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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SOME NOVEL α, β -UNSATURATED KETONES

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Abstract
In the present work we synthesized some novel α, β-unsaturated ketones with an aim to obtain some new potent antibacterial and antioxidant agents. The α, β-unsaturated ketones were synthesized by base catalysed Claisen-Schmidt condensation of ketones having α, α' hydrogens with various aldehydes. Three new series of α, β-unsaturated ketones were synthesized by reacting γ-*t*-butylcyclohexanone, γ-phenylcyclohexanone and γ-ethylcyclohexanone with different substituted benzaldehydes. The classes of the synthesized compounds are: α,α'-*Bis*(substitutedarylidene)-γ-*t*-butylcyclohexanones[1a-j],α,α' B*is*(substitutedarylidene)-γ-phenylcyclohexanones [2a-h], The formation of the compounds [1a-j] and [2a-h] are indicated by their UV spectra. The functional groups in the compounds [1a-j] and [2a-h] are characterized by their IR spectra. The number and positions of protons in the compounds [1a-j] and [2a-h] are confirmed by their ^{1}H-NMR spectra. The structures of compounds [1a-j] and [2a-h] are confirmed by their Mass spectra. The structures of the compounds [1a-j] and [2a-h] are further confirmed by their elemental analysis. All the synthesized compounds were screened for their *in vitro* antibacterial properties against human pathogenic Gram positive and gram negative bacteria. Ampicillin was used as the standard. The observations reveal that α,α'-*Bis*(2,4-dichlorobenzylidene)- γ-phenylcyclohexanone [2b] shows highest activity against *Bacillus subtilis*. α,α' -*Bis*(2,3-dichlorobenzylidene)-γ-*t*-butylcyclohexanone [1a] is most active against *Klebsiella pneumonia.* All the synthesized compounds were also screened for their antioxidant activity using ascorbic acid as the standard by DPPH method. The observations show that α,α'-*Bis*(2,4-dimethoxybenzylidene)-γ-phenylcyclohexanone [2d] exhibits highest antioxidant activity at an EC_{50} of 505 µg/mL.

Keywords: *chalcones, antioxidant activity, α, β-unsaturated ketones*

INTRODUCTION

The search for 'better medicines for a better world' is a never-ending process to help the suffering mankind from dreadful and fatal ailments. The process of new drug discovery is driven by the requirement to synthesize novel molecules having good potential with high therapeutic index[1].

α, β-Unsaturated ketones also known as chalcones have recently attracted the attention of many medicinal chemists owing to the ease of their synthesis and wide array of pharmaceutical and medicinal applications. Chalcones are abundant in the plant kingdom[2]. They are considered to be the precursors of flavanoids and isoflavanoids. Chemically they consist of two aromatic rings joined by a three carbon α, β-unsaturated carbonyl system. Synthesis of chalcones is generally accomplished by a simple base catalysed Claisen-Schmidt condensation of a ketone and a suitably substituted aldehyde.

It is now well known that most natural and synthetic chalcones have shown extensive pharmacological activities such as antiprotozoal, antifungal, anti-inflammatory, antileishmanial, nitric oxide inhibition, inhibition of the production of the interleukin-1, anticancer, antibacterial and antioxidant[3]. Keeping in view these diverse therapeutic activities, it was contemplated to synthesize a novel series of chalcones. In the present work attention has been focused on the synthesis of chalcones with different ketone moieties and their antibacterial and antioxidant properties.

Antibacterial Activity

Antimicrobial drugs are the greatest contribution of 20th century to therapeutics. They are one of the few curative drugs. Their importance is magnified in the developing countries, where infective diseases predominate.

The modern era of chemotherapy was ushered by Domagk in 1935 by demonstrating the therapeutic effect of *Prontosil*, a sulphonamide dye, in pyogenic infections[4]. Since then numerous classes of antibacterial agents have been discovered, and literally hundreds of drugs are available for use today.

With the first successful clinical trial of crude Penicillin (1941) and the requirements of the war time, an explosion of successful activity ensued which continues over 50 years later. In the rapid succession deliberate searches of the metabolic products of a wide variety of soil microbes led to the discovery of streptomycin(1943), chloramphenicol (1947),chlortetracycline (1948), neomycin (1949), erythromycin (1952) and more and this resulted in the age of the miracle drugs[5]. Though thousands of antimicrobial agents are available, the infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. The impact is more acute in the developing countries due to non availability of the desired medicines and emergence of widespread drug resistance.

Staphylococcus aureus was reported in 2003[6]. *Enterococcus faecium* is another superbug found in hospitals. Penicillin-resistant *Enterococcus* was seen in 1983, Vancomycin-resistant *Enterococcus* (VRE) in 1987, and Linezolid-resistant *Enterococcus* (LRE) in the late 1990s[6]. Resistance of *Streptococcus pneumoniae* to penicillin and other beta-lactams is increasing worldwide[6]. In November 2004, the Center for Disease Control and Prevention (CDC) reported an increasing number of *Acinetobacter baumannii* bloodstream infections in patients at military medical facilities. Most of these showed multidrug resistance, with a few isolates resistant to all drugs tested[6].

Until recently, research and development (R&D) efforts have provided new drugs in time to treat bacteria that became resistant to older antibiotics. That is no longer the case. The potential crisis at hand is the result of a marked decrease in industry R&D, and the increasing prevalence of resistant bacteria. Physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics[6].

Literature quotes a variety of α, β-unsaturated ketones with various substitutions to have been found active against a number of bacteria, hence these present a potential source to develop the much needed more efficacious antibacterial agents.

Antioxidant Activity

WHO defines health positively as "a state of complete physical, mental, and social well-being and not merely the absence of disease." Foods high in antioxidants are reliably associated with human health and well being[7].

Chronic diseases like diabetes, heart disease, arthritis, cognition diseases and cancer have been linked to "oxidation" of "cellular molecules" such as proteins, lipids and DNA. This is where damaging modified oxygen molecules attach to molecules in cells and cause damage and inflammation. This is why "antioxidants" have received so much media attention because they help to prevent or remove the damaging oxygen molecules from interacting with cellular molecules before they cause damage and lead to disease[8].

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves [6].

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells[6].

The importance of Antioxidants
Free Radicals

In the most scientific of understandings, the body runs on a series of daily, complex, chemical reactions. Our stomach breaks down food. This is used by the various organs to power cellular regeneration. This process occurs in the mitochondria in a series of complex chemical reactions able to occur because of enzymes, a protein or a protinaceous substance. As a byproduct to this key process, known as oxidative metabolism, the mitochondria produce potentially damaging reactive oxygen species[9].

