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# Synthesis, Characterization and Biological Activities of 1,2,3-Triazole Containing Substituted Cyclohexenones

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## ABSTRACT

*A novel series of Cyclohexenones carrying 1, 2, 3-Triazole core at 4th position and aryl group at 6th position **(5a-l)** have been synthesized starting from 4-Nitroaniline **(1)** in four steps. The structures of these newly synthesized compounds were elucidated by elemental analysis, 1H NMR and mass spectral data. Also these compounds were evaluated for their antibacterial activity against Pseudomonas, Staphylococcus aureus, Enterococcus, Escherichia coli, antifungal activity against Candida albicans and antioxidant activity by DPPH radical scavenging assay method. Compounds 5c, 5e, 5f, 5g, 5h, 5j and 5f showed activity comparable or higher than that of BHA.*

**Keywords:** Robinsons Annulation, 1, 2, 3-Triazole derivatives, Antibacterial, Antifungal, Antioxidant activity.

## INTRODUCTION

Substituted 1, 2, 3-triazoles are important five member heterocycles with wide variety of applications. Although 1, 2, 3-triazole was not present in nature as such, it could not be cleaved hydrolytically and was stable to metabolic degradation. Recent studies on 1, 2, 3-triazoles demonstrated that the hydrogen bonding and dipole interactions of the triazole core could favour in binding of bio molecular targets and there by improve the solubility. Further 1, 2, 3-triazoles and its derivatives exhibit antimicrobial [1-3], hypoglycaemic[4], antiprotozoal [5], anticonvulsant [6], anti-allergic [7], antihistamine [8] properties.

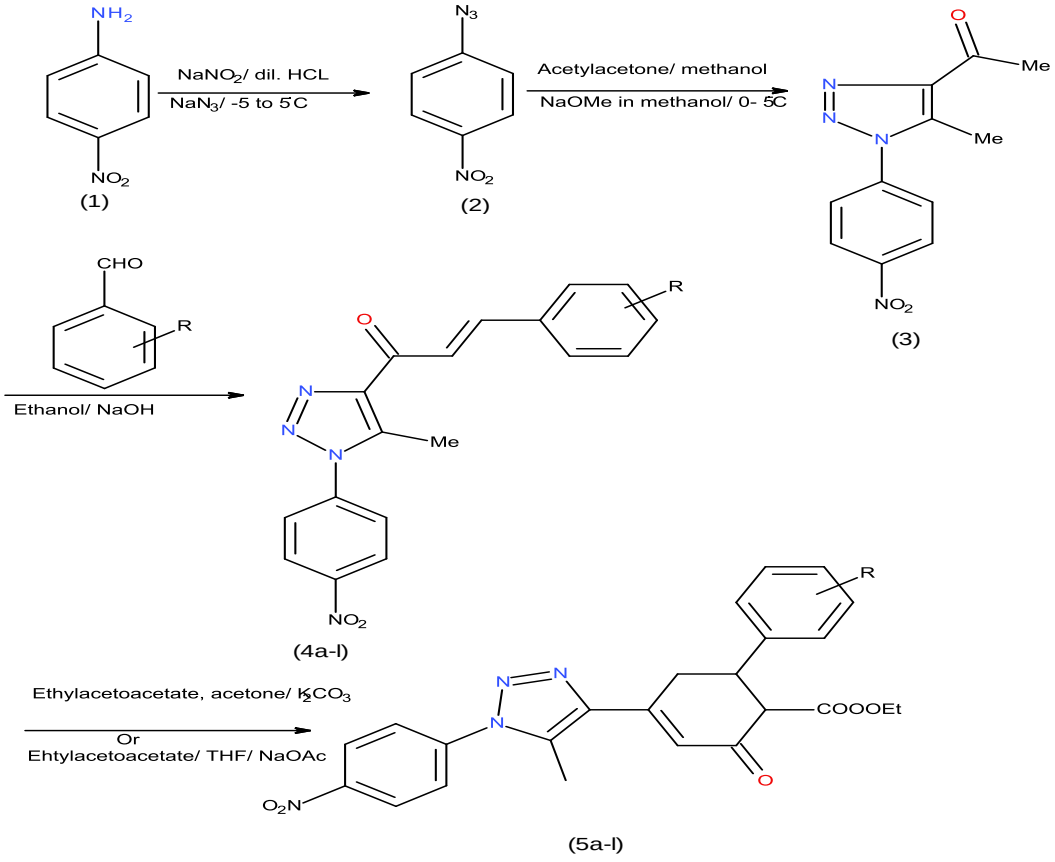
The Robinsons annulation is one of the key methods in the construction of six membered ring compounds. It is a combination of two reactions-Michael addition followed by Aldol condensation. Formation of cyclohexenone and its derivatives is an important strategy in synthetic chemistry since they find application as starting materials in the synthesis of many natural products and other interesting organic compounds including antibiotics and steroids. Many of such compounds are reported to possess pharmacological activities [9] such as antitubercular [10] and also useful for the treatment of inflammation and autoimmune diseases [11]. Cyclohexenone derivatives produced by an Endophytic fungus reported to exhibit antimicrobial activities [12].

Prompted by these observations and in continuation of our search [13-16] for biologically active heterocycles we planned to synthesize novel series of Cyclohexenones carrying 1, 2, 3-Triazole core at 4th position and aryl group at 6th position **(5a-l)** and to evaluate their biological activity.

## MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. $^1$H NMR spectra were recorded on a Bruckner AMX-400 (400 MHz) spectrometer using DMSO-$d_6$/$CDCl_3$ as solvent and TMS as an internal standard. All chemical shifts values are reported in δ scale downfield from TMS. Mass spectra of these compounds were recorded on Agilent mass spectrometer operating at 20eV/30eV and IR spectra of the compounds were recorded on Thermo Nicolet iS5- FTIR spectrophotometer. C, H, N analysis was carried out on a Vario-EL (Elementar-III) model. Homogeneity of the compounds was checked by TLC on silica gel plates (Merck).

**General procedure for the synthesis of 3-aryl-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)prop-2-en-1-ones (4a-l):** To a solution of 1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)ethanone **(3)** (0.006 mol) in ethanol (15mL), appropriate aldehyde (0.0066 mol) in ethanol (10mL) was added at 65-75ºC, followed by 10% sodium hydroxide solution (1 mL) drop wise by maintaining the same temperature with stirring. The stirring was continued for 30-60 min at 65-75ºC. The reaction mixture was cooled to room temperature and the solid obtained was collected by filtration, washed with water and dried. Further purification was done by recrystalization from ethanol. The propenones (4a-l) so prepared were characterized by reference to literature [3].



**General procedure for the synthesis of ethyl-6-(aryl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-2-oxocyclohex-3-ene carboxylate (5a-l):**

**Method A:** To a stirred solution of 3-aryl-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4) (0.004 mol) and ethylacetoacetate (0.005 mol) in tetrahydrofuran (20 mL), anhydrous sodium acetate (0.012 mol) was added at room temperature. The reaction mixture was stirred for ~ 15-20 h. The reaction mixture was poured into ice; the resultant solid was filtered, washed the product with water, and then recrystallized with ethanol (Table-1).

**Method B:** To a stirred solution of 3-aryl-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4) (0.004 mol) and ethylacetoacetate (0.005 mol) in acetone (20 mL), anhydrous potassium carbonate (0.01 mol) was added at room temperature. The reaction mixture was stirred for ~ 15-20 h. The reaction mixture was poured in to ice, the resultant solid was filtered, washed the product with water, recrystallized with ethanol.

The products obtained by both the methods are found to be identical. However method B was found to be superior with respect to yield of the compound.

**Table- 1:** Characterization of ethyl-6-(aryl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl) -2-oxocyclohex-3-ene carboxylate (**5a-l**)

| Compound No. | R | Mol. Formula<br>Mol. Wt. | Yield (%)<br>Melting point($^0$C) | % CHN Analysis<br>Found (Calc.) | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| 5a | 4-Cl | $C_{24}H_{21}ClN_4O_5$<br>480.90 | 83<br>152-157 | 59.99<br>(59.94) | 4.51<br>(4.40) | 11.74<br>(11.65) |
| 5b | 2-Cl | $C_{24}H_{21}ClN_4O_5$<br>480.90 | 81<br>136-140 | 60.02<br>(59.94) | 4.49<br>(4.40) | 11.76<br>(11.65) |
| 5c | 3,4 di-Cl | $C_{24}H_{20}Cl_2N_4O_5$<br>515.34 | 85<br>175-180 | 55.99<br>(55.93) | 3.99<br>(3.91) | 10.92<br>(10.87) |
| 5d | 2,6 di-Cl | $C_{24}H_{20}Cl_2N_4O_5$<br>515.34 | 85<br>164-168 | 55.98<br>(55.93) | 3.97<br>(3.91) | 10.95<br>(10.87) |
| 5e | 2,5 di-F | $C_{24}H_{20}F_2N_4O_5$<br>482.43 | 75<br>160-165 | 59.81<br>(59.75) | 4.29<br>(4.18) | 11.66<br>(11.61) |
| 5f | 3,5 bis(-$CF_3$) | $C_{26}H_{20}F_6N_4O_5$<br>582.42 | 81<br>181-184 | 53.67<br>(53.61) | 3.55<br>(3.46) | 9.68<br>(9.62) |
| 5g | 2-F | $C_{24}H_{21}FN_4O_5$<br>464.44 | 80<br>158-163 | 62.14<br>(62.06) | 4.66<br>(4.56) | 12.12<br>(12.06) |
| 5h | 3-F | $C_{24}H_{21}FN_4O_5$<br>464.44 | 82<br>168-172 | 62.11<br>(62.06) | 4.64<br>(4.56) | 12.15<br>(12.06) |
| 5i | 4-Me | $C_{25}H_{24}N_4O_5$ | 79 | 65.31 | 5.34 | 12.23 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 460.48 | 166-170 | (65.21) | (5.25) | (12.17) |
| 5j | 3-$NO_2$ | $C_{24}H_{21}N_5O_7$<br>491.45 | 78<br>158-163 | 58.76<br>(58.65) | 4.39<br>(4.31) | 14.33<br>(14.25) |
| 5k | 4-$NO_2$ | $C_{24}H_{21}N_5O_7$<br>491.45 | 74<br>165-170 | 58.74<br>(58.65) | 4.41<br>(4.31) | 14.35<br>(14.25) |
| 5l | 2-$CF_3$ | $C_{25}H_{21}F_3N_4O_5$<br>514.45 | 85<br>171-175 | 58.44<br>(58.37) | 4.18<br>(4.11) | 10.95<br>(10.89) |

**Biological Activity**

**Antibacterial activity:** Bacterial strains were inoculated into peptone-water and incubated for 4 h at 37°C and adjusted to a turbidity of 0.5 McFarland standards (10^8 CFU $mL^{-1}$). The suspension was used to inoculate into MHA Petri plates by lawn culture. Well (diameter 6mm) were punched in the agar and filled with 60µL of dissolved samples (200µg $mL^{-1}$). Plates were incubated in incubator at 37°C for 24 h and measured the growth inhibition zone diameters in mm, which was compared with that of the standard drug Ciprofloxacin. Plain DMSO (60µL) solution was used as control, which showed no inhibition.

