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# SYNTHESIS OF SOME NEW 1,2,4-TRIAZOLES DERIVED FROM CYCLOHEX-2-ENONE HYDRAZIDE AS POTENTIAL ANTIMICROBIAL AND ANTITUMOR AGENTS

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**ABSTRACT**

6-(1,2,4-triazol-3-thioxo-5-yl)-3,5-diaryl cyclohex-2-enone **(4)** was obtained from the reaction of cyclohexenone acid hydrazide **(2)** with phenyl isothiocyanate. 2-substituted oxadiazole derivatives **(9), (12)** have been synthesized. The treatment of **(8)** with carbonyl compounds was investigated. The propionitrile derivative **(5)** was synthesised. The antimicrobial activity of **(6, 9, 10 and 12)** against fungi, Gram-positive and Gram-negative bacteria was screened and the results were encouraging. The antitumor activity of against human cell lines was investigated. The structures of compounds **(4-13)** were elucidated by means of microanalysis as well as spectral measurements such as IR, $^{1}$H-, $^{13}$C-NMR and MS.

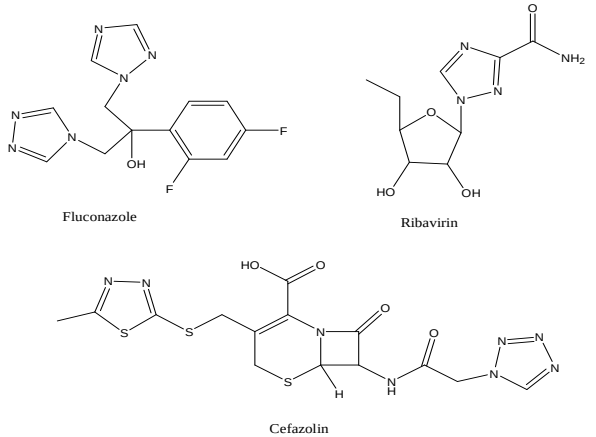
**KEYWORDS:** 1,2,4-triazol-2-thione; 1,3,4-oxadiazole; Cyclohex-2-enone.

## 1. INTRODUCTION

Ribavirin, fluconazole and cefazolin are antiviral, antifungal and antibacterial drugs which contain 1,2,4-triazole and 1,3,4-thiadiazole rings (Fig. 1).

Moreover, owing to the increasing biological importance of 1,3,4-oxadiazoles, particularly in the field of chemotherapy and the potent antimicrobial activity of 1,2,4-triazoles[1,2] and 1,3,4-thiadiazoles[3,4], they have occupied a unique position in heterocyclic chemistry. Derivatives of

1,3,4-thiadiazole and 1,2,4-triazole are known to exhibit anti-inflammatory, antiviral, antimicrobial[5-7] and antidepressant[8] activity.



Fluconazole

Ribavirin

Cefazolin

Fig. 1

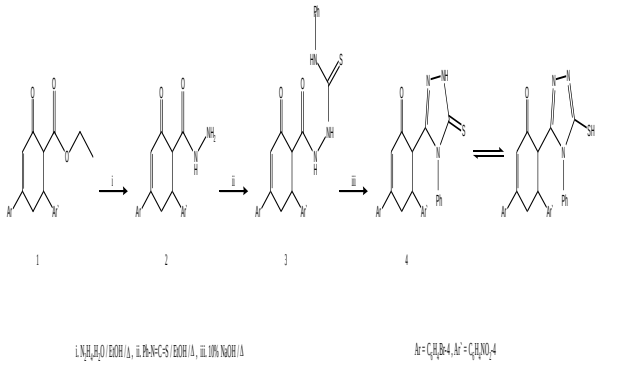
A large number of compounds containing 1,2,4-triazole system have been investigated as therapeutically interesting drug candidates because of their properties as antimicrobial and antimycotic agents.[9-11]

Among the 1,2,4-triazole derivatives are the mercapto- and the thione-substituted 1,2,4-triazole system have been studied and so far a variety of antitumor properties have been reported for a large number of these compounds.[12-14]