Oxygen molecules are generally found in pairs and with a full set of outer electrons strongly bond together. However, these reactive oxygen species such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite has a weak set of bonds, which emits unstable free electron particles, or free radicals. In an attempt to gain stability, free radicals react quickly with surrounding compounds, often "stealing" an electron from the nearest stable molecule. As this displaces the electron of the attacked molecule becomes a free radical starting a process which can cascade to the fatal disruption and damage to a living cell[9].

Reactions that generate these species are exemplified as follows[10]:

$$O_2 + QH \longrightarrow O_{2^{\cdot}} + Q^{\cdot} + 2H^+$$

(where Q is quinone, found in ubiquinone, or COQ)

$$O_2 + FADH_2 \qquad H_2O_2 + FAD$$

$$H_2O_2 + Fe^{++} \qquad 2HO^{\cdot} + Fe^{+++}$$

MATERIAL AND METHODS

Chemicals and Reagents:

The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-media, Lancaster, Loba, Merck, NR chem. Qualigens, Rolex, Reachchem, S.d– Fine Chem. Ltd, and Sigma.

Analytical Techniques
Physical data

Melting points of the synthesized compounds were determined using Thiele's melting point apparatus and were uncorrected.
Thin Layer Chromotography (TLC)

Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and various combinations of ethyl acetate: cyclohexane as mobile phase. The spots resolved were visualized as brown colored spots by using iodine chamber.

Instrumentation
The techniques employed for the characterization of the synthesized compounds were UV spectra, IR spectra, ^{1}H-NMR spectra, Mass spectra and Elemental analysis.
UV Spectra

The UV spectra of the synthesized compounds were recorded on UV–Visible spectrophotometer (model Shimadzu 8700) using chloroform and the values of wave length (λ max) are reported in nm.
Infrared Spectra

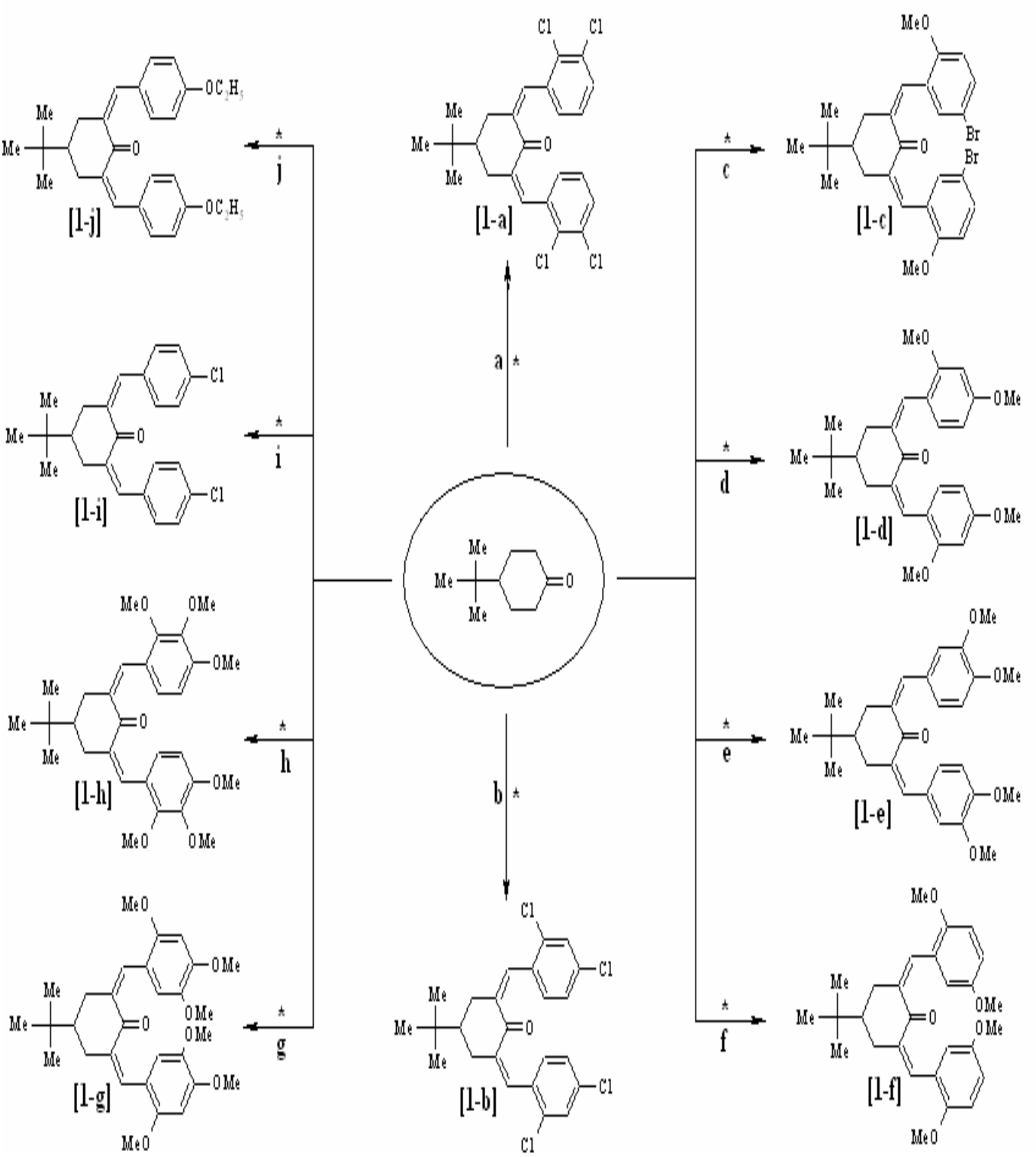
The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr and the values of ν_{max} are reported in cm^{-1}.
^{1}H-NMR Spectra

^{1}HNMR spectra were recorded on Amx-400 MHz NMR spectrometer and Amx-100 MHz NMR spectrometer using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethyl silane (TMS).
Mass Spectra
Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by LC-MS.

Elemental Analysis
Elemental analysis was performed, and the reports were obtained on Thermo Finnigan flash EA 1112 CHNS analyzer.