**Antifungal activity:** ATCC Candida *albicans* were freshly sub cultured on Sabouraud dextrose agar plates for 24 h at 35°C. An inoculums was prepared by suspending a single isolated colony in about 5mL of 0.9 %w/v of normal saline and the turbidity was adjusted ($10^{\wedge}8$ CFU m $L^{-1}$; 0.5 McFarland). A sterile cotton swab was moistened in the inoculums suspension. Sabouraud's dextrose agar plates were streaked in 4 different directions (at 90º angles), so as to cover the entire surfaces. The plates were kept to dry and a sterile borer (6mm in diameter) was then used to punch wells in the agar medium. Subsequently, wells were filled with 60µl of the compound (200µg m $L^{-1}$). The plates were incubated at 35°C for 24h. And the values of zone of inhibition were recorded (in mm). Plain DMSO used as negative control, and showed no zone of inhibition. Fluconazole was used in the assay as positive control.

**Antioxidant activity:** DPPH radical scavenging activity was determined following the modified method of Brand-Williams et al [17] using ethanol in DPPH as the control. The absorbance was measured at 518 nm using UV-visible spectrophotometer. The percentage of radical scavenging was calculated by using the following formula:

$$\% \text{ Inhibition} = \left( \frac{(Ac - As)}{Ac} \times 100 \right)$$

Where $A_C$ is the absorbance of the control (blank, without test sample) and
$A_S$ is the absorbance of the test samples

## RESULTS AND DISCUSSION

A novel series of 4,6-disubstituted cyclohexenones **(5a-l)** were synthesized in four steps starting from *p*-Nitroaniline as outlined in Scheme-1. The *p*-Nitroaniline on diazotization with sodium nitrite/dilute hydrochloric acid followed by reaction with sodium azide gave *p*-Nitrophenylazide **(2)**. Azide **(2)** on reaction with acetylacetone gave 1,2,3-triazole having acetyl group at $4^{th}$ position **(3)**. This scaffold **(3)** on Claisen-Schmidt condensation with different aromatic aldehyde gave propenones **(4a-l)**, which when treated with ethylacetoacetate in the presence of suitable base underwent Robinson's annulation to give the title compounds **(5a-l)**.

This Robinsons annulation reaction was tried by using different bases such as anhydrous sodium acetate, sodium bicarbonate, anhydrous potassium carbonate, cesium carbonate, and strong bases like sodium *tert*-butoxide, potassium *tert*-butoxide, sodium ethoxide and sodium methoxide. But reaction with strong bases was not successful due to formation of multiple numbers of impurities. Potassium carbonate or sodium acetate was found to be most suitable bases.

The formation of 4,6-disubstituted 2-oxocyclohex-3-enes **(5a-l)** (Scheme-I) were confirmed by spectral and analytical data. In the $^1$H NMR spectrums the olefinic proton appeared as a singlet at δ around 6 ppm integrated for one proton at C-3 of Cyclohexenone ring. The proton attached to the C-1 carbon appeared as doublet in the region of δ 3.4 to 4 integrating for one proton. The proton at C-6 carbon appeared as doublet of doublet in the region of δ 3.6 to 4.02 for single proton. However in few cases signals due to C-1 and C-6 proton overlapped with each other and appeared as multiplet in the region of δ 3.7 to 4.1. The two protons at C-5 which are pro-chiral hence are magnetically non-equivalent and so appeared as two distinct doublet of doublets due to geminal and vicinal coupling with coupling constant around 3Hz, 18Hz in the region of δ 3.4 to 3.8 ppm and around 11 Hz, 18 Hz in the region of δ 2.7 to 3.2 ppm. Protons of -$CH_3$ group attached to triazole ring appeared as singlet in the region of ~δ 2.5 ppm integrating for three protons, -$CH_3$ of ester attached to C-1 of Cyclohexenone ring appeared as triplet in the region of δ 0.9 to 1.2 ppm, and -$CH_2$ protons of ester came into resonance in the region of δ 3.97 to 4.15 ppm as quartet. The signal due to the aromatic protons appeared in the region of δ 6.94 to 8.5 ppm.

In the IR spectra of these compounds the ester carbonyl absorption band was seen around 1690-1740 cm$^{-1}$, while the α,β-unsaturated carbonyl band was seen around 1650-1670 cm$^{-1}$, the alkene absorption band was seen around 1600-1620 cm$^{-1}$. The asymmetric and symmetric stretching band of the nitro group was observed around 1520 and 1340 cm$^{-1}$.

