{1}H-^{1}H COSY correlations for compound **16** is given in Table 2.

Analysis of ^{1}H-^{13}C HSQC spectrum of *bis* cyclohexenone ester 16

In the HSQC spectrum of **16**, one bond correlation (14.3/0.92 ppm) is observed between ester methyl carbon at C-1 and ester methyl proton at C-1. The two ^{13}C resonances at 60.4 and 60.3 ppm have correlation with the proton signal around 3.92–3.89 ppm, which is obviously due to ester methylene protons at C-1. Another one bond correlation (35.5 & 35.6/32.99–2.95 & 3.13–3.04 ppm) is observed between C-5 and H-5a & H-5e. The ^{13}C resonance at 43.9 & 44.0 ppm has one bond correlation with a multiplet around 3.68–3.57 ppm. Hence, the signal at 43.9 & 44.0 ppm corresponds to C-6 carbon, whereas the multiplet at 3.68–3.57 ppm is assigned to H-6 proton.

Another aliphatic carbon, which resonances at 59.1 ppm, shows one bond correlation with a doublet at 4.09 ppm. From this correlation, it is revealed that the doublet at 4.09 ppm corresponds to H-1 proton of the cyclohexenone moiety and the ^{13}C signal at 59.1 ppm is assigned to C-1 carbon. The ^{13}C resonance at 123.8/122.5 ppm has correlations with singlet at 6.52 ppm. So the signal at 6.52 ppm is conveniently assigned to H-3 proton and the carbon signal at 123.8/122.5 ppm is assigned to C-3. In the HSQC, the ^{13}C resonances at 159.6/158.9, 169.8/169.7, and 194.7 ppm have no correlations with protons and hence it is due to quaternary carbon C-4, ester C=O at C-1 and C-2 carbons, respectively. Among the quaternary carbons, the ^{13}C resonances at 143.02, 141.00, 140.83, 140.53, 135.54, 134.87 ppm are due to *ipso* carbons. All the ^{1}H-^{13}C HSQC correlations for compound **16** is given in Table 3. Therefore with reference to ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC correlations in compound **16**, the tentative assignments made for the protons and carbons are confirmed. Based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC correlations of **16**, the ^{1}H and ^{13}C chemical shifts of **16** are assigned unambiguously.

Antibacterial activity of novel *bis* cyclohexenone ester derivatives 14–19 by disc diffusion method

A series of novel *bis* cyclohexanone ester derivatives **14–19** are tested for their antimicrobial activity by disc diffusion method against tested bacterial strains. From the zone of inhibitions, it is inferred that compound **14**, which have no substitution at the phenyl rings show good zone of inhibition activity against *β-H. streptococcus*, *M. luteus*, *and S. flexneri* whereas it shows moderate zone of inhibition activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *K. pneumonia* and shows poor activity against *V. cholerae* even at a very higher concentration of 500 ppm. Compound **15**, which has electron withdrawing fluoro substituent at the *para* position of the two phenyl rings, exhibits excellent zone of inhibition antibacterial activity against all the tested bacterial strains and shows moderate activity against *S. typhii*. It shows poor zone of inhibition activity against *S. flexneri* even at a very higher concentration of 500 ppm. Compound **16**, which has electron donating methyl substituent at the *para* position of the two phenyl rings, possesses good zone of inhibition antibacterial activity against *S. aureus*, *β-H. streptococcus*, and *P. aeruginosa* whereas it possesses modest activity against bacterial strains namely *M. luteus, B. subtilis, S. flexneri, V. cholerae*, and *E. coli*. Compound **17,** which has electron withdrawing chloro functional group present at the *para* positions of the two phenyl rings, exerts moderate zone of inhibition activity against *S. aureus, β-H. streptococcus*, and *B. subtilis* and exhibits good activities against *S. typhii, S. flexneri, V. cholerae, E. coli, P. aeruginosa*. Electron withdrawing bulky bromo substituted compound **18** reveals good antibacterial activity against *S. aureus, M. luteus, B. subtilis, S. typhii,* and *K. pneumonia,* and exhibits moderate activity against *β-H. streptococcus, S. flexneri* and *V. cholerae*. Moreover, it exerts very poor zone of inhibition activity against *E. coli* even at a very higher concentration of 500 ppm. Electron

Table 2. 1H-1H COSY correlations for 16.

