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Research Article

# A FACILE ROUTE FOR THE SYNTHESIS OF ETHYL N-ARYL-2,6-DIOXO-PIPERID-3-ENE-4-CARBOXYLATES AND THEIR BIOLOGICAL ACTIVITY

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**ABSTRACT**

Cyclopropyl derivatives and piperidinediones have known to exhibit enormous amount of biological activities. In the present study the synthesis of biologically potent ethyl N-aryl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylates (**2**), N-aryl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylic acids (**3**) and ethyl 2,6-dioxo-N-aryl-piperid-3-ene-4-carboxylates (**4**) were described. The compounds (**2, 4**) were evaluated in vitro for their antibacterial, antifungal, antioxidant and reducing ability. The findings revealed that compounds (**2**) may be used as control measures against different bacterium and fungi, and (4) can be used as reference standard for antioxidant activities.

**Keywords**: Maleimides, Cyclopropane, Piperidinedione, Antibacterial, Antifungal, Antioxidant.

## INTRODUCTION

Maleimide compounds are an important class of substrates for biological and chemical application[1]. In organic chemistry the maleimide functionality can be used as a synthetic platform in total synthesis due to its Michael-accepting ability and dienophillic nature[2,3]. Compounds containing cyclopropyl system possess wide variety of pharmacological activities. The usual synthesis of cyclopropyl esters involves the cyclopropanation of chalcones with cyclopropylating agents such as sulfonium methylide[4] and reactive methylide[5], ethyl cyanoacetate in aprotic solvents[6]. The stereoselective cyclopropanation of several glycols and alkenes was reported using bromoform or chloroform in the presence of the TEBAC and aqueous NaOH at room temperature [7].

Cyclopropanation was also achieved by treating haloesters with sodium in presence of dimethylsilylchloride and enolether with diiodomethane in presence of diethylzinc in ether[8]. Phenacylmethyl(dimethyl)selenonium bromide reacts with aqueous potassium hydroxide in chloroform to form the corresponding selenium ylide, which on irradiation or heating decomposes to 1,2,3-triphenacylcyclopropane[9]. Phenyliodonium ylides provide easy access to a variety of useful 1,1-cyclopropane diesters using rhodium or copper catalysis[10]. The cyclopropane acetic acid ethyl esters have conveniently prepared in one step from cyclopropylalkylketones by reacting with lead (IV) acetate in triethylorthoformate and perchloric acid[11].

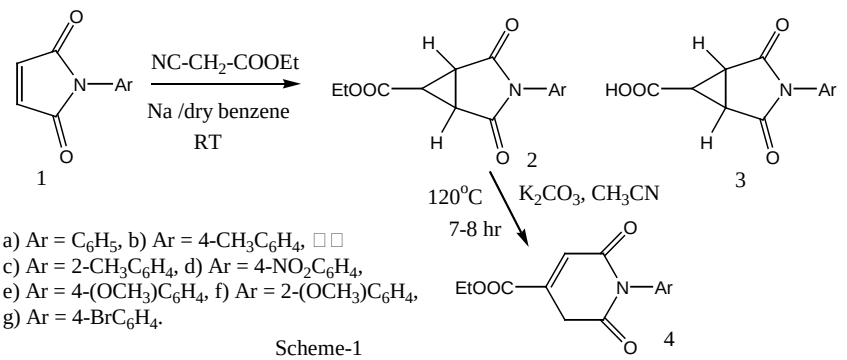
Indeed the three membered rings have a great deal of angle strain, ring opening relieve the strain to form more stable molecules. N-methyl-3-piperidyl and N-methyl-4-piperidyl esters of 1-benzanilidocyclohexane carboxylic acids have known to exhibit analgesic properties[12]. Oxepines are obtained by the ring expansion of cyclopropanes with several equivalents of potassium carbonate in methanol under reflux conditions[13,14]. $Cu(SbF_6)_2$ ligand catalyzed reaction of 2-substituted cyclopropane-1,1-dicarboxylates with enolsilylethers readily undergoes cycloaddition to provide substituted cyclopentane derivatives[15]. Amidoester on Dieckmann cyclisation using potassium t-butoxide in toluene produced piperidinediones[16]. The scanty of information on the ring expansion of cyclopropanes into piperidones prompted us to work in this area.