SCHEME 1:

Synthesis of α,α'-*Bis*(substitutedarylidene)-γ-*t*-butylcyclohexanones[1a-j]



a) **2,3-$Cl_2C_6H_3CHO$** (b) **2,4-$Cl_2C_6H_3CHO$** (c) **5-Br-2-$OCH_3C_6H_3CHO$** d) **2,4-$(OCH_3)_2C_6H_3CHO$** (e) **3,4-$(OCH_3)_2C_6H_3CHO$** (f) **2,5-$(OCH_3)_2C_6H_3CHO$** (g) **2,4,5-$(OCH_3)_3C_6H_2CHO$** (h) **2,3,4-$(OCH_3)_3C_6H_2CHO$** (i) ***p*-ClC_6H_4CHO** (j) ***p*-$OC^2H^5C_6H_4CHO$**
*** EtOH/ OH^-/ 10-20°C**

α,α' -*Bis*(2,3-dichlorobenzylidene)-γ-*t*-butylcyclohexanone [1a].
An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,3-dichloro benzaldehyde (3.5 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.178-179 ^{0}C, was 83%.
λ_{max} (nm): 310.0, **IR spectral data ν_{max} (cm^{-1}) :**2956(Alp C-H str), 1155(C-C str), 1666(C=O str), 3087(Ar C-H str), 1577, 1590(Ar C=C str), 759(C-Cl str). **^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.5-1.7, 2.2-2.4 & 2.8-3.0(m, CH_2CHCH_2, **5H**), 7.1-7.3 & 7.4-7.6(m, ArH, **6H**), 7.8(s, 2 x Methine H, **2H**). **Mass spectral data (M+1): 469, Eemental analysis: %C** 61.53 (61.52), % H 4.70 (5.05)
α,α'-*Bis*(2,4-dichlorobenzylidene)-γ-*t*-butylcyclohexanone [1b].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4-dichlorobenzaldehyde (3.5 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.196-197 ^{0}C, was 83%.
λ_{max} (nm): 321.0, **IR spectral data ν_{max} (cm^{-1}) :** 2958(Alp C-H str), 1149(C-C str), 1675(C=O), 3068(Ar C-H str), 1548, 1600(Ar C=C str), 730(C-Cl str)**^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.6, 2.2-2.4 & 2.7-3.0(m, CH_2CHCH_2, **5H**), 7.0-7.2 & 7.3-7.5(m, ArH, **6H**), 7.8(s, 2 x Methine H, **2H**), **Mass spectral data (M+1): 469, Elemental analysis: %C** 61.53 (61.42), % H 4.70 (5.05)
α,α'-*Bis*(5-bromo-2-methoxybenzylidene)-γ-*t*-butylcyclohexanone [1c].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 5-bromo-2-methoxybenzaldehyde (4.3 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g,mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.138-139 ^{0}C, was 82%.

λ_{max} (nm): 350.0, **IR spectral data ν_{max} (cm^{-1}) :** 2956(Alp C-H str), 1174(C-C str), 1660(C=O str), 3050(Ar C-H str),1450, 1479(Ar C=C str), 1251(C-O str)) **^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.7, 2.2-2.5 & 2.9-3.1(m, CH_2CHCH_2, **5H**), 3.8 (s, 2 x OMe, **6H**), 6.7-6.9(s, *o*-ArH, **2H**), 7.3-7.5(d, ArH, **4H**), 7.8(s, 2 x Methine H, **2H**) . **Mass spectral data (M+1):** 549**, Eemental analysis: %C** 56.93 (56.79), % H 5.10 (5.30) α,α'-*Bis*(2,4-dimethoxybenzylidene)-γ-*t*-butylcyclohexanone [1d].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.190-191 ^{0}C, was 87%.

λ_{max} (nm): 365.0, **IR spectral data ν_{max} (cm^{-1}) :** 2960(Alp C-H str), 1157(C-C str), 1658(C=O str), 3050(Ar C-H str),1463, 1480(Ar C=C str), 1241(C=O str)**^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.6, 2.3-2.4 &3.0-3.2(m, CH_2CHCH_2, **5H**), 3.8(s, 4 x OMe, **12H**), 6.3-6.6 & 7.2-7.4(m, ArH, **6H**), 7.9(s, 2 x Methine H, **2H**). **Mass spectral data (M+1):** 451**, Eemental analysis: %C** 74.66 (74.59), % H 7.55 (7.80)

α,α'-*Bis*(3,4-dimethoxybenzylidene)-γ-*t*-butylcyclohexanone [1e]. An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 3,4-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.118-119 ^{0}C, was 82%.

λ_{max} (nm): 310.0, **IR spectral data ν_{max} (cm^{-1}) :** 2950(Alp C-H str), 1143(C-C str), 1656(C=O str), 1430, 1465(Ar C=C str), 1255(C-O str)**^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.6, 2.3-2.5 & 3.1-3.3(m, CH_2CHCH_2, **5H**), 3.9 (s, 4 x OMe, **12H**), 6.8-7.2(m, ArH, **6H**), 7.7(s, 2 x Methine H, **2H**), **Mass spectral data (M+1):** 451**, Elemental analysis: %C** 74.66 (74.46), % H 7.55 (7.88) **α,α'-*Bis*(2,5-dimethoxybenzylidene)-γ-*t*-butylcyclohexanone [1f].**

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,5-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.119-120 ^{0}C, was 83%.

λ_{max} (nm): 371.5, **IR spectral data ν_{max} (cm^{-1}) :** 2958(Alp C-H str), 1049(C-C str), 1658(C=O str), 3050 (Ar C-H str), 1400, 1450, 1490(Ar C=C str), 1222(C-O str) **^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.6, 2.3-2.5 & 2.9-3.1(m, CH_2CHCH_2, **5H**), 3.9 (s, 6 x OMe, **18H**), 6.4-6.6 & 6.8-7.0(m, ArH, **4H**), 8.0(s, 2 x Methine H, **2H**) **Mass spectral data (M+1):** 451**, Elemental analysis: %C** 74.66 (74.65), % H 7.55 (7.84)
α,α'-*Bis*(2,4,5-trimethoxybenzylidene)-γ-*t*-butylcyclohexanone [1g].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4,5-trimethoxybenzaldehyde (3.9 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.160-161^{0}C, was 75%.

λ_{max} (nm): 391.5, **IR spectral data ν_{max} (cm^{-1}) :** 2956(Alp C-H str), 1150(C-C str), 1660(C=O str), 1480, 1509(Ar C=C str), 1211(C-O str)**^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.4-1.6, 2.4-2.6 & 3.0-3.2(m, CH_2CHCH_2, **5H**), 7.3-7.4(m, ArH, **8H**), 7.8(s, 2 x Methine H, **2H**), **Mass spectral data (M+1):** 511**, Elemental analysis: %C** 70.58 (70.37), % H 7.45 (7.67)
α,α'-*Bis*(2,3,4-trimethoxybenzylidene)-γ-*t*-butylcyclohexanone [1h].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,3,4-trimethoxybenzaldehyde (3.9 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.122-123^{0}C , was 84%.
λ_{max} (nm): 391.0, **IR spectral data ν_{max} (cm^{-1}) :**2956(Alp C-H str), 1155(C-C str), 1666(C=O str), 3087(Ar C-H str), 1577, 1590(Ar C=C str), 759(C-Cl str). **^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.5-1.7, 2.2-2.4 & 2.8-3.0(m, CH_2CHCH_2, **5H**), 7.1-7.3 & 7.4-7.6(m, ArH, **6H**), 7.8(s, 2 x Methine H, **2H**). **Mass spectral data (M+1):** 511**, Elemental analysis: %C** 70.58 (70.46), % H 7.45 (7.67)

α,α'-*Bis*(*p*-chlorobenzylidene)-γ-*t*butylcyclohexanone [1i]
An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of *p*-chlorobenzaldehyde (2.8 g, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice- cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.186-187^{0}C, is 90%.