		H-1	H-3	H-5a	H-5e	H-6	CH2 at ester	CH3 at ester	Ar-CH3	Aromatic protons
Proton	ppm	4.09	6.52	2.99–2.95	3.13–3.04	3.68–3.57	3.92–3.89	0.92	2.33	7.63–7.24
H-1	4.09					Coupled				
H-3	6.52				Coupled					
H-5a	2.99–2.95				Coupled	Coupled				
H-5e	3.13–3.04		Coupled	Coupled		Coupled				
H-6	3.68–3.57	Coupled		Coupled	Coupled					
CH2 at ester	3.92–3.89							Coupled		
CH3 at ester	0.92						Coupled			
Ar-CH3	2.33									Coupled
Aromatic protons	7.63–7.24								Coupled	Coupled

donating methoxy substituted compound **19** possesses good zone of inhibition antibacterial activity against *S. aureus*, *β-H. streptococcus, M. luteus*, and *K. pneumonia*, whereas it reveals moderate activity against *S. typhii, S. flexneri, E. coli*, and *P. aeruginosa*.

Antibacterial activity of novel *bis* cyclohexenone ester derivatives 14–19 by two-fold serial dilution method

In vitro antibacterial activity results by two-fold serial dilution method of novel *bis* cyclohexanone ester derivatives **14–19** is shown in Table 4. Ciprofloxacin is used as standard drug. All the tested compounds **14–19** exhibit good antibacterial activity at an MIC value range of 200–6.25 μg/ml. But Compound **14** against *V. cholerae,* **15** against *S. flexneri*, **18** against *E. coli* do not show any activity even at a maximum concentration of 200 μg/ml. Compound **14** exhibits excellent antibacterial activity against *β-H. streptococcus* and *S. flexneri* at an MIC value of 6.25 μg/ml, whereas it shows good activity against *M. luteus* at an MIC value of 12.5 μg/ml. Compound **15** exhibits excellent antibacterial activity against *S. aureus, β-H. streptococcus, M. luteus, E. coli,* and *K. pneumonia* at an MIC value of 6.25 μg/ml, whereas it shows promising activity against *B. subtilis, V. cholerae*, and *P. aeruginosa* at an MIC value of 12.5 μg/ml. Compound **16** against *S. aureus* and *P. aeruginosa* displays superior activity at an MIC value of 6.25 μg/ml, whereas it reveals fine activities against *β-H. streptococcus* at an MIC value of 12.5 μg/ml. Compound **17** displays good antibacterial activity against *S. typhii* and *S. flexneri* at an MIC value of 6.25 μg/ml and shows good activities against *V. cholerae, E. coli,* and *P. aeruginosa* at an MIC value of 12.5 μg/ml. Poor antibacterial activity at an MIC value of 200 μg/ml is noted against *K. pneumonia* by compound **17**. Compound **18** exhibits good microbial activity against *S. aureus, S. typhii,* and *K. pneumonia* at an MIC value of 6.25 μg/ml, whereas it reveals fine activities against *M. luteus* and *B. subtilis* at an MIC value of 12.5 μg/ml. Likewise, compound **19** against *S. aureus* and *β-H. streptococcus* shows activity at an MIC value of 12.5 μg/ml, whereas excellent antibacterial activity is shown against *M. luteus* and *K. pneumonia* at an MIC value of 6.25 μg/ml. Electron withdrawing

Table 3. H-13C HSQC correlations for 16.