## MATERIALS AND METHODS

The chemicals used were purchased from Aldrich chemicals (India), and Merck Chemicals (India). Melting points were taken in open capillaries using Thomus Hoover melting point apparatus and are uncorrected. IR spectra were recorded in Nujol mull on Shimadzu 8300 spectrometer. The $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker Supercon 400 MHz spectrophotometer in $CDCl_3$; chemical shifts are expressed in □ppm. The coupling constant (*J*) is expressed in Hz. Mass spectra were obtained on Maspec MSW 9629 spectrophotometer, important fragments are given with the relative abundance in the bracket. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using benzene: ethyl acetate (8:1 v/v) as eluent.

### Synthesis

In a typical procedure, N-aryl maleimides (**1**) were converted to a mixture of ethyl N-aryl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylates (**2**) and N-aryl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylic acids (**3**) by the reaction of ethyl cyanoacetate and dried sodium metal in dry benzene at room temperature. The compounds (**2**) on ring expansion reaction under reflux conditions for 7-8 hours in acetonitrile in the presence of $K_2CO_3$ produced title compounds (**4**) in relatively good yield (Scheme 1).




a) Ar = $C_6H_5$, b) Ar = 4-$CH_3C_6H_4$, □□
c) Ar = 2-$CH_3C_6H_4$, d) Ar = 4-$NO_2C_6H_4$,
e) Ar = 4-$(OCH_3)C_6H_4$, f) Ar = 2-$(OCH_3)C_6H_4$,
g) Ar = 4-$BrC_6H_4$.

Scheme-1

**Typical procedure for the preparation of Ethyl N(phenyl)-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylate (2a) and N(phenyl)-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylic acids (3a):** Freshly distilled ethyl cyanoacetate (5 mmol) was added into a stirred suspension of powdered sodium (2 mmol) in dry benzene (100 mL) at room temperature. To this, N-aryl maleimide 1a (5 mmol) was added, and stirred for 8 hours at room temperature. The progress of the reaction was monitored by TLC. After the completion of the reaction, salts were filtered off and the filtrate was successively washed with water, brine solution (1 X 10 mL) and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a gummy mass, and was crystallized from chloroform: petroleum ether (1:5 v/v) gave white crystalline solid 2a in 64% yield. The aqueous layer on neutralization with dilute hydrochloric acid gave 3a as white crystalline solid in 30% yield.

**Typical procedure for the preparation of ethyl 2,6-dioxo-N-phenyl-piperid-3-ene-4-carboxylate 4a:** A solution of ethyl N-phenyl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylate 2a (2 mmol) in acetonitrile, $K_2CO_3$ (1 equivalent) was refluxed for 7-8 hours at 120˚C. After the completion of the reaction, mixture was cooled and filtered. The filtrate was extracted into ether (30 mL), washed successively with water and brine solution, the solvent was evaporated in vacuo. The resultant mass was crystallized from chloroform: petroleum ether (1:5 v/v), which afforded the white crystalline solid in 72% yield.

**Biological activity**

Antimicrobial activity of the synthesized compounds was done by paper disc diffusion method[17,18]. The compounds (**2a-g, 4a-g**) at the concentration of 50 µg/mL in methanol in the nutrient agar media were screened for their antibacterial activity against Gram-negative bacteria species *Escherichia coli, Salmonella typhimurium,* Gram-positive bacteria species *Bacillus substilis*, *Staphylococus aureus* and for their antifungal activity against *Aspergillus niger, Aspergillus flavus, C. albicans, Fusarium oxysporium*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The screening tests were performed in triplicate and the results were taken as a mean of three determinations (Table-1, Table-2).

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound and controls were inoculated with approximately 5 x $10^5$ c.f.u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 hrs at $37^0$C and fungi cultures were incubated for 72 hrs at $37^0$C, the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC). The experiments were carried out in triplicate; the results were taken as a mean of three determinations (Table-1, Table-2).

The antioxidant activity of synthesized compounds (**4a-g**) was carried out by DPPH radical scavenging assay using butylated hydroxyl toluene (BHT) as standard antioxidant[19,20]. Samples dissolved in methanol (0-50 µg/mL; 0-5 µg/mL for BHT) in 200 µL aliquot was mixed with 100 mM tris-HCl buffer (800 µL, pH 7.4) and then added 1 mL of 500 µM DPPH in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The experiments were performed in triplicates; the results are expressed as mean ± standard deviation (SD) (Table-3).