λ_{max} (nm): 356.5, **IR spectral data ν_{max} (cm^{-1}) :** 2971(Alp C-H str), 1114(C-C str), 1670(C=O str), 1570, 1598(Ar C=C str), 1251(C-O str)**^{1}H NMR spectral data (δ):** 0.9(s, *t*-Butyl H, **9H**), 1.5-1.7, 2.2-2.4 & 2.8-3.0(m, CH_2CHCH_2, **5H**), 7.1-7.3 & 7.4-7.6(m, ArH, **6H**), 7.8(s, 2 x Methine H, **2H**). **Mass spectral data (M+1):** 399**, Elemental analysis: %C** 72.18 (72.07), % H 6.01 (6.29)

α,α'-*Bis*(*p*-ethoxybenzylidene)-γ-*t*-butylcyclohexanone [1j]. An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of *p*-ethoxybenzaldehyde (3.0 mL, 0.02 mol) and γ-*t*-butylcyclohexanone (1.5 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.166-167 ^{0}C, was 88%.

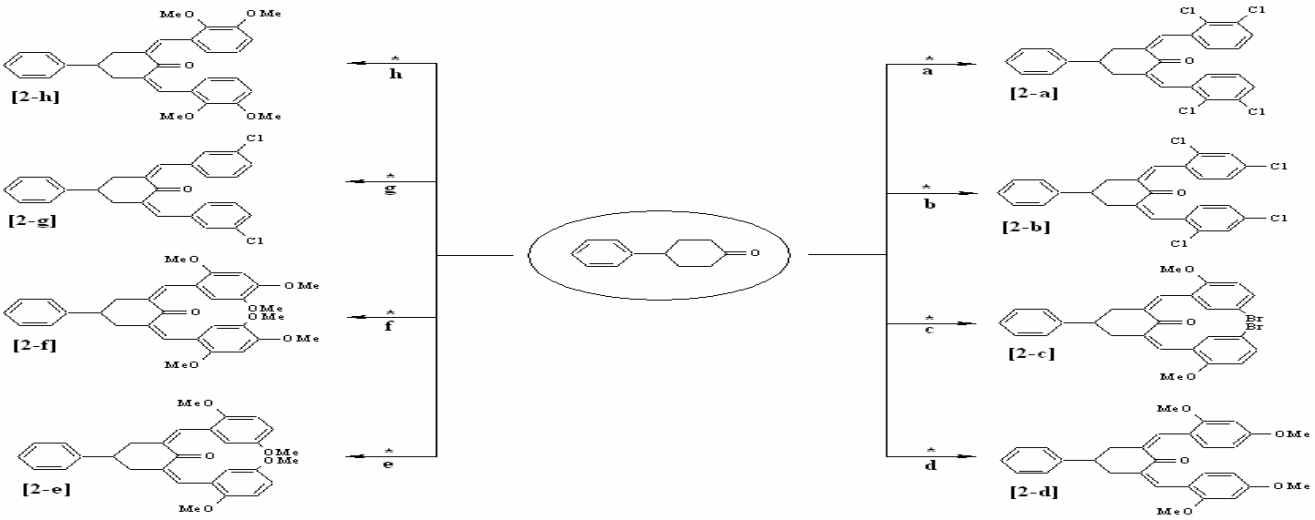
λ_{max} (nm): 333.5, **IR spectral data ν_{max} (cm^{-1}) :** 2950(Alp C-H str), 1101(C-C str), 1633(C=O str), 3108(Ar C-H str), 1550, 1536(Ar C=C str), 765(C-Cl str)**^{1}H NMR spectral data (δ):** 0.6(s, *t*-Butyl H, **9H**), 1.3-1.7, 2.2-2.5 & 2.9-3.0(m, CH_2CHCH_2, **5H**), 7.2-7.3 & 7.4-7.6(m, ArH, **6H**), 7.8(s, 2 x Methine H, **2H**). **Mass spectral data (M+1):** 399**, Elemental analysis: %C** 80.38 (80.06), % H 8.13 (8.43)

Table 1: Characteristics of α,α'-*bis*(arylidene)-γ-*t*-butylcyclohexanones [1a-j]

Compound No.	Ar	Mol. formula	Mol. wt	TLC solvent system(x:y)*	R_f
[1a]	2,3-$Cl_2C_6H_3$	$C_{24}H_{22}OCl_4$	468	5:1	0.92
[1b]	2,4-$Cl_2C_6H_3$	$C_{24}H_{22}OCl_4$	468	6:1	0.81
[1c]	5-Br-2-OMeC_6H_3	$C_{26}H_{28}O_3Br_2$	548	6:1	0.65
[1d]	2,4-$(OMe)_2C_6H_3$	$C_{28}H_{34}O_5$	450	4:1	0.63
[1e]	3,4-$(OMe)_2C_6H_3$	$C_{28}H_{34}O_5$	450	4:1	0.31
[1f]	2,5-$(OMe)_2C_6H_3$	$C_{28}H_{34}O_5$	450	4:1	0.28
[1g]	2,4,5-$(OMe)_3C_6H_2$	$C_{30}H_{38}O_7$	510	5:5	0.29
[1h]	2,3,4-$(OMe)_3C_6H_2$	$C_{30}H_{38}O_7$	510	4:1	0.24
[1i]	*p*-ClC_6H_4	$C_{24}H_{24}OCl_2$	399	4:1	0.87
[1j]	*p*-$OC_2H_5C_6H_4$	$C_{28}H_{34}O_3$	418	6:1	0.93

*** (x:y) = Cyclohexane : Ethyl acetate**

SCHEME 2: Synthesis of α,α' B*is*(substitutedarylidene)-γ-phenylcyclohexanones [2a-h]



a) 2,3-$Cl_2C_6H_3CHO$ **b)** 2,4-$Cl_2C_6H_3CHO$ **c)** 5-Br-2-$OCH_3C_6H_3CHO$ **d)** 2,4-$(OCH_3)_2C_6H_3CHO$
e) 2,5-$(OCH_3)_2C_6H_3CHO$ **f)** 2,4,5-$(OCH_3)_3C_6H_2CHO$ **g)** *m*-ClC_6H_4CHO **h)** 2,3-$(OCH_3)_2C_6H_3CHO$

* EtOH/ OH^-/ 10-20°C

***α,α'-Bis*(2,3-dichlorobenzylidene)- γ-phenylcyclohexanone [2a].**

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,3-dichlorobenzaldehyde (3.5 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by

ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.91-92 ^{0}C, was 82%.