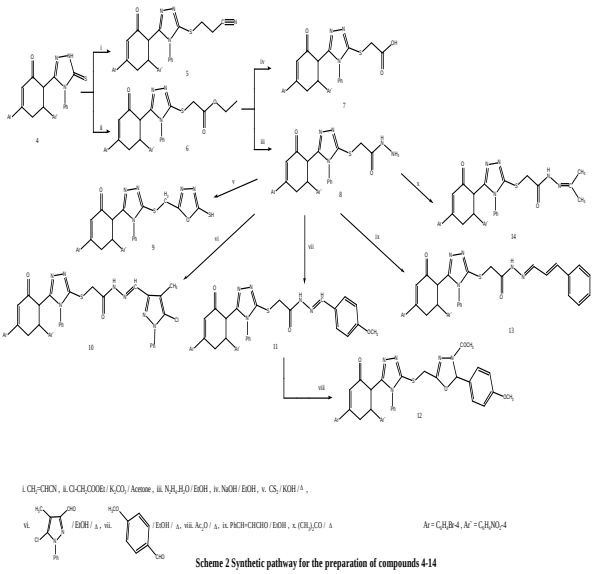
The similarity in the structure of the 2-amino-1,3,4-thiadiazole and the mercapto- and thione-substituted 1,2,4-triazole ring systems assumes similar biological properties and so far a variety of antitumor properties have been reported for a large number of these compounds.

We report here the synthesis, the antimicrobial and the cytotoxicity of some 1,2,4-triazolyl cyclohexenone derivatives against tumor cells. The choice of these structures was in accordance with the fact that cyclohexenone moiety takes part in the structure of substances possessing antimicrobial and antitubercular activity[15,16], therefore, it was of pharmacological interest to incorporate a cyclohexenone moiety in the structure of 5-mercapto-1,2,4-triazole[17] emphasizing in particular the strategy of combining two chemically different but pharmacologically compatible molecules (the cyclohexenone and the five-membered heterocycles) in one frame.[18]

In view of the pharmacological profiles of these two chemical moieties, as described above, we considered it interesting to further design and explore the synthesis as well as the biological properties of compounds **(4-14)**.



Scheme 1 Synthetic pathway for the preparation of the target molecule 4



Scheme 2 Synthetic pathway for the preparation of compounds 4-14

## 2. Chemistry

The synthetic pathway followed for the preparation of the target molecules **(1-14)** is depicted in schemes (1,2). 6-carbethoxy-3,5-diaryl cyclohex-2-enone **(1)** was synthesized according to the procedure described in ref.[18]

The interaction of 6-ethoxy carbonyl cyclohex-2-enone with hydrazine hydrate in ethanol medium led to the hydrazide **(2)** in 73% yield. The hitherto unknown thiosemicarbazide **(3)** was obtained upon the reaction of the acid hydrazide **(2)** with phenyl isothiocyanate in ethanol. Cyclization of **(3)** with sodium hydroxide resulted in the formation of target molecule **(4)**.

The acetic acid ethyl ester derivative **(6)** was obtained by treating **(4)** with ethyl chloroacetate in boiling acetone containing anhydrous potassium carbonate (for 24 hrs.). By heating this ethyl ester with hydrazine hydrate in ethanol, it yielded the acid hydrazide **(8)**. The MS agreed well with the proposed structure (Fig. 2). Alkaline hydrolysis of compound **(6)** using alcoholic sodium hydroxide afforded the corresponding acid **(7)**. The reactivity of compound **(4)** was also tested against active olefinic compounds. Thus, reaction of **(4)** with acrylonitrile in boiling pyridine afforded the corresponding propionitrile **(5)**. However in this study we sought to combine the 1,2,4-triazole nucleus and the 1,3,4-oxadiazole nucleus to study the effect of this combination on the biological activity of the product. Thus, reacting the acid hydrazide **(8)** with carbon disulphide in the presence of potassium hydroxide affected cyclization to the corresponding 1,3,4-oxadiazole derivative **(9)**.

The reaction of the hydrazide derivative **(8)** with carbonyl compounds namely, 5-chloro-4-methyl-1-phenyl-1H-pyrazol-3-formyl, o-methoxy benzaldehyde, cinnamaldehyde and/or acetone yielded the corresponding arylidene products **(10, 11 and 13)** and the isopropylidene derivative **(14)**. The MS spectrum of the arylidene hydrazide derivative **(10)** agreed well with the proposed structure (Fig. 3). Interaction of the benzylidene hydrazide derivative **(11)** with acetic anhydride affected cyclization to the corresponding [1,3,4] oxadiazol-2-methylsulpharyl-4-phenyl-4H-[1,2,4] triazolyl derivative **(12)**.