Further support for the proposed structures was obtained by recording the mass spectra of these compounds. In all these cases molecular ion peak are in agreement with the molecular formulae proposed.

**Analytical and Spectral Data of synthesized compounds**

**3-(4-chlorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4a):** Yield: 88%; m.p. 218-223 ºC; Anal. calcd. for $C_{18}H_{13}ClN_4O_3$: C, 58.62; H, 3.55; N, 15.19 %. Found: C, 58.55, H, 3.68, N, 15.25 %; IR (cm$^{-1}$): 1670 (C=O stretching), 1610 (C=C stretching of Cyclohexenone), 1527 & 1347 (asymmetric and symmetric stretching of nitro); MS (m/z): 369.1 ($M^+$+1).

**3-(2-chlorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4b):** Yield: 86%; m.p. 225-230 ºC; Anal. calcd. for $C_{18}H_{13}ClN_4O_3$: C, 58.62; H, 3.55; N, 15.19 %. Found: C, 58.50, H, 3.65, N, 15.22 %; IR (cm$^{-1}$): 1669 (C=O stretching), 1612 (C=C stretching of Cyclohexenone), 1528 & 1348 (asymmetric and symmetric stretching of nitro); MS (m/z): 369.1 ($M^+$+1).

**3-(3, 4-dichlorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4c):** Yield: 94%; m.p. 194-199 ºC; Anal. calcd. for $C_{18}H_{12}Cl_2N_4O_3$: C, 53.62; H, 3.00; N, 13.89 %. Found: C, 53.58, H, 3.10, N, 13.92 %; IR (cm$^{-1}$): 1671 (C=O stretching), 1614 (C=C stretching of Cyclohexenone), 1530 & 1349 (asymmetric and symmetric stretching of nitro); MS (m/z): 403.1 ($M^+$+1).

**3-(2, 6-dichlorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4d):** Yield: 80%; m.p. 230-235 ºC; Anal. calcd. for $C_{18}H_{12}Cl_2N_4O_3$: C, 53.62; H, 3.00; N, 13.89 %. Found: C, 53.68, H, 3.12, N, 13.96 %; IR (cm$^{-1}$): 1670 (C=O stretching), 1613 (C=C stretching of Cyclohexenone), 1529 & 1350 (asymmetric and symmetric stretching of nitro); MS (m/z): 403.1 ($M^+$+1).

**3-(2, 5-difluorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4e):** Yield: 79%; m.p. 250-255 ºC; Anal. calcd. for $C_{18}H_{12}F_2N_4O_3$: C, 58.38; H, 3.27; N, 15.13 %. Found: C,

58.25, H, 3.32, N, 15.26 %; IR ($cm^{-1}$): 1665 (C=O stretching), 1610 (C=C stretching of Cyclohexenone), 1524 & 1345 (asymmetric and symmetric stretching of nitro); MS (m/z): 371.1 ($M^+$+1).

**3-(3, 5-bis(trifluoromethyl)phenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4f):** Yield: 81%; m.p. 221-226 ºC; Anal. calcd. for $C_{20}H_{12}F_6N_4O_3$: C, 51.07; H, 2.57; N, 11.91 %. Found: C, 51.17, H, 2.66, N, 11.96%; IR ($cm^{-1}$): 1667 (C=O stretching), 1610 (C=C stretching of Cyclohexenone), 1530 & 1347 (asymmetric and symmetric stretching of nitro); MS (m/z): 469.2 ($M^+$-1).

**3-(2-fluorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4g):** Yield: 76%; m.p. 179-184 ºC; Anal. calcd. for $C_{18}H_{13}FN_4O_3$: C, 61.36; H, 3.72; N, 15.90 %. Found: C, 61.25, H, 3.82, N, 15.95 %; IR ($cm^{-1}$): 1664 (C=O stretching), 1611 (C=C stretching of Cyclohexenone), 1528 & 1350 (asymmetric and symmetric stretching of nitro); MS (m/z): 353.2 ($M^+$+1).

**3-(3-fluorophenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4h):** Yield: 77%; m.p. 198-203 ºC; Anal. calcd. for $C_{18}H_{13}FN_4O_3$: C, 61.36; H, 3.72; N, 15.90 %. Found: C, 61.22, H, 3.80, N, 15.97 %; IR ($cm^{-1}$): 1670 (C=O stretching), 1611 (C=C stretching of Cyclohexenone), 1525 & 1345 (asymmetric and symmetric stretching of nitro); MS (m/z): 353.1 ($M^+$+1).

**1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-3-p-tolylprop-2-en-1-one (4i):** Yield: 75%; m.p. 132-137 ºC; Anal. calcd. for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08 %. Found: C, 65.35, H, 4.75, N, 16.15 %; MS (m/z): 349.1 ($M^+$+1).
1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3-nitrophenyl)prop-2-en-1-one **(4j):** Yield: 84%; m.p. 209-214 ºC; Anal. calcd. for $C_{18}H_{13}N_5O_5$: C, 56.99; H, 3.45; N, 18.46 %. Found: C, 57.05, H, 3.55, N, 18.50 %; MS (m/z): 380.1 ($M^+$+1).

**1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-3-(4-nitrophenyl)prop-2-en-1-one (4k):** Yield: 83%; m.p. 230-235 ºC; Anal. calcd. for $C_{18}H_{13}N_5O_5$: C, 56.99; H, 3.45; N, 18.46 %. Found: C, 57.1, H, 3.60, N, 18.52 %; MS (m/z): 380.1 ($M^+$+1).