λ_{max} (nm): 314.5, **IR spectral data ν_{max} (cm^{-1}) :** 908(Alp C-H str), 1178(C-C str), 1673(C=O str), 3098(Ar C-H str), 1550, 1594(Ar C=C str), 763(C-Cl), **^{1}H NMR spectral data (δ):** 2.7-3.2(m, CH_2CHCH_2, **5H**), 7.0-7.4 (m, ArH, **11H**), 7.9 (s, 2 x Methine H, **2H**), **Mass spectral data (M+1):** 481**, Elemental analysis: %C** 63.93 (64.06), % H 3.68 (3.72)

α,α'-*Bis*(2,4-dichlorobenzylidene)-γ-phenylcyclohexanone [2b].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4-dichlorobenzaldehyde (3.5 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.119-120 ^{0}C, was 80%.

λ_{max} (nm): 318.5, **IR spectral data ν_{max} (cm^{-1}) :** 1143(C-C str), 1664(C=O str), 3064(Ar C-H str), 1430, 1465, 1500(Ar C=C str), 775(C-Cl str), **^{1}H NMR spectral data (δ):** 2.6-3.0(m, CH_2CHCH_2, **5H**), 6.9-7.3(m, ArH, **9H**), 7.4-7.6(s, *m*-ArH, **2H**), 7.9(s, 2 x Methine H, **2H**)

Mass spectral data (M+1): 481**, Elemental analysis: %C** 63.93 (64.00), % H 3.68 (3.79)

α,α'-*Bis*(5-bromo-2-methoxybenzylidene)-γ-phenylcyclohexanone [2c].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 5-bromo-2-methoxybenzaldehyde (4.3 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.140-141 ^{0}C, was 77%.

λ_{max} (nm): 346.5, **IR spectral data ν_{max} (cm^{-1}) :** 2938(Alp C-H str), 1147(C-C str), 1660(C=O str), 3029(Ar C-H str), 1460, 1481(Ar C=C str), 1253(C-O str), **^{1}H NMR spectral data (δ**2.7-3.1(m, CH_2CHCH_2, **5H**), 3.8 (s, 2 x OMe, **6H**), 6.8-7.0(s, *m*-ArH, **2H**), 7.3-7.5(m, ArH, **9H**), 7.9(s, 2 x Methine H, **2H**)

Mass spectral data (M+1): 569**, Elemental analysis: %C** 76.59 (76.53), % H 6.38 (6.40)

α,α'-*Bis*(2,4-dimethoxybenzylidene)-γ-phenylcyclohexanone [2d].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.127-128 ^{0}C, was 79%.

λ_{max} (nm): 333.5, **IR spectral data ν_{max} (cm^{-1}) :** 2967(Alp C-H str), 1153(C-C str), 1658(C=O str), 3006(Ar C-H str), 1460, 1470,1498(Ar C=C str), 1211(C-O str), **^{1}H NMR spectral data (δ):** 2.8-2.9 & 3.0-3.1(m, CH_2CHCH_2,**5H**), 3.7 (s, 4 x OMe, **12H**), 6.3-6.5 &7.0-7.1(m, ArH, **11H**), 7.9(s, 2 x Methine H, **2H**). **Mass spectral data (M+1):** 471**, Elemental analysis: %C** 76.59 (76.50), % H 6.38 (6.44)

α,α'-*Bis*(2,5-dimethoxybenzylidene)-γ-phenylcyclohexanone [2e].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,5-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.122-123 ^{0}C, was 75%.

λ_{max} (nm): 372.5, **IR spectral data ν_{max} (cm^{-1}) :** 2992(Alp C-H str), 1155(C-C str), 1656(C=O str), 3054(Ar C-H str), 1470, 1492(Ar C=C str), 1286(C-O str), **^{1}H NMR spectral data (δ):** 2.8-3.0 & 3.2-3.4(m, CH_2CHCH_2,**5H**), 3.8 (s, 4 x OMe, **12H**), 6.8-7.0 & 7.2-7.4(m, ArH, **11H**), 8.0(s, 2 x Methine H, **2H**)

Mass spectral data (M+1): 471**, Elemental analysis: %C** 72.45 (72.51), % H 6.41 (6.50)

α,α'-*Bis*(2,4,5-trimethoxybenzylidene)-γ-phenylcyclohexanone [2f].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,4,5-trimethoxybenzaldehyde (3.9 g, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.115-116 ^{0}C, was 78%.

λ_{max} (nm): 404.5, **IR spectral data ν_{max} (cm^{-1}) :** 2996(Alp C-H str), 1147(C-C str), 1650(C=O str), 1467, 1506(Ar

C=C str), 1274(C-O str), **^{1}H NMR spectral data (δ):** 2.8-3.0 & 3.1-3.2(m, CH_2CHCH_2, **5H**), 3.9(s, 6 x OMe, **18H**), 6.5-6.8(s, ArH, **4H**), 7.1-7.4(m, ArH, **5H**), 8.0(s, 2 x Methine H, **2H**), **Mass spectral data (M+1):** 531**, Elemental analysis: %C** 74.46 (74.48)% H 4.77 (4.87)

α,α'-*Bis*(*m*-chlorobenzylidene)- γ-phenylcyclohexanone [2g].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of *m*-chlorobenzaldehyde (2.8 mL, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.127-128 ^{0}C, was 75%.

λ_{max} (nm): 329.0, **IR spectral data ν_{max} (cm^{-1}) :** 2950(Alp C-H str), 1193(C-C str), 1662(C=O str), 3060(Ar C-H str), 1577, 1590(Ar C=C str), 763(C-Cl str),**^{1}H NMR spectral data (δ):** 2.9-3.1 & 3.1-3.4(m, CH_2CHCH_2,**5H**), 7.0-7.4(m, ArH, **13H**), 7.8(s, 2 x Methine H, **2H**)

Mass spectral data (M+1): 419**, Elemental analysis: %C** 76.59 (76.52), % H 8.13 (8.43)

α,α'-*Bis*(2,3-dimethoxybenzylidene)- γ-phenylcyclohexanone [2h].

An aqueous solution of sodium hydroxide (10% w/v, 30 mL) was added to a solution of 2,3-dimethoxybenzaldehyde (3.3 mL, 0.02 mol) and γ-phenylcyclohexanone (1.7 g, 0.01 mol) in ethanol (50 mL). The reaction mixture was stirred at 10-20 ^{0}C for 2 hr and left overnight in an ice chest to get a yellow colored solid. The product was filtered, washed with ice-cold water (100 mL) followed by ice-cold ethanol (20 mL), dried and recrystallized from alcohol. The yield of product, m.p.99-100 ^{0}C, was 71 %.