The reducing power of test samples (**4a-g**) was determined by the method of Yen and Chen[21]. The samples (0-50 µg/mL) were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. The equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 5000 rpm for 10 min., the upper layer was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2. The absorbance was measured at 700 nm. The experiments were performed in triplicates; the results are expressed as mean ± standard deviation (Table-3).

## RESULTS AND DISCUSSION

**Chemistry**

***Ethyl N-phenyl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylate* (2a):** M.P. 93-94˚C. IR (nujol): 1730, 1785cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.26 (t, 3H, $CH_3$), 1.52 (t, 1H, $C_6$-H), 2.02-2.05 (m, 2H, $C_1$-, $C_5$-H), 4.04 (q, 2H, $CH_2$), 7.20-7.44 (m, 5H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 13.82 (1C), 22.36 (2C), 25.12 (1C), 62.36 (1C), 127.66 (2C), 129.52 (1C), 129.78 (2C), 133.02 (1C), 169.52 (1C), 171.92 (2C). MS (relative abundance) m/z: for $C_{14}H_{13}NO_4$, 259($M^+$, 16), 231(30), 230(24), 187(16), 159(22), 119(42), 93(100). Anal. Calcd: C, 64.86, H, 5.05, N, 5.40%. Found: C, 64.79, H, 4.97, N, 5.34%.

***Ethyl 2,4-dioxo-3-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2b:*** Obtained from N-(4-methylphenyl)maleimide (5 mmol) as colourless crystalline solid in 56% yield, m.p. 119-120˚C. IR (nujol): 1745, 1800 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.30 (t, 3H, $CH_3$), 1.60 (t, 1H, $C_6$-H), 2.08 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.2 Hz), 2.40 (t, 3H, $CH_3$), 4.10 (q, 2H, $CH_2$), 7.21 (dd, 2H, Ar-H), 7.34 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 13.98 (1C), 22.52 (2C), 21,40 (1C), 25.28 (1C), 62.45 (1C), 127.80 (2C), 129.43 (1C), 129.88 (2C), 133.12 (1C), 169.59 (1C), 171.99 (2C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_4$, 273($M^+$, 18), 245(28), 244(26), 201(14), 173(24), 133(48), 107(100). Anal. Calcd: C, 65.92, H, 5.53, N, 5.13%. Found: C, 65.86, H, 5.44, N, 5.05%.

***Ethyl 2,4-dioxo-3-(2-methylphenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2c:*** Obtained from N-(2-methylphenyl)maleimide (5 mmol) as colourless crystalline solid in 48% yield, m.p. 73-74˚C. IR (nujol): 1735, 1795 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.32 (t, 3H, $CH_3$), 1.62 (t, 1H, $C_6$-H), 2.05 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.1 Hz), 2.36 (t, 3H, $CH_3$), 4.15 (q, 2H, $CH_2$), 7.10-7.65 (m, 4H, Ar-H). Anal. Calcd: C, 65.92, H, 5.53, N, 5.13%. Found: C, 65.86, H, 5.44, N, 5.05%.

***Ethyl 2,4-dioxo-3-(4-nitrophenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2d*:** Obtained from N(4-nitrophenyl)maleimide (5 mmol) as colourless amorphous solid in 56%, m.p. 121-122˚C. IR (nujol): 1740, 1790 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.28 (t, 3H, $CH_3$), 1.65 (t, 1H, $C_6$-H), 2.10 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.0 Hz), 4.09 (q, 2H, $CH_2$), 7.62 (dd, 2H, Ar-H), 7.98 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 14.04 (1C), 22.52 (2C), 21.52 (1C), 25.20 (1C), 61.91 (1C), 124.22 (2C), 129.36 (2C), 137.86 (1C), 142.60 (1C), 170.08 (1C), 172.14 (2C). Anal. Calcd for $C_{14}H_{12}N_2O_6$: C, 55.27, H, 3.98, N, 9.21%. Found: C, 55.17, H, 3.93, N, 9.13%.

***Ethyl 2,4-dioxo-3-(4-methoxyphenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2e*:** Obtained from N(4-methoxyphenyl)maleimide (5 mmol) as orange red oil in 61%. IR (nujol): 1738, 1792 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.30 (t, 3H, $CH_3$), 1.66 (t, 1H, $C_6$-H), 2.07 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.2 Hz), 3.85 (s, 3H, $OCH_3$), 4.18 (q, 2H, $CH_2$), 7.02 (dd, 2H, Ar-H), 7.18 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 13.94 (1C), 22.40 (2C), 21.30 (1C), 25.15 (1C), 62.62 (1C), 128.56 (2C), 156.94 (1C), 114.92 (2C), 123.86 (1C), 169.40 (1C), 172.70 (2C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_5$, 289($M^+$, 18), 261(24), 260(42), 217(38), 149(50), 119(24), 93(100). Anal. Calcd: C, 62.28, H, 5.23, N, 4.84%. Found: C, 62.21, H, 5.19, N, 4.77%.