**3-(2-(trifluoromethyl)phenyl)-1-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)prop-2-en-1-one (4l):** Yield: 78%; m.p. 186-191 ºC; Anal. calcd. for $C_{19}H_{13}F_3N_4O_3$: C, 56.72; H, 3.26; N, 13.93 %. Found: C, 56.8, H, 3.35, N, 14.02 %; IR ($cm^{-1}$): 1668 (C=O stretching), 1613 (C=C stretching of Cyclohexenone), 1528 & 1350 (asymmetric and symmetric stretching of nitro); MS (m/z): 403.1 ($M^+$+1).

**Ethyl-6-(4-chlorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-ene carboxylate (5a)**: Yield:83%; m.p. 152-157 ºC; Anal. calcd. for $C_{24}H_{21}ClN_4O_5$ : C, 59.94; H, 4.40; N, 11.65 %. Found: C, 59.99, H, 4.51, N, 11.74 %; IR ($cm^{-1}$): 1735 (C=O stretching of ester), 1664 (C=O stretching of Cyclohexenone), 1612 (C=C stretching of Cyclohexenone), 1523 & 1346 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.471-8.492 (d, 2H, ArH, J-8.4 Hz), 7.717-7.738 (d, 2H, ArH, J-8.4 Hz), 7.322-7.343 (d, 2H, ArH, J-8.4 Hz), 7.271-7.292 (d, 2H, ArH, J-8.4 Hz), 6.453 (s, 1H,=CH-), 4.059-4.112 (q, 2H, -$CO_2CH_2$-), 3.769-3.87 (m, 2H, -CH-, -CH-), 3.607-3.663 (dd, 1H, Ha of -$CH_2$-, J-3.4 Hz, 18.7 Hz), 2.986-3.057 (dd, 1H, Hb of -$CH_2$-, J-10.0 Hz, 18.2 Hz), 2.575 (s, 3H, -$CH_3$), 1.075-1.11 (t, 3H, -$CH_3$); MS (m/z): 481.2 ($M^+$+1).

**Ethyl-6-(2-chlorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-ene carboxylate (5b):** Yield:81%; m.p. 136-140 ºC; Anal. calcd. for $C_{24}H_{21}ClN_4O_5$: C, 59.94; H, 4.40; N, 11.65 %. Found: C, 60.02, H, 4.49, N, 11.76 %; IR ($cm^{-1}$): 1738 (C=O stretching of ester), 1667 (C=O stretching of Cyclohexenone), 1614 (C=C stretching of Cyclohexenone), 1525 & 1347 (asymmetric and symmetric stretching of nitro); 1H-NMR (400 MHz, DMSO-$d_6$, δ/ ppm): 8.418-8.441 (dd, 2H, ArH, J-2.4 Hz, 7.2 Hz), 7.871-7.894 (dd, 2H, ArH, J-2.0 Hz, 8.0 Hz), 7.782-7.805 (d, 1H, ArH, J-9.2 Hz), 7.343-7.438 (m, 2H, ArH), 7.249-7.269 (d, 1H, ArH, J-8.0 Hz), 5.933 (s, 1H,=CH-), 4.462-4.494 (q, 2H, -$CO_2CH_2$-),

3.895-3.972 (m, 2H, -CH-, -CH-), 3.489-3.545 (dd, 1H, Ha of $-CH_2-$), 2.76-2.833 (dd, 1H, Hb of $-CH_2-$), 2.478 (s, 3H, $-CH_3$), 0.964-1.000 (t, 3H, $-CH_3$); MS (m/z): 481.2 ($M^+$+1).

**Ethyl-6-(3, 4-dichlorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex -3-enecarboxylate (5c) :** Yield:85%; m.p. 175-180 ºC; Anal. calcd. for $C_{24}H_{20}Cl_2N_4O_5$: C, 55.93; H, 3.91; N,10.87 %. Found: C, 55.99, H, 3.99, N, 10.92 %; IR ($cm^{-1}$): 1733 (C=O stretching of ester), 1665 (C=O stretching of Cyclohexenone), 1612 (C=C stretching of Cyclohexenone), 1520 & 1346 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.475-8.496 (d, 2H, ArH, J-8.4 Hz), 7.717-7.737 (d, 2H, ArH, J-8.0 Hz), 7.43-7.449 (m, 2H, ArH), 7.186 (s, 1H, ArH), 6.453 (s, 1H,=CH-), 4.096-4.148 (q, 2H, $-CO_2CH_2-$), 3.747-3.857 (m, 2H, -CH-, -CH-), 3.627-3.681 (dd, 1H, Ha of $-CH_2-$, J-3.2 Hz, 18.4 Hz), 2.986-3.057 (dd, 1H, Hb of $-CH_2-$, J-10.6 Hz, 18.6 Hz), 2.577 (s, 3H, $-CH_3$), 1.116-1.151 (t, 3H, $-CH_3$); MS (m/z): 513.0 ($M^+$-1).