λ_{max} (nm): 325.0, **IR spectral data ν_{max} (cm^{-1}) :** 2944(Alp C-H str), 1149(C-C str), 1666(C=O str), 1427, 1471(Ar C=C str), 1274(C-O str)**^{1}H NMR spectral data (δ):** 2.8-3.0 & 3.0-3.2(m, CH_2CHCH_2, **5H**), 3.8 (s, 4 x OMe, **12H**), 6.9-7.2 & 7.4-7.6(m, ArH, **11H**), 8.1(s, 2 x Methine H, **2H**)

. **Mass spectral data (M+1):** 471**, Elemental analysis: %C** 80.38 (80.06), % H 6.38 (6.4

Table 2: Characteristics of α, α'-*bis*(arylidene)-γ-phenylcyclohexanones 2a-h].

Compound No.	Ar	Mol. formulae	Mol. wt	TLC solvent system (x:y)*	R_f
[2a]	2,3-$Cl_2C_6H_3$	$C_{26}H_{18}OCl_4$	488	4:1	0.88
[2b]	2,4-$Cl_2C_6H_3$	$C_{26}H_{18}OCl_4$	488	4:1	0.85
[2c]	5-Br-2-OMeC_6H_3	$C_{28}H_{24}O_3Br_2$	568	4:1	0.71
[2d]	2,4-$(OMe)_2C_6H_3$	$C_{30}H_{30}O_5$	470	4:1	0.15
[2e]	2,5-$(OMe)_2C_6H_3$	$C_{30}H_{30}O_5$	470	4:1	0.30
[2f]	2,4,5-$(OMe)_3C_6H_2$	$C_{32}H_{34}O_7$	530	1:1	0.30
[2g]	*m*-ClC_6H_4	$C_{26}H_{20}OCl_2$	419	2:1	0.77
[2h]	2,3-$(OMe)_2C_6H_3$	$C_{30}H_{30}O_5$	470	2:1	0.38

*** (x:y) = Cyclohexane : Ethyl acetate**

Antibacterial activity

A volume of 25 ml of sterile hot agar medium was poured in each plate and allowed to harden on a level surface. The agar plates were inoculated with 24 hr test cultures by spreading uniformly with sterile cotton swabs. The plates were then allowed to dry in the inverted position in an incubator for 30 min. Afterwards they were removed and bores were made on the medium using sterile borer. A volume of 0.1 mL of test solution was added to the respective bores. Ampicillin at a concentration of 100 □g/ 0.1 mL was taken as standard reference. A control having only DMF in the cup was maintained in each plate. The petri plates were kept in the refrigerator at 4 ^{0}C for 15 min for diffusion to take place. Afterwards they were incubated at 37 ^{0}C for 24 hr and zones of inhibition were observed and measured using a scale. Each experiment was carried out in triplicate and the mean diameter of inhibition zone was recorded. .The various antibacterial results of **[1a-j]** & **[2a- h]** are shown in the Table 3 , 4 respectively.

Table 3: Antibacterial activities of α, α'-*bis*(substitutedarylidene)-γ-*t*-butylcyclohexanones [1a-j]

Compound No.	Ar	Antibacterial activity (zone of inhibition in mm)			
		S.a	*B.s*	*E c*	*K.p*
[**1a**]	2,3-$Cl_2C_6H_3$	11	13	11	22
[**1b**]	2,4-$Cl_2C_6H_3$	18	14	20	18
[**1c**]	5-Br-2-$OMeC_6H_3$	12	15	12	10
[**1d**]	2,4-$(OMe)_2C_6H_3$	13	11	15	20
[**1e**]	3,4-$(OMe)_2C_6H_3$	11	10	13	18
[**1f**]	2,5-$(OMe)_2C_6H_3$	10	12	10	12
[**1g**]	2,4,5-$(OMe)_3C_6H_2$	14	14	14	13
[**1h**]	2,3,4-$(OMe)_3C_6H_2$	15	14	19	19
[**1i**]	*p*-ClC_6H_4	16	14	19	19
[**1j**]	*p*-$OC_2H_5C_6H_4$	15	14	15	15
Std	Ampicillin	31	32	30	29

***S.a**: Staphylococcus aureus, **B.s**: Bacillus subtilis, **E.c**: Escherichia coli, **K.p**: Klebsiella pneumoniae*

Table 4: Antibacterial activities of α,α'-*bis*(substitutedarylidene)-γ-phenylcyclohexanones [2a-j]

Compound No.	Ar	Antibacterial activity (zone of inhibition in mm)			
		S.a	*B.s*	*E.c*	*K.p*
[**2a**]	2,3-$Cl_2C_6H_3$	11	19	17	21
[**2b**]	2,4-$Cl_2C_6H_3$	16	23	24	21
[**2c**]	5-Br-2-$OMeC_6H_3$	13	14	10	12
[**2d**]	2,4-$(OMe)_2C_6H_3$	17	18	15	17
[**2e**]	2,5-$(OMe)_2C_6H_3$	13	11	16	17
[**2f**]	2,4,5-$(OMe)_3C_6H_2$	15	15	15	18
[**2g**]	*m*-ClC_6H_4	17	18	18	18
[**2h**]	2,3-$(OMe)_2C_6H_3$	16	12	21	14
Std	Ampicillin	30	33	31	31

***S.a**: Staphylococcus aureus, **B.s:** Bacillus subtilis, **E.c**: Escherichia coli,**K.p**: Klebsiella pneumoniae*

Antioxidant Activity

Working Procedure:

D PPPH solution

A working solution of DPPH having an absorbance of 0.9 at 516 nm was used. This was Prepared by taking 95 µL of stock solution containing 12.9 mg of DPPH in 10 mL of methanol.

Standard solution

Ascorbic acid was used as a standard free radical scavenger. This was prepared by dissolving 50 mg of ascorbic acid in 50 mL of methanol.

Test solution

Test solutions of the compounds (10mg/10mL) were prepared by dissolving them in 1 mL DMSO and volume was made to 10 mL with methanol.

Procedure

To 95 µL DPPH solution in methanol, different concentrations of ascorbic acid were added, and the volumes were made up to 4 mL with methanol. DPPH diluted to 4 mL was taken as blank. Decrease of absorbance in the presence of ascorbic acid was noted down after 15 minutes. Linear regression was applied for concentration and percentage inhibition and EC_{50} was calculated. To the different concentrations of test solutions (0.4, 0.8, 1.2, 1.6 mL), 95 µL of DPPH solution was added and volume made up to 4 mL with methanol. Decrease in absorbance of DPPH was noted after 15 minutes. Linear regression was

applied for concentration and percentage inhibition and EC_{50} was calculated from graph.