## 3. Experimental section

### 3.1. General instrumentation

Melting points were taken in glass capillary tubes on a Gallen Kamp apparatus and are uncorrected. Infra red spectra were measured as potassium bromide pellets using a Perkin-Elmer 883 spectrophotometer. The $^{1}$HNMR and $^{13}$CNMR spectra were recorded on Bruker Ac 200 and 300 MHz spectrometers. Chemical shift (δ) values were expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained for compounds from a JEOLJMS. 700 magnetic sector instrument (70 eV). Microanalysis of compounds were within ±0.4% of theoretical values and the spectral data (IR, NMR and mass) were compatible with the assigned structures.

### 3.2. Chemistry

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid ethyl ester (6).**

A mixture of the triazolyl derivative **(4)** (0.01 mol.), ethyl chloroacetate (0.02 mol.) and anhydrous potassium carbonate (0.04 mol.) in 50 ml of dry acetone was heated on a water bath for 24 hours, then filtered while hot. After concentration and cooling, the product was collected, washed well with dilute alcohol and recrystallized from acetone to give **(6)** in 40% yield; m.p. 101-102˚C. IR (KBr, cm$^{-1}$): 1730 (C=O); 1672 (C=O); 1629 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 15.3 ($CH_3$); 33.5 (<u>CH</u>-$CH_2$); 40.1 (<u>$CH_2$</u>-CH); 40.7 (S-<u>$CH_2$</u>-CO); 52.9 (<u>CH</u>-CH); 55.9 (O-<u>$CH_2$</u>-$CH_3$); 116.8 (CH=C); 121.1, 121.7, 122.3, 123.7, 127.2, 128.4, 129.1, 129.4, 129.7

(CH-aromatic); 133.7 (C-C aromatic); 149.1 (N=C-S); 149.6 (C-$NO_2$); 155.3 (C-Br); 176.1 (C=O); 189.6 (C=O).

Anal. Calcd. (%) for $C_{30}H_{25}N_4O_5SBr$ (633). C, 56.87; H, 3.95; N, 8.85; S, 5.06; Br, 12.64. Found: C, 56.9; H, 4.0; N, 8.9; S, 5.1; Br, 12.8.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid hydrazide (8).**
A mixture of the ester derivative **(6)** (0.01 mol.) and hydrazine hydrate (0.01 mol.) in 30 ml of absolute ethanol was heated under reflux for 6 hours then filtered off while hot. After concentration and cooling, the product was collected by filtration and washed well with dilute ethanol then recrystallized from ethanol to give **(8)** in 74% yield; m.p. 156˚C. IR (KBr, $cm^{-1}$): 3333 (NH); 3220 ($NH_2$); 1679 (C=O); 1661 (C=O); 1598 (C=N). MS: molecular ion peak at m/e 619 (3.15%); molecular fragments at m/e 77 (100%) for $C_6H_5^{\bullet}$; m/e 92 (24.7%) for $C_6H_4O^{\bullet}$; m/e 76 (35.5%) for $C_2H_4OS^{\bullet}$; m/e 130 (6.7%) for $C_3H_4N_3OS^{\bullet}$; m/e 158 (6.6%) for $C_4H_6N_4OS^{\bullet}$; m/e 249 (5.9%) for $C_{10}H_{11}N_5OS^{\bullet}$.

Anal. Calcd. (%) for $C_{28}H_{23}N_6O_4SBr$ (619). C, 54.28; H, 3.72; N, 13.57; S, 5.17; Br, 12.92. Found: C, 54.3; H, 3.8; N, 13.7; S, 5.3; Br, 13.1.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid (7).**
To a suspension of the ester **(6)** (0.01 mol.) in 30 ml of absolute ethanol, was added 15 ml of 10% aqueous sodium hydroxide and the mixture was refluxed for 6 hours, filtered while hot then acidified with dilute hydrochloric acid and left to cool. The product was collected, washed well with dilute alcohol then recrystallized from pet. ether-ethanol mixture to give **(7)** in 67% yield; m.p. 155˚C. IR (KBr, $cm^{-1}$): 3402.6 (OH); 1686 (C=O); 1662 (C=O); 1595 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 33.3 (CH-$CH_2$); 40.7 ($CH_2$-CH); 41.9 (S-$CH_2$-CO); 55.1 (CH-CH); 116.9 (CH=C); 121.1, 121.3, 122.7, 123.3, 126.7, 127.8, 129.1 (CH-aromatic); 133.9 (C-C aromatic); 146.9 (N=C-S); 147.1 (C-$NO_2$); 155.3 (C-Br); 186.9 (C=O); 197.6 (C=O).