**Ethyl-6-(2, 6-dichlorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-enecarboxylate (5d):** Yield:84%; m.p. 164-168 ºC; Anal. calcd. for $C_{24}H_{20}Cl_2N_4O_5$: C, 55.93; H, 3.91; N,10.87 %. Found: C, 55.98, H, 3.97, N, 10.95 %; IR ($cm^{-1}$): 1734 (C=O stretching of ester), 1664 (C=O stretching of Cyclohexenone), 1612 (C=C stretching of Cyclohexenone), 1522 & 1345 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.428-8.448 (d, 2H, ArH, J-8.0 Hz), 7.675-7.696 (d, 2H, ArH, J-8.4 Hz), 7.298-7.318 (d, 2H, ArH, J-8.0 Hz), 7.191-7.211 (d, 1H, ArH, J-8.0 Hz), 6.43 (s, 1H, =CH-), 3.978-4.032 (q, 2H, $-CO_2CH_2-$), 3.762-3.876 (m, 2H, -CH-, -CH-), 3.445-3.499 (dd, 1H, Ha of $-CH_2-$, J-3.6 Hz, 17.4 Hz), 2.747-2.817 (dd, 1H, Hb of $-CH_2-$, J-11.0 Hz, 17.4 Hz), 2.559 (s, 3H, $-CH_3$), 1.021-1.057 (t, 3H, $-CH_3$); MS (m/z): 513.2 ($M^+$-1).

**Ethyl-6-(2, 5-difluorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-enecarboxylate (5e)*:*** Yield:75%; m.p. 160-165 ºC; Anal. calcd. for $C_{24}H_{20}F_2N_4O_5$: C, 59.75; H, 4.18; N, 11.61 %. Found: C,59.81, H, 4.29, N, 11.66 %; IR ($cm^{-1}$): 1710 (C=O stretching of ester), 1680 (C=O stretching of Cyclohexenone), 1610 (C=C stretching of Cyclohexenone), 1525 & 1343 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.47-8.491 (d, 2H, ArH, J-8.4 Hz), 7.73-7.751 (d, 2H, ArH, J-8.4 Hz), 6.969-7.088 (m, 3H, ArH), 6.461 (s, 1H,=CH-), 4.088-4.14 (q, 2H, $-CO_2CH_2-$), 4.029-4.068 (dd, 1H, -CH-, J-4 Hz, 11.8 Hz), 3.917-3.95 (d, 1H, -CH-, J-13.2 Hz), 3.621-3.678 (dd, 1H, Ha of $-CH_2-$, J-4 Hz, 18.8 Hz), 3.088-3.163 (dd, 1H, Hb of $-CH_2-$, J-11.4 Hz, 18.5 Hz), 2.588 (s, 3H, $-CH_3$), 1.09-1.125 (t, 3H, $-CH_3$); MS (m/z): 483.2 ($M^+$+1).

**Ethyl-6-(3, 5-bis(trifluoromethyl)phenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxo cyclohex-3-enecarboxylate (5f):** Yield:81%; m.p. 181-184 ºC; Anal. calcd. for $C_{26}H_{20}F_6N_4O_5$: C, 53.61; H, 3.46; N, 9.62 %. Found: C, 53.67, H, 3.55, N, 9.68 %; IR ($cm^{-1}$): 1720 (C=O stretching of ester), 1682 (C=O stretching of Cyclohexenone), 1615 (C=C stretching of Cyclohexenone), 1527 & 1339 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.481-8.502 (d, 2H, ArH, J-8.4 Hz), 7.824 (m, 3H, ArH), 7.722-7.743 (d, 2H, ArH, J-8.4 Hz), 6.482 (s, 1H,=CH-), 4.081-4.131 (q, 2H, $-CO_2CH_2-$), 3.989-4.028 (dd, 1H, -CH-, J-3.6 Hz, 12 Hz), 3.859-3.892 (d, 1H, -CH-, J-13.2 Hz), 3.717-3.772 (dd, 1H, Ha of $-CH_2-$, J-3.4 Hz, 18.5 Hz), 3.049-3.124 (dd, 1H, Hb of $-CH_2-$, J-11.4 Hz, 18.5 Hz), 2.593 (s, 3H, $-CH_3$), 1.067-1.102 (t, 3H, $-CH_3$); MS (m/z): 581.0 ($M^+$-1).

**Ethyl-6-(2-fluorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-ene carboxylate (5g):** Yield:80%; m.p. 158-163 ºC; Anal. calcd. for $C_{24}H_{21}FN_4O_5$: C,62.06; H, 4.56; N, 12.06 %. Found: C, 62.14, H, 4.66, N, 12.12 %; IR ($cm^{-1}$): 1728 (C=O stretching of ester), 1675 (C=O stretching of Cyclohexenone), 1614 (C=C stretching of Cyclohexenone), 1532 & 1340 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.466-8.487 (d, 2H, ArH, J-8.4 Hz), 7.725-7.746 (d, 2H, ArH, J-8.4 Hz), 7.254-7.332 (m, 2H, ArH), 7.057-7.149 (m, 2H, ArH), 6.468 (s, 1H,=CH-), 3.98-4.1 (m, 4H, $-CO_2CH_2-$, -CH-, -CH-), 3.608-3.665 (dd, 1H, Ha of $-CH_2-$, J-4.0 Hz, 18.8 Hz), 3.128-3.203

(dd, 1H, Hb of -$CH_2$-, J-11.2 Hz, 18.8 Hz), 2.582 (s, 3H, -$CH_3$), 1.036-1.071 (t, 3H, -$CH_3$); MS (m/z): 465.2 ($M^+$+1).