***Ethyl 2,4-dioxo-3-(2-methoxyphenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2f*:** Obtained from N(2-methoxyphenyl)maleimide (5 mmol) as white crystalline solid in 53%, m.p. 116-117˚C. IR (nujol): 1745, 1795 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.35 (t, 3H, $CH_3$), 1.61 (t, 1H, $C_6$-H), 2.04 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.3 Hz), 3.90 (s, 3H, $OCH_3$), 4.21 (q, 2H, $CH_2$), 6.96-7.25 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 14.10 (1C), 22.44 (2C), 21.56 (1C), 25.20 (1C), 62.64 (1C), 115.56 (1C), 115.90 (1C), 122.08 (1C), 124.80 (1C), 128.02 (1C), 152.80 (1C), 169.85 (1C), 172.20 (2C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_5$, 289($M^+$, 18), 261(24), 260(42), 217(38), 149(50), 119(24), 93(100). Anal. Calcd: C, 62.28, H, 5.23, N, 4.84%. Found: C, 62.19, H, 5.16, N, 4.79%.

***Ethyl 2,4-dioxo-3-(4-bromophenyl)-3-azabicyclo[3.1.0]hexane-6-carboxylate 2g*:** Obtained from N(4-bromophenyl)maleimide (5 mmol) as white crystalline solid in 48%, m.p. 234-236˚C. IR (nujol): 1736, 1794 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.42 (t, 3H, $CH_3$), 1.66 (t, 1H, $C_6$-H), 2.09 (dd, 2H, $C_1$, $C_5$-H, *J*= 8.1 Hz), 4.15 (q, 2H, $CH_2$), 7.52 (dd, 2H, Ar-H), 7.98 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 13.85 (1C), 22.50 (2C), 21.65 (1C), 25.12 (1C), 62.66 (1C), 121.96 (1C), 128.55 (2C),

130.30 (1C), 130.85 (2C), 170.06 (1C), 172.46 (2C). MS (relative abundance) m/z: for $C_{14}H_{12}BrNO_4$, 339($M^+$, $^{81}Br$, 29), 337($M^+$, $^{79}Br$, 30), 309(24), 308(18), 265(28), 237(20), 197(36), 173(100). Anal. Calcd: C, 49.73, H, 3.58, N, 4.14%. Found: C, 49.65, H, 3.60, N, 4.09%.

***N-Aryl-2,4-dioxo-3-azabicyclo[3.1.0]hexane-6-carboxylic acids*** **3a**: M.P. 112-114˚C. $^1$H NMR ($CDCl_3$): δ 5.56(dd, 2H, $C_1$,$C_5$-H), 6.28($C_6$-H) 67.84(s, 5H, Ar-H). MS (relative abundance) m/z: for $C_{12}H_9NO_4$, 231($M^+$, 42), 187(28), 159(18), 119(36), 93(100).

***Ethyl 2,6-dioxo-N-phenyl-piperid-3-ene-4-carboxylate*** **4a:** M.P. 80-82C. IR (nujol): 1670, 1735 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.31 (q, 3H, $CH_3$), 2.90 (s, 2H, $C_5$-H), 3.98 (t, 2H, $OCH_2$), 5.80 (s, 1H, $C_3$-H), 7.20-7.35 (m, 5H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 14.28 (1C), 31.12 (1C), 61.90 (1C), 128.50 (2C), 128.30 (1C), 128.96 (2C), 132.82 (1C), 138.16 (1C), 140.20 (1C), 163.60 (1C), 170.60 (1C), 170.65 (1C). MS (relative abundance) m/z: for $C_{14}H_{13}NO_4$, 259($M^+$, 27), 231(42), 230(16), 187(44), 159(36), 119(66), 93(100). Anal. Calcd: C, 64.86, H, 5.05, N, 5.40%. Found: C, 64.79, H, 4.98, N, 5.32%.