	Carbon	C-1	C-2	C-3	C-4	C-5	C-6	CH2 at ester	CH3 at ester	Ester C=O	Ar-CH3	Aromatic carbons	Ipso carbons
Proton	ppm	59.1	194.7	123.8, 122.5	159.6, 158.9	35.5, 35.6	43.9, 44.0	60.4, 60.3	14.3	169.8, 169.7	21.32, 21.34	129.9–126.7	143.0, 141.0, 140.8, 140.5, 135.5, 134.8
H-1	4.09	Bonded											
H-3	6.52			Bonded									
H-5a	2.99–2.95					Bonded							
H-5e	3.13–3.04					Bonded							
H-6	3.68–3.57						Bonded						
CH2 at ester	3.92–3.89							Bonded					
CH3 at ester	0.92								Bonded				
Aromatic CH3	2.33										Bonded		
Aromatic protons	7.63–7.24											Bonded	

Table 4. *In vitro* antibacterial activities of compounds 14–19 by two-fold serial dilution method.

	Minimum inhibitory concentration (MIC) in μg/ml						
Microorganisms	14	15	16	17	18	19	Ciprofloxacin
Staphylococcus aureus	100	6.25	6.25	25	6.25	12.5	25
β-Hemolytic streptococcus	6.25	6.25	12.5	25	25	12.5	25
Micrococcus luteus	12.5	6.25	25	100	12.5	6.25	12.5
Bacillus subtilis	50	12.5	25	25	12.5	100	12.5
Salmonella typhii	100	25	100	6.25	6.25	25	25
Shigella flexneri	6.25	—	25	6.25	25	50	12.5
Vibreo cholerae	—	12.5	25	12.5	50	100	25
Escherichia coli	50	6.25	50	12.5	—	50	25
Pseudomonas aeruginosa	25	12.5	6.25	12.5	100	50	25
Klebsiella pneumonia	25	6.25	25	200	6.25	6.25	12.5

'—' No activity even at a maximum concentration of 200 μg/ml.

substituted fluoro compound **15** against *S. aureus, β-H. streptococcus, M. luteus, E. coli, K. pneumonia, M. gypseum,* chloro substituted compound **17** against *S. typhii, S. flexneri, A. niger, M. gypseum,* bulky bromo substituted compound **18** against *S. aureus, S. typhii, K. pneumonia, M. indicus, M. gypseum* exhibit excellent antibacterial activity at an MIC value of 6.25 μg/ml. Compound **15** against *B. subtilis, V. cholerae, P. aeruginosa*, **17** against *V. cholerae, E. coli, P. aeruginosa*, **18** against *M. luteus* and *B. subtilis* exhibits good antibacterial activity at an MIC value of 12.5 μg/ml.

Antifungal activity of novel *bis* cyclohexenone ester derivatives 14–19 by disc diffusion method

Novel *bis* cyclohexanone ester derivatives **14–19** are tested for their antifungal activity by disc diffusion method against tested fungal strains. From the zone of inhibitions, it is inferred that compound **14** shows good zone of inhibition activity against *A. niger,* whereas it exhibits moderate activity against *A. flavus* and *M. gypseum*. Compound **14** against *R. arrhizus* reveals poor activity even at a higher concentration of 500 ppm. Compound **15** exhibits fine zone of inhibition activity against *M. gypseum,* whereas it shows modest activity against *A. flavus, A. niger*, and *M. indicus*. Excellent antifungal zone of inhibition is noted for compound **16** against the fungal strains *A. flavus* and *M. indicus*, whereas it exhibits moderate activity against *A. niger* and *R. arrhizus*. Compound **17** displays superior zone of inhibition activity against *A. flavus A. niger,* and *M. gypseum* and displays moderate activity against *M. indicus*. Compound **18** possesses superior zone of inhibitions activity against *M. indicus* and *M. gypseum* and displays modest zone of inhibition activity against *A. flavus, A. niger,* and *R. arrhizus*. Compound **19** displays excellent antifungal zone of inhibition activity against *A. flavus, A. niger,* and *M. gypseum* and exerts moderate activity against *M. indicus*.