Anal. Calcd. (%) for $C_{28}H_{21}N_4O_5SBr$ (605). C, 55.54; H, 3.47; N, 9.26; S, 5.30; Br, 13.22. Found: C, 55.4; H, 3.4; N, 9.3; S, 5.5; Br, 13.3.

**3-{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} propionitrile (5).**
To a suspension of **(4)** (0.01 mol.) in 30 ml of dry pyridine, was added acrylonitrile (0.01 mol.) and the mixture was refluxed for 6 hours, filtered while hot then neutralized with ice-conc. hydrochloric acid. The product was collected, washed well with water then dilute alcohol, collected by filtration and recrystallized from acetic acid to give **(5)** in 80% yield; m.p. 121-122˚C. IR (KBr, $cm^{-1}$): 1715.7 (C=O); 2223 (C≡N); 1600.1 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 19.3 ($CH_2$-CN); 30.7 (S-$CH_2$-$CH_2$); 33.5 (CH-$CH_2$); 41.1 ($CH_2$-CH); 54.3 (CH-CO); 117.9 (C≡N); 118.1 (CH=C); 122.3, 123.3, 126.3, 126.6, 127.2, 128.2, 128.4, 129.1, 131.7 (CH-aromatic); 133.9 (C-C aromatic); 145.6 (C-$NO_2$); 149.6 (N=C-S); 154.3 (C-Br); 155.6 (C-C aromatic); 196.7 (C=O).

Anal. Calcd. (%) for $C_{29}H_{22}N_5O_3SBr$ (600). C, 58.00; H, 3.67; N, 11.67; S, 5.33; Br, 13.33. Found: C, 58.01; H, 3.7; N, 11.8; S, 5.3; Br, 13.5.

**3-(4-bromophenyl)-6-[5-(5-mercapto-[1,3,4]oxadiazole-2-yl methyl sulphanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-5-(4-nitrophenyl)-cyclohex-2-enone (9).**
To a suspension of **(8)** (0.01 mol.) in 30 ml of absolute ethanol, was added 10 ml of carbon disulphide and potassium hydroxide (0.01 mol., 5 g dissolved in 5 ml of water) and the mixture was heated on a water bath for 6 hours, filtered while hot, left to cool then neutralized with few drops of hydrochloric acid, extracted with ether and the ethereal layer was washed well with water then dried (over anhydrous magnesium sulphate) and the solvent was evaporated. The residue was recrystallized from petroleum ether-ethanol mixture to give **(9)** in 55% yield; m.p. 145-146˚C. IR (KBr, $cm^{-1}$): 3330 (NH); 1630 (C=N); 2640 (w) (SH). $^{13}$C-NMR (DMSO-d6, δ ppm): 33.2 (CH-$CH_2$); 34.6 (CH2-S); 41.7 ($CH_2$-CH); 116.7 (CH=C); 121.6, 122.3, 126.5, 127.2, 128.4, 129.7 (CH-aromatic); 133.8 (C-C aromatic); 145.9 (N-C-S); 146.7 (C-$NO_2$); 155.9 (C-Br); 160.7 (C-O); 162.1 (O-C-SH); 196.7 (C=O).

Anal. Calcd. (%) for $C_{29}H_{21}N_6O_4S_2Br$ (661). C, 52.65; H, 3.18; N, 12.71; S, 9.68; Br, 12.10. Found: C, 52.7; H, 3.2; N, 12.8; S, 9.8; Br, 12.3.