***Ethyl N-(4'-methylphenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4b:** Obtained from 2b (0.54g, 2.0mmol) as a white solid in 80% yield, m.p. 93-94˚C. IR (nujol): 1685, 1744 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.35 (q, 3H, $CH_3$), 2.30 (s. 3H, $CH_3$), 3.12 (s, 2H, $C_5$-H), 4.10 (t, 2H, $OCH_2$), 5.86 (s, 1H, $C_3$-H), 7.21 (dd, 2H, Ar-H), 7.29 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 14.20 (1C), 32.24 (1C), 61.74 (1C), 128.54 (2C), 129.32 (2C), 132.16 (1C), 136.65 (1C), 138.30 (1C), 141.10 (1C), 163.45 (1C), 170.16 (1C), 170.25 (1C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_4$, 273($M^+$, 28), 245(38), 244(46), 201(52), 173(39), 133(62), 107(100). Anal. Calcd: C, 65.92, H, 5.53, N, 5.13%. Found: C, 65.90, H, 5.48, N, 5.14%.

***Ethyl N-(2'-methylphenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4c:** Obtained from 2c (0.54g, 2.0mmol) as a white solid in 74% yield, m.p. 104-05˚C. IR (nujol): 1690, 1745 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.28 (q, 3H, $CH_3$), 2.24 (s. 3H, $CH_3$), 2.95 (s, 2H, $C_5$-H), 4.16 (t, 2H, $OCH_2$), 5.98 (s, 1H, $C_3$-H), 7.10-7.54 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$): $\delta_c$ 14.22 (1C), 31.50 (1C), 61.78 (1C), 125.78 (1C), 128.04 (1C), 129.36 (1C), 130.25 (1C), 133.88 (1C), 136.56 (1C), 138.80 (1C), 140.90 (1C), 163.40 (1C), 170.34 (1C), 170.40 (1C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_4$, 273($M^+$, 32), 245(34), 244(49), 201(46), 173(37), 133(66), 107(100). Anal. Calcd: C, 65.92, H, 5.53, N, 5.13%. Found: C, 65.88, H, 5.45, N, 5.06%.

***Ethyl N-(4'-nitrophenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4d:** Obtained from 2d (0.58g, 2.0mmol) as a white solid in 76% yield, m.p. 122-123˚C. IR (nujol): 1680, 1736 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.38 (q, 3H, $CH_3$), 3.02 (s, 2H, $C_5$-H), 4.12 (t, 2H, $OCH_2$), 5.90 (s, 1H, $C_3$-H), 7.74 (dd, 2H, Ar-H), 8.02 (dd, 2H, Ar-H). Anal. Calcd for $C_{14}H_{12}N_2O_6$, m/z 304: C, 55.27, H, 3.98, N, 9.21%. Found: C, 55.21, H, 3.96, N, 39.16%.

***Ethyl N-(4'-methoxyphenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4e:** Obtained from 2e (0.58g, 2.0mmol) as a white solid in 76% yield, m.p. 132-134˚C. IR (nujol): 1690, 1740 cm$^{-1}$. $^1$H NMR ($CDCl_3$): δ 1.30 (q, 3H, $CH_3$), 2.99 (s, 2H, $C_5$-H), 3.85 (s. 3H, $OCH_3$), 4.28 (t, 2H, $OCH_2$), 5.85 (s, 1H, $C_3$-H), 7.04 (dd, 2H, Ar-H), 7.22 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): 14.10 (1C), 31.95 (1C), 55.20 (1C), 61.54 (1C), 114.60 (2C), 128.14 (2C), 156.65 (1C), 126.50 (1C), 138.66 (1C), 140.56 (1C), 163.15 (1C), 170.75 (1C), 170.83 (1C). MS (relative abundance) m/z: for $C_{15}H_{15}NO_5$, 289($M^+$, 22), 261(228, 260(46), 217(36), 149(54), 119(26), 93(100). Anal. Calcd: C, 62.28, H, 5.23, N, 4.84%. Found: C, 62.25, H, 5.15, N, 4.76%.