Table 5: Antioxidant activities of α, α'-*bis* (substitutedarylidene)-γ-*t*-butylcyclohexanones [1a-j]

Compound No.	Ar	Antioxidant activity (EC_{50} in µg/mL)
[**1a**]	2,3-$Cl_2C_6H_3$	579
[**1b**]	2,4-$Cl_2C_6H_3$	544
[**1c**]	5-Br-2-$OMeC_6H_3$	577
[**1d**]	2,4-$(OMe)_2C_6H_3$	705
[**1e**]	3,4-$(OMe)_2C_6H_3$	540
[**1f**]	2,5-$(OMe)_2C_6H_3$	605
[**1g**]	2,4,5-$(OMe)_3C_6H_2$	518
[**1h**]	2,3,4-$(OMe)_3C_6H_2$	558
[**1i**]	*p*-ClC_6H_4	525
[**1j**]	*p*-$OC_2H_5C_6H_4$	587
Std	Ascorbic acid	63

Table 6: Antioxidant activities of α, α'-*bis* (substitutedarylidene)-γ-phenylcyclohexanones [2a-j]

Compound No.	Ar	Antioxidant activity (EC_{50} in µg/mL)
[**2a**]	2,3-$Cl_2C_6H_3$	597
[**2b**]	2,4-$Cl_2C_6H_3$	590
[**2c**]	5-Br-2-$OMeC_6H_3$	828
[**2d**]	2,4-$(OMe)_2C_6H_3$	505
[**2e**]	2,5-$(OMe)_2C_6H_3$	798
[**2f**]	2,4,5-$(OMe)_3C_6H_2$	524
[**2g**]	*m*-ClC_6H_4	657
[**2h**]	2,3-$(OMe)_2C_6H_3$	880
Std	Ascorbic acid	63

RESULT AND DISCUSSION:

The formation of α,α'-*bis*(2,3-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1a]** from **[1]** has been indicated by its UV spectrum. The substrate **[1]** exhibited λ_{max} at 289 nm. The compound **[1a]** exhibited λ_{max} at 310 nm. This change in λ_{max} indicates that the bathochromic shift which is due to $C{=}CHC_6H_5$ chromophores at α and α ′positions. Similarly, the formations of other cyclohexanones **[1b-j]** have been indicated by their UV spectra. The formation of α,α'-*bis*(2,4-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1b]** from **[1]** has been indicated by its IR spectrum. The substrate **[1]** exhibited ν_{max} due to carbonyl group (C=O) at 1715 cm^{-1}. The compound **[1b]** exhibited ν_{max} due to carbonyl group (C=O) at 1675 cm^{-1}. The appearance of a band at 1675 cm^{-1} is mainly due to the presence of C=CH chromophores at α and α ′positions. This indicates the formation of **[1b]**. Similarly, the formations of other compounds **[1a]** and **[1c-j]** have been indicated by their IR spectra. The structure of α,α'-*bis*(5-bromo-2-methoxybenzylidene)-γ-*t*-butylcyclohexanone **[1c]** has been confirmed by its ^{1}H NMR spectrum. The presence of signals at δ 0.9(s, *t*-butyl H, **9H**); 1.4-1.7, 2.2-2.5, 2.9-3.1(m, CH_2CHCH_2, **5H**); 3.8 (s, 2 x OMe, 6H); 6.7-6.9(s, *o*-ArH, **2H**); 7.3-7.5(m, ArH, **4H**); 7.8(s, 2 x methine H, **2H**) establish the structure of [**1c**]. Similarly the numbers of protons in other compounds **[1a-1b]** and **[1d-1j]** have been confirmed by their ^{1}H NMR spectra.The structure of α,α'-*bis*(2,4-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1b]** is also confirmed by its mass spectrum. The molecular ion peak of **[1b]** by LCMS in the positive mode has been observed at 469, which is in good agreement with the calculated molecular weight. The compound **[1b]** also has shown an additional peak at 477 which proves the isotopic nature of 4 chlorine atoms. Similarly, the structures of other compounds **[1a]** and **[1b-j]** have also been confirmed by their mass spectra. The structures of all the compounds **[1a-j]** have been further confirmed by their elemental analysis and the found values are with in ± 0.3 ٪ of required values.

The formation of α,α'-*bis*(2,4-dimethoxybenzylidene)-γ-phenylcyclohexanone [**2d**] from **(b)** has been indicated by its UV spectrum. The substrate **[2]** exhibited λ_{max} at 285 nm. The compound **[2d]** exhibited λ_{max} at 376 nm. This change in λ_{max} indicates that bathochromic shift is due to $C{=}CHC_6H_5$ chromophores at α and α ′positions. Similarly, the formations of other cyclohexanones **[2a-2c]** and **[2e-2h]** have been indicated by their UV spectra. The formation of α,α'-*bis*(2,5-dimethoxybenzylidene)-γ-phenylcyclohexanone **[2e]** from **(b)** has been indicated by its IR spectrum. The substrate **[2]** exhibited ν_{max} due to carbonyl group (C=O) at 1715 cm^{-1}. The compound **[2e]** exhibited ν_{max} at 1656 cm^{-1} which is due to carbonyl group (C=O). The appearance of a band at 1656 cm^{-1} is mainly due to the presence of C=CH chromophores at α and α ′ positions. This indicates the formations of **[2e]**. Similarly, the formation of other compounds **[2a-2d]** and **[2f-2h]** have been indicated by their IR spectra. The structure of α,α'-*bis*(2,4,5-trimethoxybenzylidene)-γ-phenylcyclohexanone **[2f]** has been confirmed by its ^{1}H NMR spectrum. The presence of signals at δ 2.8-3.0, 3.1-3.2(m, CH_2CHCH_2, **5H**); 3.9(s, 6 x OMe, **18H**); 6.5-6.8(s, ArH, **4H**); 7.1-7.4(m, ArH, **5H**); 8.0(s, 2 x methine H, **2H**) establish the structure of **[2f]**. Similarly, the numbers of protons in other compounds **[2a-2e]** and **[2g-2h]** have been confirmed by their ^{1}H NMR spectra. The structure of α,α'-*bis*(*m*-chlorobenzylidene)-γ-phenylcyclohexanone **[2g]** is confirmed by its mass spectrum. The molecular ion peak of [**2g**] by LCMS in the positive mode has been observed at 419, which is in good agreement with the calculated molecular weight. The compound **[1b]** also has shown an additional peak at 423 which proves the isotopic nature of 2 chlorine atoms. Similarly, the structures of other compounds **[2a-2f]** and **[2h]** have also been confirmed by their mass spectra. The structures of all the compounds **[2a-h]** have been further confirmed by their elemental analysis and the found values are with in ± 0.3 ٪ of required values

The antibacterial activity of newly synthesized α, β-unsaturated ketones has been evaluated against gram-positive *Staphylococcus aureus and Bacillus subtilis and* gram-negative *Escherichia coli* and *Klebsiella pneumoniae* species

The zone of inhibition exhibited by α,α'-*bis*(2,4-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1b]** against *Staphylococcus aureus* is 18 mm. The observations of other results indicate that replacement of 2,4-dichlorophenyl by other groups such as 2,3-dichlorophenyl, 5-bromo-2-methoxyphenyl, 2,4-dimethoxyphenyl, 3,4-dimethoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, 2,3,4-trimethoxyphenyl, *p*-chlorophenyl or *p*-ethoxyphenyl decreases the activity