**Ethyl-6-(3-fluorophenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclohex-3-ene carboxylate (5h):** Yield:82%; m.p. 168-172 ºC; Anal. calcd. for $C_{24}H_{21}FN_4O_5$: C,62.06; H, 4.56; N, 12.06 %. Found: C, 62.11, H, 4.64, N, 12.15 %; IR ($cm^{-1}$): 1727 (C=O stretching of ester), 1676 (C=O stretching of Cyclohexenone), 1612 (C=C stretching of Cyclohexenone), 1533 & 1341 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.473-8.494 (d, 2H, ArH, J-8.4 Hz), 7.717-7.738 (d, 2H, ArH, J-8.4 Hz), 7.303-7.356 (m, 1H, ArH), 6.948-7.14 (m, 3H, ArH), 6.463 (s, 1H,=CH-), 4.07-4.125 (q, 2H, -$CO_2CH_2$-), 3.827-3.862 (dd, 2H, -CH-, J-3.6 Hz, 10.4 Hz), 3.762-3.793 (d, 1H, -CH-, J-12.4 Hz), 3.635-3.692 (dd, 1H, Ha of -$CH_2$-, J-4.2 Hz, 18.5 Hz), 3.006-3.08 (dd, 1H, Hb of -$CH_2$-, J-10.8 Hz, 18.4 Hz), 2.578 (s, 3H, -$CH_3$), 1.071-1.104 (t, 3H, -$CH_3$); MS (m/z): 465.2 ($M^+$+1).

**Ethyl-6-(p-tolyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxo-cyclohex-3-ene carboxy late (5i):** Yield:79%; m.p. 166-170 ºC; Anal. calcd. for $C_{25}H_{24}N_4O_5$: C, 65.21; H, 5.25; N, 12.17 %. Found: C, 65.31, H, 5.34, N, 12.23 %; IR ($cm^{-1}$): 1743 (C=O stretching of ester), 1662 (C=O stretching of Cyclohexenone), 1610 (C=C stretching of Cyclohexenone), 1532 & 1343 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.467-8.488 (d, 2H, ArH, J-8.4 Hz), 7.712-7.734 (d, 2H, ArH, J-8.8 Hz), 7.21-7.229 (d, 2H, ArH, J-7.6 Hz), 7.14-7.16 (d, 2H, ArH, J-8.0 Hz), 6.46 (s, 1H,=CH-), 4.049-4.101 (q, 2H, -$CO_2CH_2$-), 3.809-3.899 (m, 2H, -CH-, -CH-), 3.592-3.642 (dd, 1H, Ha of -$CH_2$-, J-1.2 Hz, 18.8 Hz), 3.01-3.076 (dd, 1H, Hb of -$CH_2$-, J-8.0 Hz, 18.4 Hz), 2.567 (s, 3H, -$CH_3$), 2.337 (s, 3H, -$CH_3$), 1.059-1.094 (t, 3H, -$CH_3$); MS (m/z): 461.2 ($M^+$+1).

**Ethyl-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-6-(3-nitrophenyl)-2-oxocyclohex-3-ene carboxylate (5j):** Yield:78%; m.p. 158-163 ºC; Anal. calcd. for $C_{24}H_{21}N_5O_7$: C, 58.65; H, 4.31; N, 14.25 %. Found: C, 58.76, H, 4.39, N, 14.33 %; IR ($cm^{-1}$): 1741 (C=O stretching of ester), 1660 (C=O stretching of Cyclohexenone), 1609 (C=C stretching of Cyclohexenone), 1538 & 1345 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.477-8.498 (d, 2H, ArH, J-8.4 Hz), 8.164-8.252 (m, 2H, ArH), 7.726-7.748 (d, 2H, ArH, J-8.8 Hz), 7.702 (s, 1H, ArH), 7.569-7.589 (d, 1H, ArH, J-8.0 Hz) 6.482 (s, 1H,=CH-), 4.066-4.117 (q, 2H, -$CO_2CH_2$-), 3.845-4.025 (m, 2H, -CH-, -CH-), 3.694-3.75 (dd, 1H, Ha of -$CH_2$-, J-4.0 Hz, 18.4 Hz), 3.067-3.141 (dd, 1H, Hb of -$CH_2$-, J-11.6 Hz, 18.0 Hz), 2.597 (s, 3H, -$CH_3$), 1.078-1.113 (t, 3H, -$CH_3$); MS (m/z): 492.2 ($M^+$+1).

**Ethyl-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-6-(4-nitrophenyl)-2-oxocyclohex-3-ene carboxylate (5k):** Yield:74%; m.p. 165-170 ºC; Anal. calcd. for $C_{24}H_{21}N_5O_7$: C, 58.65; H, 4.31; N, 14.25 %. Found: C, 58.74, H, 4.41, N, 14.35 %; IR ($cm^{-1}$): 1740 (C=O stretching of ester), 1661 (C=O stretching of Cyclohexenone), 1610 (C=C stretching of Cyclohexenone), 1536 & 1344 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.479-8.5 (d, 2H, ArH, J-8.4 Hz), 8.230-8.251 (d, 2H, ArH, J-8.4 Hz), 7.722-7.743 (d, 2H, ArH, J-8.4 Hz), 7.4-7.421 (d, 2H, ArH, J-8.4 Hz), 6.485 (s, 1H,=CH-), 4.07-4.121 (q, 2H, -$CO_2CH_2$-), 3.755-3.897 (m, 2H, -CH-, -CH-), 3.566-3.623 (dd, 1H, Ha of -$CH_2$-, J-4.0 Hz, 18.8 Hz), 3.092-3.166 (1H, Hb of -$CH_2$-, J-11.6 Hz, 18.0 Hz), 2.599 (s, 3H, -$CH_3$), 1.087-1.122 (t, 3H, -$CH_3$); MS (m/z): 492.2 ($M^+$+1).