***Ethyl N-(2'-methoxyphenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4f:** Obtained from 2f (0.58g, 2.0mmol) as a white solid in 71% yield, m.p. 111-113˚C. IR (nujol): 1692, 1741 cm$^{-1}$. $^1$H NMR ($CDCl_3$): 1.35 (q, 3H, $CH_3$), 3.08 (s, 2H, $C_5$-H), 3.82 (s. 3H, $OCH_3$), 4.30 (t, 2H, $OCH_2$), 5.92 (s, 1H, $C_3$-H), 7.02-7.26 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$): 14.02 (1C), 32.20 (1C), 55.08 (1C), 61.42 (1C), 115.20 (1C), 116.24 (1C), 120.98 (1C), 124.52 (1C), 128.66 (1C), 153.25 (1C), 138.86 (1C), 140.40 (1C), 163.38 (1C), 170.56 (1C), 170.62 (1C). Anal. Calcd: for $C_{15}H_{15}NO_5$; 289.10: C, 62.28, H, 5.23, N, 4.84%. Found: C, 62.30, H, 5.21, N, 4.77%.

***Ethyl N-(4'-bromophenyl)-2,6-dioxo-piperid-3-ene-4-carboxylate*** **4g:** Obtained from 2g (0.54g, 2.0 mmol) as a white solid in 68% yield. IR (nujol): 1685, 1740 cm$^{-1}$. $^1$H NMR ($CDCl_3$): 1.30 (q, 3H, $CH_3$), 2.95 (s, 2H, $C_5$-H), 4.18 (t, 2H, $OCH_2$), 5.95 (s, 1H, $C_3$-H), 7.61 (dd, 2H, Ar-H), 8.22 (dd, 2H, Ar-H). $^{13}$C NMR ($CDCl_3$): 14.24 (1C), 31.72 (1C), 61.54 (1C), 123.06 (1C), 128.64 (2C), 129.94 (2C), 132.28 (1C), 138.46 (1C), 141.00 (1C), 163.45 (1C), 170.41 (1C), 170.48 (1C). Anal. Calcd: C, 49.73, H, 3.58, N, 4.14%. Found: C, 49.71, H, 3.53, N, 4.07%.

The general synthetic pathway employed is depicted in the scheme-1. The structure proof of the products was provided by IR, $^1$H NMR, $^{13}$C NMR, MS studies, and elemental analysis. For instance, in IR spectrum, all the products (**4**) showed the shift in the IR absorption due to C=O str frequencies in the region 1670-1690cm$^{-1}$, while the precursors showed in the region 1785-1800cm$^{-1}$. Further all showed the COO frequencies in the region 1730-1745cm$^{-1}$. In $^1$H NMR spectrum cyclopropane esters (**2**) showed a characteristic of doublet of doublet corresponding to $C_1$, $C_5$-H in the region δ 2.02-2.10 ppm., the $C_1$-H was coupled with $C_5$-H (*J*=8.2 Hz). $C_6$-protons showed triplet in the region δ 1.60-1.66 ppm. Ethyl 2,6-dioxo-N-aryl-piperid-3-ene-4-carboxylates (**4**) gave singlet in the region δ 5.80-5.98 ppm. due to $C_3$-H, and singlet for $C_5$-methylne protons in the region δ 2.90-3.12 ppm. Further all showed the signals due to aromatic and substituent protons at the expected region.

In $^{13}$C NMR, the compounds (**2**) showed signals for two carbons ($C_1$, $C_5$) in the region $\delta_c$ 22.36-22.52 ppm., and one carbon signal due to $C_6$ carbon at $\delta_c$ 25.12-25.28 ppm. The compounds (**4**) showed the signals due to $C_4$-carbons in the region $\delta_c$ 140.20-141.10 ppm., $C_5$-carbons in the region $\delta_c$ 31.12-32.24 ppm., and $C_3$-carbons in the region $\delta_c$ 138.16-138.90 ppm. The signals due to $C_2$ and $C_6$ carbons appeared in the region $\delta_c$ 161.80.162.30 ppm., and $\delta_c$ 165.40-166.30 ppm. respectively. Further all showed the signals due to aromatic and substituent carbons at the expected region.

All new compounds gave significantly stable molecular ion peaks with a relative abundance ranging from 14-50%. The common possible fragmentation pattern involves some rearrangement with the removal of smaller molecules viz. CO, $C_2H_5OH$, $C_2H_2$, $CO_2$ etc. The satisfactorily elemental analysis further supports structure of the products.