Antifungal activity of novel *bis* cyclohexenone ester derivatives 14–19 by two-fold serial dilution method

In vitro antifungal activity results by two-fold serial dilution method (Table 5) of novel *bis* cyclohexanone ester derivatives **14–19** show that compound **14** displays admirable activities against *A. niger* at an MIC value of 12.5 μg/ml, whereas it displays low activity against *A. flavus* and *M. gypseum* at an MIC value of 50 μg/ml. Fluconazole is used as a standard drug. Compound **15** displays good activity against *M. gypseum* at an MIC value of 6.25 μg/ml and possesses modest activity against *A. niger* and *M. indicus* at an MIC value of 25 μg/ml. It displays low activity against *A. flavus* at an MIC value of 50 μg/ml. Compound **16** exhibits excellent antifungal activity against *A. flavus* and *M. indicus* at an MIC value of 6.25 μg/ml and displays poor activity against *M. gypseum* at an MIC value of 200 μg/ml. Compound **17** displays fine activity against *A. flavus* at an MIC value of 12.5 μg/ml, whereas it shows superior activity against *A. niger* and *M. gypseum* at an MIC value of 6.25 μg/ml. Excellent antifungal activity is noted against *M. indicus* and *M. gypseum* by compound **18** at an MIC value of 6.25 μg/ml. Compound **19** displays good activity against *A. flavus* and *A. niger* at an MIC value of 12.5 μg/ml and displays excellent antifungal activity against *M. gypseum* at an MIC value of 6.25 μg/ml. From the above observed antifungal activity, it is known that electron withdrawing substituted compound **15** against *M. gypseum,* compound **17** against *A. niger, M. gypseum,* compound **18** against *M. indicus, M. gypseum,* exhibit excellent antifungal activity at an MIC value of 6.25 μg/ml, whereas compound **17** against *A. flavus* reveals good antifungal activity at an MIC value of 12.5 μg/ml.

Conclusion

Synthesis and their spectral characterization of novel diethyl 6,6'-(1,4-phenylene)*bis*(4-aryl-2-oxo-cyclohex-3-enecarboxylates), a new *bis* cyclohexanone ester derivative is described. This reaction may have wide applicability in building a variety of heterocycles by choosing *bis* cyclohexanone esters as synthon, which has three versatile functional groups, that is ketone, olefin, and ester for the synthesis of structurally diverse organic compounds. The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the synthesized novel *bis* cyclohexanone ester derivatives **14–19** are clearly known from Tables 4 and 5. Compound **14** against *β-H. streptococcus, S. flexneri,* compound **15** against *S. aureus, β-H. streptococcus, M. luteus, E. coli, K. pneumonia, M. gypseum,* compound **16** against *S. aureus, P. aeruginosa, A. flavus, M. indicus,* compound **17** against *S. typhii, S. flexneri, A. niger, M. gypseum,* compound **18** against *S. aureus, S. typhii, K. pneumonia, M. indicus, M. gypseum,* compound **19** against *M. luteus, K. pneumonia,* and *M. gypseum* exhibits excellent antimicrobial activity at an MIC value of 6.25 μg/ml. A close inspection of the *in vitro* antibacterial and antifungal activity profile

Table 5. *In vitro* antifungal activities of compounds 14–19 by two-fold serial dilution method.

	Minimum inhibitory concentration (MIC) in μg/ml						
Microorganisms	14	15	16	17	18	19	Fluconazole
Aspergillus flavus	50	50	6.25	12.5	50	12.5	12.5
Aspergillus niger	12.5	25	25	6.25	50	12.5	12.5
Mucor indicus	100	25	6.25	25	6.25	25	25
Rhizopus arrhizus	—	100	25	100	25	100	25
Microsporum gypseum	50	6.25	200	6.25	6.25	6.25	12.5

—, No activity even at a maximum concentration of 200 μg/ml.

in differently electron donating (CH_3 and OCH_3) and electron withdrawing –F, -Cl, Br) functional group substituted phenyl rings of novel target compounds exerted strong antibacterial and antifungal activity against all the tested bacterial strains. Among the all tested compounds, electron withdrawing substituted compounds **15**, **17**, and **18** exerted potent antimicrobial activity, since electron withdrawing substitutent increases the lipophilicity due to the strong electron withdrawing capability[27]. To improve metabolic stability, bioavailability, and protein ligand interactions[28], fluorine substitution is commonly used in contemporary medicinal chemistry. The methods of action of these compounds were unknown. These observations may promote a further development of our research in this field. Further development of this group of cyclohexanone ester derivatives may lead to compounds with better pharmacological profile than standard antibacterial and antifungal drugs.

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