**Ethyl-6-(2-(trifluoromethyl)phenyl)-4-(5-methyl-1-(4-nitrophenyl)-1H-1, 2, 3-triazol-4-yl)-2-oxocyclo hex-3-enecarboxylate(5l):** Yield:85%; m.p. 171-175 ºC; Anal. calcd. for $C_{25}H_{21}F_3N_4O_5$: C, 58.37; H, 4.11; N, 10.89 %. Found: C, 58.44, H, 4.18, N, 10.95 %; IR ($cm^{-1}$): 1736 (C=O stretching of ester), 1669 (C=O stretching of Cyclohexenone), 1617 (C=C stretching of Cyclohexenone), 1533 & 1341 (asymmetric and symmetric stretching of nitro); $^1$H-NMR (400 MHz, $CDCl_3$, δ/ ppm): 8.476-8.497 (d, 2H, ArH, J-8.4 Hz), 7.735-7.756 (d, 2H, ArH, J-8.4 Hz), 7.263-7.345 (m, 2H, ArH), 7.069-7.163 (m, 2H, ArH), 6.479 (s, 1H,=CH-), 3.97-4.022 (q, 2H, -$CO_2CH_2$-), 3.782-3.885 (m, 2H, -CH-, -CH-), 3.618-3.675 (dd, 1H, Ha of -

$CH_2$-, J-4.0 Hz, 18.8 Hz), 3.137-3.212 (dd, 1H, Hb of -$CH_2$-, J-11.2 Hz, 18.8 Hz), 2.595 (s, 3H, -$CH_3$), 1.056-1.091 (t, 3H, -$CH_3$); MS (m/z): 515.2 ($M^+$+1).

## APPLICATIONS

**Antimicrobial activity:** The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains, namely *Pseudomonas, Escherichia coli* (Gram negative), *Enterococcus, Staphylococcus aureus* (Gram positive) by agar well diffusion method [18] by dissolving compounds in DMSO (Dimethyl sulfoxide). Ciprofloxacin was used as the standard. Also the compounds were screened for antifungal studies against fungus Candida *albicans* using Flucanazol as standard drug. The antibacterial and antifungal activities were evaluated by measuring zone of inhibition surrounding the compounds at a concentration of 200µg $mL^{-1}$. The results of antibacterial and antifungal activities are summarized in table-2.

**Table-2:** Antimicrobial and antifungal activities of compounds 5a-j

| Compound No | Antibacterial activity | | | | Antifungal activity |
|---|---|---|---|---|---|
| | Diameter of zone of inhibition (at 200µg $mL^{-1}$) | | | | |
| | **Pseudomonas** | **S *aureus*** | **Enterococcus** | **E *coli*** | **C albicans** |
| 5a | - | 9mm | - | - | - |
| 5b | - | - | 11mm | 9mm | 9mm |
| 5c | 12mm | - | 8mm | - | - |
| 5d | 10mm | 11mm | 10mm | 8mm | - |
| 5e | - | - | 11mm | 8mm | - |
| 5f | 11mm | - | - | - | - |
| 5g | 10mm | - | 11mm | - | - |
| 5i | 11mm | 10mm | 12mm | 8mm | - |
| 5j | 11mm | - | 13mm | - | - |
| Ciprofloxacin (Std) | 24mm | 24mm | 23mm | 23mm | - |
| Flucanazol (Std) | - | - | - | - | 25mm |
| Solvent control | - | - | - | - | - |

**Antioxidant activity:** The free radical-scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The molecules capable of donating H atoms, neutralizes the radical character [19]. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. This was expressed as the inhibition percentage, and butylated hydroxyanisole (BHA) was employed as standard. All tests were performed in triplicate at a concentration of 200µg $mL^{-1}$ and the results were expressed as mean values ± standard deviations. Results are shown in table-3.

**Table-3** - Percentage inhibition of compounds 5a-l on DPPH radical (200µg m $L^{-1}$)

| Compound No | Percentage inhibition |
|---|---|
| 5b | 15.82±0.12 |

| | |
|---|---|
| 5c | 23.82±0.23 |
| 5d | 18.92±0.31 |
| 5e | 23.90±0.59 |
| 5f | 38.44±0.64 |
| 5g | 31.73±0.15 |
| 5h | 24.76±0.09 |
| 5i | 3.10±0.05 |
| 5j | 45.92±0.19 |
| 5l | 32.93±0.14 |
| BHA | 25.02±0.02 |

## CONCLUSIONS

Most of the newly synthesized cyclohexenones (**5c, 5e-h, 5j, 5l**) displayed significant antioxidant property comparable with that of standard BHA. The data of antibacterial studies showed moderate to low activity by most of these molecules against all the four bacteria, and **5b** showed moderate activity against fungus Candida *albicans*.

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