**Biological activity**

The study revealed that compounds 2a-g and 4a-g exhibits moderate to good antibacterial and antifungal activity against all the tested organisms. These compounds showed remarkable activity against the bacterium *E.coli, S. aureus* and fungi species *A. niger, C. Albicans,* and showed moderate activity against the bacterium *B. substilis, S. typhimurium* and fungi *A. flavus*. The compounds 2b, 2c, 2e, 2f, 4b, 4c, 4e, 4f have found more active, which is attributed to the presence of electron donating substituents on the benzene ring. The results indicate that these compounds may be used as control measures against different bacterium and fungi. The compounds 2d, 2g, 4d, 4g were less active which may be attributed to the presence of electron withdrawing group and steric reasons. The compounds 2a-g has showed relatively higher activity in comparison with their ring expansion products 4a-g. This reveals that the presence of cyclopropyl ring system might be the cause of the higher activity of 2a-g.

The compounds 4a-g showed promising free radical scavenging ability and their reducing power ability to reduce ferric chloride and potassium ferricyanide complex, but of lesser activity compared with the standard antioxidant. No much significant variations in the free radical scavenging ability and reducing power ability were observed at the initial concentrations of (10-20 µg/mL). However, at the higher concentrations (30-50 µg/mL) all showed a remarkable activity. The compounds 4a, 4d, 4g showed radical scavenging ability up to 60%, 4b, 4c, 4e and 4f showed radical scavenging ability up to 38% with reference to the standard antioxidant. The compounds 4a, 4d showed greater and 4b, 4c, 4e, 4f, 4g moderate reducing power ability. The experimental results indicate that these synthesized compounds possess potential electron donating ability and reducing power ability. The studies revealed that the compounds 2a-g have

insignificant free radical scavenging ability and reducing power compared to the standard BHT.

**Table 1: Zone of Inhibition (diameter) at 50 µg/mL concentrations (X) in mm and MICs (Y) in µg/mL of the synthesized compounds tested against bacterial stains by disc diffusion method and micro dilution method respectively.**

| Compound | *Escherichia coli* | | *Salmonella typhimurium* | | *Bacillus substilis* | | *Staphylococus aureus* | |
|---|---|---|---|---|---|---|---|---|
| | X | Y | X | Y | X | Y | X | Y |
| 2a | 24 | 28 | 17 | 30 | 20 | 28 | 25 | 24 |
| 2b | 28 | 29 | 20 | 28 | 22 | 26 | 30 | 28 |
| 2c | 22 | 33 | 16 | 31 | 18 | 34 | 22 | 22 |
| 2d | 18 | 31 | 14 | 30 | 15 | 32 | 19 | 18 |
| 2e | 30 | 30 | 21 | 32 | 24 | 30 | 31 | 30 |
| 2f | 23 | 26 | 19 | 34 | 18 | 28 | 24 | 23 |
| 2g | 15 | 27 | 14 | 27 | 14 | 27 | 18 | 17 |
| 4a | 20 | 28 | 15 | 29 | 18 | 24 | 21 | 36 |
| 4b | 23 | 30 | 17 | 25 | 20 | 30 | 24 | 29 |
| 4c | 18 | 31 | 14 | 26 | 16 | 29 | 18 | 26 |
| 4d | 15 | 26 | 12 | 31 | 13 | 26 | 15 | 30 |
| 4e | 24 | 23 | 19 | 25 | 21 | 30 | 27 | 32 |
| 4f | 19 | 26 | 16 | 28 | 16 | 28 | 20 | 24 |
| 4g | 13 | 24 | 12 | 25 | 12 | 36 | 15 | 28 |
| Streptomycin | 36 | 20 | 35 | 22 | 32 | 24 | 38 | 21 |

Streptomycin sulphate (50 µg per disc) was used as positive reference standard drug (n=3).

**Table 2: Zone of Inhibition (diameter) at a 25 µg/mL concentrations (X) in mm and MICs in µg/mL (Y) measured in mm of the synthesized compounds tested against fungi stains by disc diffusion method and micro dilution method respectively.**

| Compound | *Aspergillus niger* | | *Aspergillus flavus* | | *C. albicans* | |
|---|---|---|---|---|---|---|
| | X | Y | X | Y | X | Y |
| 2a | 24 | 26 | 20 | 27 | 25 | 25 |
| 2b | 28 | 25 | 24 | 28 | 27 | 29 |
| 2c | 26 | 26 | 20 | 30 | 24 | 31 |
| 2d | 14 | 30 | 13 | 32 | 13 | 30 |
| 2e | 25 | 28 | 23 | 29 | 24 | 27 |
| 2f | 22 | 26 | 21 | 24 | 21 | 26 |
| 2g | 12 | 24 | 12 | 25 | 12 | 28 |
| 4a | 20 | 28 | 19 | 30 | 16 | 28 |
| 4b | 22 | 31 | 20 | 24 | 17 | 25 |
| 4c | 21 | 24 | 19 | 21 | 16 | 30 |
| 4d | 14 | 25 | 14 | 26 | 12 | 24 |
| 4e | 20 | 23 | 21 | 20 | 18 | 25 |
| 4f | 18 | 23 | 20 | 24 | 17 | 36 |
| 4g | 12 | 26 | 13 | 21 | 11 | 23 |
| Nystatin | 35 | 20 | 30 | 18 | 32 | 22 |