**General procedure for the reaction of {5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid hydrazide (8) with carbonyl compounds.**
A mixture of the hydrazide derivative **(8)** (0.01 mol.) and carbonyl compounds namely, 5-chloro-4-methyl-1-phenyl-1H-pyrazol-3-carbaldehyde, anisaldehyde, cinnamaldehyde and/or acetone (0.01 mol.) in 30 ml of absolute ethanol was heated under reflux for 6 hours then filtered while hot, left to cool and the solvent was evaporated (under reduced pressure). The product that separated was collected, washed well with dilute alcohol then recrystallized from the proper solvent to give **(10, 11, 13 and 14)**.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid (5-chloro-4-methyl-1-phenyl-1H-pyrazol-3-yl methylene) hydrazide (10).**
Yield 65%; m.p.176˚C. IR (KBr, $cm^{-1}$): 3320 (NH); 1681 (C=O); 1667 (C=O); 1606 (C=N). MS: $M^{\bullet}$ at m/e 822

(1.13%) for $C_{39}H_{30}N_8O_4SClBr$ (0.13%) and the base peak m/e 77 (100%) for $C_6H_5^{\neg\ddagger}$; m/e 268 for $C_{14}H_{10}N_3OS^{\neg\ddagger}$; m/e 282 for $C_{14}H_{10}N_4OS^{\neg\ddagger}$; m/e 341 for $C_{16}H_{13}N_4O_3S^{\neg\ddagger}$; m/e 383 for $C_{16}H_{15}N_5O_4S^{\neg\ddagger}$.

Anal. Calcd. (%) for $C_{39}H_{30}N_8O_4SClBr$ (821.5). C, 56.97; H, 3.65; N, 13.63; S, 3.90; Cl, 4.32; Br, 9.74. Found: C, 57.1; H, 3.7; N, 13.5; S, 4.1; Cl, 4.2; Br, 9.8.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid (2-methoxy benzylidene) hydrazide (11).**
Yield 69%; m.p. 167˚C. IR (KBr, $cm^{-1}$): 3330 (NH); 1681 (C=O); 1661 (C=O); 1624 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 33.1 (CH-$CH_2$); 41.7 ($CH_2$-CH); 43.7 (S-$CH_2$-CO); 55.1 (CH-CO); 56.7 (O-$CH_3$); 117.8 (CH=C); 118.8, 120.9, 123.2, 127.2, 128.4, 129.1, 131.1, 131.7 (CH-aromatic); 134.9 (C-C aromatic); 152.3 (C-Br); 154.7 (CH=N); 161.1 (C=N); 161.7 (C-$NO_2$); 162.5 (C-O); 173.1 (C=O); 189.8 (C=O).

Anal. Calcd. (%) for $C_{36}H_{29}N_6O_5SBr$ (737). C, 58.62; H, 3.93; N, 11.40; S, 4.34; Br, 10.85. Found: C, 58.6; H, 4.0; N, 11.5; S, 4.3; Br, 10.9.

**6-{5-[4-acetyl-5-(2-methoxyphenyl)-4,5-dihydro-[1,3,4]oxadiazole-2-yl methyl sulphanyl]-4-phenyl-4H-[1,2,4]triazol-3-yl}-3-(4-bromophenyl)-5-(4-nitrophenyl)-cyclohex-2-enone (12).**
A suspension of compound **(11)** in 15 ml of acetic anhydride was heated on a water bath for 2 hours (till clear solution), cooled and the product was filtered off, washed well with dilute alcohol then recrystallized from ethanol as brown crystals. Yield 63%; m.p179˚C. IR (KBr, $cm^{-1}$): 1737.2 (C=O, acetyl); 1666 (C=O); 1630 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 19.9 ($CH_3$-CO); 33.3 (CH-$CH_2$); 41.7 ($CH_2$-CH); 42.7 ($CH_2$-S); 53.1 (CH-CO); 56.7 (O-$CH_3$); 66.3 (N-CH-O); 117.8, 120.5, 127.5, 128.1, 128.4, 129.2, 130.3, 133.1 (CH-aromatic); 145.8 (C-$NO_2$); 155.3 (N=C-O); 155.7 (C-Br); 160.6 (C-O); 197.6 (C=O).