The zone of inhibition exhibited by α,α'-*bis*(5-bromo-2-methoxybenzylidene)-γ-*t*-butylcyclohexanone **[1c]** against *Bacillus subtilis* is 15 mm. The observations of other results indicate that replacement of 5-bromo-2-methoxyphenyl by other groups such as 2,3-dichlorophenyl, 2,4-dichlorophenyl, 2,4-dimethoxyphenyl, 3,4-dimethoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, 2,3,4-trimethoxyphenyl, *p*-chlorophenyl or *p*-ethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(2,4-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1b]** against *Escherichia coli* is 20 mm. The observations of other results indicate that replacement of 2,4-dichlorophenyl by other groups such as 2,3-dichlorophenyl, 5-bromo-2-methoxy phenyl, 2,4-dimethoxyphenyl, 3,4-dimethoxyphenyl, 2,5-

dimethoxyphenyl, 2,4,5-trimethoxyphenyl, 2,3,4-trimethoxyphenyl, *p*-chlorophenyl or *p*-ethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(2,3-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1a]** against *Klebsiella pnueumoniae* is 22 mm. The observations of other results indicate that replacement of 2,3-dichlorophenyl by other groups such as 2,4-dichlorophenyl, 5-bromo-2-methoxyphenyl, 2,4-dimethoxyphenyl, 3,4-dimethoxyphenyl, 2,5-dimethoxyphenyl 2,4,5-trimethoxyphenyl, 2,3,4-trimethoxyphenyl, *p*-chlorophenyl or *p*-ethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(*m*-chlorobenzylidene)-γ-phenylcyclohexanone **[2g]** against *Staphylococcus aureus* is 17 mm. The observations of other results indicate that replacement of *m*-chlorophenyl by: 2,4-dimethoxyphenyl retains the activity. other groups such as 2,3-dichlorophenyl, 2,4-dichlorophenyl, 5-bromo-2-methoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl or 2,3-dimethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(2,4-dichlorobenzylidene)-γ-phenylcyclohexanone **[2b]** against *Bacillus subtilis* is 23 mm. The observations of other results indicate that replacement of 2,4-dichlorophenyl by other groups such as 2,3-dichlorophenyl, 5-bromo-2-methoxy phenyl, 2,4-dimethoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, *m*-chlorophenyl or 2,3-dimethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(2,4-dichlorobenzylidene)-γ-phenylcyclohexanone **[2b]** against *Escherichia coli* is 24 mm. The observations of other results indicate that replacement of 2,4-dichlorophenyl by other groups such as 2,3-dichlorophenyl, 5-bromo-2-methoxy phenyl, 2,4-dimethoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, *m*-chlorophenyl or 2,3-dimethoxyphenyl decreases the activity.

The zone of inhibition by α,α'-*bis*(2,4-dichlorobenzylidene)-γ-phenylcyclohexanone **[2b]** against *Klebsiella pneumoniae* is 21 mm. The observations of other results indicate that replacement of 2,4-dichlorophenyl by 2,3-dichlorophenyl retains the activity other groups such as 5-bromo-2-methoxyphenyl, 2,4-dimethoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, *m*-chlorophenyl or 2,3-dimethoxyphenyl decreases the activity.

The antioxidant activity of all the newly synthesized α, β-unsaturated ketones has been evaluated by DPPH method using ascorbic acid as the standard. The EC_{50} value for α,α'-*bis*(2,4,5-trimethoxybenzylidene)-γ-*t*-butylcyclohexanone **[1g]** due to DPPH radical scavenging activity is 518 µg/mL. The observations of other results indicate that replacement of 2,4,5-trimethoxyphenyl by other groups such as 2,3-dichlorophenyl, 2,4-dichlorophenyl, 5-bromo-2-methoxyphenyl, 2,4-dimethoxyphenyl, 3,4-dimethoxy, 2,5-dimethoxyphenyl, 2,3,4-trimethoxyphenyl, *p*-chlorophenyl or *p*-ethoxyphenyl decreases the activity.

The EC_{50} value for α,α'-*bis*(2,4-dimethoxybenzylidene)-γ-phenylcyclohexanone **[2d]** due to DPPH radical scavenging activity is 505 µg/mL. The observations of other results indicate that replacement of 2,4-dimethoxyphenyl by other groups such as 2,3-dichlorophenyl, 2,4-dichlorophenyl, 5-bromo-2-methoxyphenyl, 2,5-dimethoxyphenyl, 2,4,5-trimethoxyphenyl, *m*-chlorophenyl or 2,3-dimethoxyphenyl decreases the activity.

CONCLUSION:

In the present work we synthesized some novel α, β-unsaturated ketones with an aim to obtain some new potent antibacterial and antioxidant agents. The α, β-unsaturated ketones were synthesized by base catalysed Claisen-Schmidt condensation of ketones having α, α' hydrogens with various aldehydes. Three new series of α, β-unsaturated ketones were synthesized by reacting γ-*t*-butylcyclohexanone, γ-phenylcyclohexanone and γ-ethylcyclohexanone with different substituted benzaldehydes. The classes of the synthesized compounds are: α,α'-*Bis*(substitutedarylidene)-γ-*t*-butylcyclohexanones **[1a-j]** α,α'-*Bis*(substitutedarylidene)-γ-phenylcyclohexanones **[2a-h]** The formation of the compounds **[1a-j]** is indicated by their UV spectra. The functional groups in the compounds **[1a-j]** are characterized by their IR spectra. The number and positions of protons in the compounds **[1a]**, **[1b]**, **[1c]**, **[1d]**, **[1e]**, **[1g]** and **[1i]** are confirmed by their ^{1}H-NMR spectra. The structures of compounds **[1a-j]** are confirmed by their Mass spectra. The structures of the compounds **[1a-j]** are further confirmed by their elemental analysis. The formation of the compounds **[2a-h]** is indicated by their UV spectra.

All the synthesized compounds were screened for their *in vitro* antibacterial properties against human pathogenic Gram positive and gram-negative bacteria. Ampicillin was used as the standard. The observations reveal that: α,α'-*Bis*(2,4-dichlorobenzylidene)- γ-phenylcyclohexanone **[2b]** shows highest activity against *Bacillus subtilis*. α,α' -*Bis*(2,3-dichlorobenzylidene)-γ-*t*-butylcyclohexanone **[1a]** is most active against *Klebsiella pneumonia*. All the synthesized compounds were also screened for their antioxidant activity using ascorbic acid as the standard by DPPH method. The observations show that α,α'-

Bis(2,4-dimethoxybenzylidene)-γ-phenylcyclohexanone **[2d]** exhibits highest antioxidant activity at an EC_{50} of 505 µg/mL.

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