Nystatin (25 µg per disc) was used as positive reference standard drug (n=3).

**Table 3: DPPH Radical Scavenging activity and Reducing power ability of the compounds 4a-g relative to the standard antioxidant BHT.**

| Samples | Concentration (µg/mL) | % Radical Scavenging activity | Reducing power Absorbance at 700 nm (OD) |
|---|---|---|---|
| Control | 0 | 0.00 ± 0.00 | ------ |
| 4a | 10 | 19.46 ± 0.82 | 0.276 ± 0.013 |
| | 20 | 22.30 ± 0.89 | 0.293 ± 0.010 |
| | 30 | 38.81 ± 1.01 | 0.370 ± 0.009 |
| | 40 | 47.52 ± 0.98 | 0.418 ± 0.008 |
| | 50 | 54.73 ± 1.00 | 0.471 ± 0.012 |
| 4b | 10 | 13.12 ± 0.78 | 0.262 ± 0.010 |
| | 20 | 16.36 ± 0.92 | 0.281 ± 0.011 |
| | 30 | 34.42 ± 0.83 | 0.332 ± 0.008 |
| | 40 | 45.23 ± 0,78 | 0.375 ± 0.014 |
| | 50 | 31.92 ± 1.00 | 0.409 ± 0.012 |
| 4c | 10 | 9.22 ± 0.85 | 0.270 ± 0.011 |
| | 20 | 11.96 ± 0.89 | 0.298 ± 0.014 |
| | 30 | 22.61 ± 0.68 | 0.351 ± 0.010 |
| | 40 | 31.96 ± 0.98 | 0.383 ± 0.009 |
| | 50 | 32.67 ± 0.71 | 0.412 ± 0.013 |
| 4d | 10 | 22.76 ± 0.94 | 0.295 ± 0.012 |
| | 20 | 24.23 ± 0.88 | 0.314 ± 0.014 |
| | 30 | 36.90 ± 0.74 | 0.365 ± 0.010 |
| | 40 | 48.66 ± 0.98 | 0.408 ± 0.008 |
| | 50 | 59.17 ± 0.82 | 0.486 ± 0.012 |

| | | | |
|---|---|---|---|
| 4e | 10 | 10.16 ± 0.80 | 0.258 ± 0.014 |
| | 20 | 12.22 ± 1.00 | 0.285 ± 0.010 |
| | 30 | 29.90 ± 0.94 | 0.319 ± 0.009 |
| | 40 | 42.61 ± 0.95 | 0.346 ± 0.011 |
| | 50 | 36.18 ± 0.80 | 0.380 ± 0.010 |
| 4f | 10 | 18.32 ± 0.86 | 0.260 ± 0.012 |
| | 20 | 25.22 ± 0.76 | 0.284 ± 0.008 |
| | 30 | 42.26 ± 1.00 | 0.336 ± 0.014 |
| | 40 | 50.22 ± 0.90 | 0.359 ± 0.011 |
| | 50 | 37.96 ± 0.88 | 0.390 ± 0.009 |
| 4g | 10 | 19.12 ± 0.88 | 0.282 ± 0.011 |
| | 20 | 21.36 ± 0.98 | 0.324 ± 0.014 |
| | 30 | 36.80 ± 0.81 | 0.368 ± 0.012 |
| | 40 | 45.75 ± 0.78 | 0.439 ± 0.013 |
| | 50 | 56.80 ± 1.00 | 0.470 ± 0.010 |

*Values are expressed as mean ± standard deviation (n=3)

## CONCLUSION

The divergence in the biological activity of synthesized compounds validates the significance of this study. The study revealed that the most of the compounds tested showed moderate to good antimicrobial and antioxidant activity. However, the effect of compounds on the host cell and their mode of action remain to be studied.

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