Anal. Calcd. (%) for $C_{38}H_{31}N_6O_6SBr$ (779). C, 58.54; H, 4.00; N, 10.78; S, 4.11; Br, 10.27. Found: C, 58.5; H, 4.1; N, 11.1; S, 4.3; Br, 10.3.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid (3-phenyl allylidene) hydrazide (13).**
Yield 65%; m.p. 140-141˚C. IR (KBr, $cm^{-1}$): 3332 (NH); 1662 (C=O); 1628 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 33.1 (CH-$CH_2$); 41.6 ($CH_2$-CH); 42.7 (CO-$CH_2$-S); 53.9 (CH-CO); 117.8, 122.7, 126.3, 127.2, 127.7, 128.4, 129.1 (CH-aromatic); 136.6, 136.9 (2xCH=); 153.3 (C-Br); 154.7 (CH=N); 159.9 (N=C); 160.6 (C-$NO_2$); 174 (C=O); 189.7 (C=O).

Anal. Calcd. (%) for $C_{37}H_{29}N_6O_4SBr$ (733). C, 60.57; H, 3.96; N, 11.46; S, 4.37; Br, 10.91. Found: C, 60.1; H, 4.0; N, 11.5; S, 4.5; Br, 11.1.

**{5-[4-(4-bromophenyl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enyl]-4-phenyl-4H-[1,2,4]triazol-3-yl sulphanyl} acetic acid isopropylidene hydrazide (14).**
Yield 71%; m.p. 148-149˚C. IR (KBr, $cm^{-1}$): 3330 (NH); 1663 (C=O); 1626 (C=N). $^{13}$C-NMR (DMSO-d6, δ ppm): 19.6, 23.1 (2x$CH_3$); 37.1 (CH-$CH_2$); 40.1 ($CH_2$-CH); 42.7 (CO-$CH_2$-S); 54.9 (CH-CO); 117.9, 122.7, 123.8, 127.2, 128.4, 129, 129.3, 131.7 (CH-aromatic); 133.5, 145.6, 154.8 (C-C aromatic); 155.1 (C-$NO_2$); 155.8 (C=N); 161.6 (N=C); 162.7 (C-Br); 177.1 (NH-C=O); 197.8 (C=O, cyclohexyl).

Anal. Calcd. (%) for $C_{31}H_{27}N_6O_4SBr$ (659). C, 56.54; H, 4.10; N, 12.75; S, 4.86; Br, 12.14. Found: C, 56.5; H, 4.2; N, 12.8; S, 4.9; Br, 12.3.

$^1$HNMR data for compounds **(5-14)** are listed in Table 1.

**Table 1: $^1$HNMR spectral data of the 1,2,4-triazolyl derivatives (5-14).**

| Compound | $^1$HNMR |
|---|---|
| 5 | 2.25(d, 2H, $CH_2$-CH); 2.90(t, 2H, $CH_2$-CN); 3.34(t, 2H, $CH_2S$); 3.44(d, 1H, CH-CH); 4.04(d, 1H, CH-CH); 6.66(s, 1H, CH=); 7.10-8.16(m, 13H, Ar-H) |
| 6 | 1.29(t, 3H, $CH_2CH_3$); 2.46(d, 2H, $CH_2$-CH); 3.35(s, 2H, $COCH_2S$); 3.45(d, 1H, CH-CH); 4.21(q, 2H, $CH_2CH_3$); 4.61(d, 1H, COCH-CH); 6.71(s, 1H, CH=); 7.09-8.109(m, 13H, Ar-H) |
| 7 | 2.27(d, 2H, $CH_2$-CH); 3.43(d, 1H, CH-CH); 3.87(s, 2H, $COCH_2S$); 4.07(d, 1H, CH-CH); 6.67(s, 1H, CH=); 7.19-8.19(m, 13H, Ar-H); 10.99(s, 1H, OH) |
| 8 | 2.28(d, 2H, $CH_2$-CH); 3.41(s, 2H, $COCH_2S$); 3.46(d, 1H, CH-CH); 4.65(d, 1H, COCH-CH); 6.71(s, 1H, CH=); 7.19-8.11(m, 13H, Ar-H), 9.12(d, 2H, $NH_2$); 10.01(t, 1H, NH)($D_2O$ exchangeable) |

| | |
|---|---|
| 9 | 2.29(d, 2H, $\underline{CH_2}$-CH); 3.41(t, 1H, $\underline{CH}$-$CH_2$); 4.03(d, 1H, CH-CH); 4.21(s, 2H, $CH_2$-S); 4.66(s, 1H, SH); 6.65(s, 1H, CH=); 7.17-8.19(m, 13H, Ar-H); 9.91(s, 1H, NH) |
| 10 | 1.91(s, 3H, $CH_3$ pyrazole); 2.29(d, 2H, $\underline{CH_2}$-CH); 3.45(t, 1H, $\underline{CH}$-$CH_2$); 4.01(s, 2H, $COCH_2S$); 4.09(d, 1H, CH-CH); 6.65(s, 1H, CH=); 6.91(s, 1H, CH=N); 7.12-8.19(m, 18H, Ar-H); 9.21(s, 1H, NH) |
| 11 | 2.31(d, 2H, $\underline{CH_2}$-CH); 3.43(t, 1H, $\underline{CH}$-$CH_2$); 3.71(s, 3H, $OCH_3$-Ar); 3.82(s, 2H, $COCH_2S$); 4.09(d, 1H, CH-CH); 6.64(s, 1H, CH=); 6.91-8.13(m, 17H, Ar-H); 9.92(s, 1H, NH) |
| 12 | 2.19(s, 3H, $COCH_3$); 2.35(d, 2H, $\underline{CH_2}$-CH); 3.37(t, 1H, $\underline{CH}$-$CH_2$); 3.70(s, 3H, $OCH_3$-Ar); 3.72(s, 2H, $CH_2S$); 4.07(d, 1H, CH-CH); 6.64(s, 1H,CH=); 6.71(s, 1H, N-CH-O); 6.75-8.15(m, 17H, Ar-H) |
| 13 | 2.29(d, 2H, $\underline{CH_2}$-CH); 3.41(t, 1H, $\underline{CH}$-$CH_2$); 3.75(s, 2H, $COCH_2S$); 4.06(d, 1H, CH-CH); 4.35(d, 1H, CH=CH); 5.96(d, 1H, CH=CH); 6.65(s, 1H, CH=); 6.96(d, 1H, CH-$\underline{CH}$=N); 7.09-8.12(m, 18H, Ar-H); 9.91(s, 1H, NH) |
| 14 | 1.01, 1.02(2xs, 2x3H, 2x$CH_3$); 2.30(d, 2H, $\underline{CH_2}$-CH); 3.71(s, 2H, $COCH_2S$); 4.01(d, 1H, CH-CH); 4.31(d, 1H, CH-CH); 6.35(s, 1H, CH=); 7.01-8.11(m, 13H, Ar-H); 9.09(s, 1H, NH) |

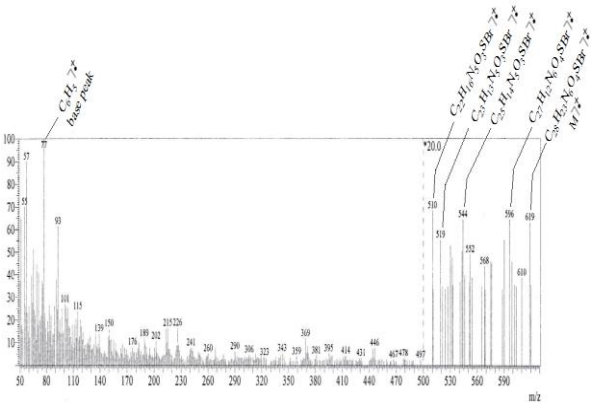


**Fig. 2: Fragmentation pattern of compound 8**

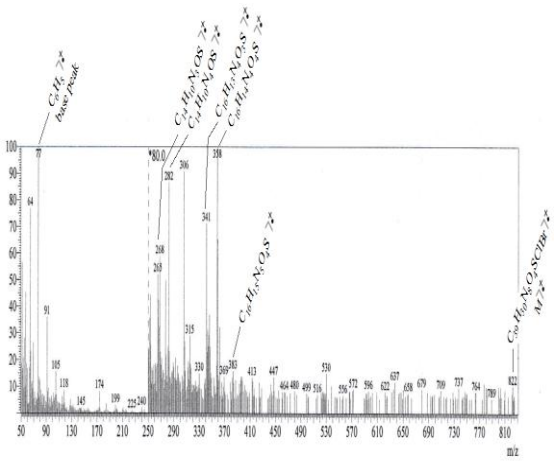


**Fig. 3: Fragmentation pattern of compound 10**

## 4. Biological assays

### 4.1. Antimicrobial evaluation

Among the synthesized triazolyl derivatives **(4-14)**, four of them **(6, 9, 10, 12)** were screened in vitro for their antimicrobial activity against laboratory strains totally microbial cultures isolates of Gram-positive, Gram-negative bacteria and fungi that used in this study by using microwell dilution assay[19,20] and MIC agar dilution assay[20], the results are given in Tables 3,4. All bacterial and yeast strains were obtained from The Regional Center for Mycology and Biotechnology (RCMB) (The Antimicrobial Activity Unit), Al-Azhar University (Nasr city, Cairo, ARE) and were as follows: E.coli (RCMBA 5003), Pseudomonas aeruginosa (RCMBA 1002), Staphylococcus aureus (RCMBA 2004), Bacillus subtilis (RCMBA 6005), Aspergillus fumigatus (RCMBA 06002) and Candida albicans (RCMBA 05035). All the tested compounds were dissolved in dimethyl sulphoxide (DMSO) to prepare chemicals stock solution of 10 mg/1 ml. Standard drugs namely, Amphotericin B, Penicillin G and Streptomycin were used for comparison purpose. Agar-well diffusion method. Simple susceptibility screening test using agar-well diffusion method[19] as adapted earlier[20] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately $10^6$ colony forming unit (cfu) per ml. They were "flood-inoculated" onto the surface of MH Agar and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) and then dried. For C.albicans and A.fumigatus, SDA was used. Five millimeter diameter wells were cut from the agar using a sterile cork-borer

and 50 µl of the extract substances were delivered into the wells. The plates were incubated for 18 hours at 35˚C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Amphotericin B, Penicillin G and Streptomycin were standard drugs. Dimethyl sulphoxide and ethanol were used as solvent control. The antimicrobial activity and the MIC results are summerized in Tables 2,3.

**Table 2: Antimicrobial activities of new synthesized compounds against the bacterial strains tested based on diffusion agar technique[a]**

| Microorganisms-Code[b] | Samples | | | | Standard Antibiotics | |
|---|---|---|---|---|---|---|
| | 6 | 9 | 10 | 12 | | |
| Aspergillus fumigatus (RCMBA 06002) | 21.9±0.58 | 22.4±1.3 | 23.7±0.58 | 17.3±0.58 | 25.9±0.53 | Amphotericin B |
| Candida albicans (RCMBA 05035) | NA | NA | NA | NA | 20.1±0.14 | |
| Staphylococcus aureus (RCMBA 2004) | 22.3±0.63 | 23.6±0.58 | 24.2±0.63 | 19.2±0.63 | 24.6±0.51 | Penicillin G |
| Bacillus subtilis (RCMBA 6005) | 23.8±1.2 | 24.2±0.63 | 26.3±1.2 | 20.3±1.2 | 26.4±0.42 | |
| Pseudomonas aeruginosa (RCMBA 1002) | 19.6±0.63 | 20.1±1.5 | 20.2±0.63 | 16.2±0.63 | 20.4±0.34 | Streptomycin |
| Escherichia coli (RCMBA 5003) | 22.5±1.5 | 23.3±2.1 | 25.3±1.5 | 18.3±1.5 | 26.7±0.19 | |

**Table 3: Antimicrobial activity as MICs* (µg/ml of tested compounds against tested microorganisms**

| Tested microorganism | Sample | | | Standard Antibiotics | |
|---|---|---|---|---|---|
| | 6 | 9 | 10 | | |
| Aspergillus fumigatus (RCMBA 06002) | 1.95 | 0.98 | 0.98 | 0.98 | Amphotericin B |
| Candida albicans (RCMBA 05035) | NA | NA | NA | 1.95 | |
| Staphylococcus aureus (RCMBA 2004) | 0.98 | 0.98 | 0.98 | 0.98 | Penicillin G |
| Bacillus subtilis (RCMBA 6005) | 0.98 | 0.49 | 0.49 | 0.49 | |
| Pseudomonas aeruginosa (RCMBA 1002) | 3.9 | 3.9 | 3.9 | 3.9 | Streptomycin |
| Escherichia coli (RCMBA 5003) | 0.98 | 0.98 | 0.49 | 0.49 | |

*Minimum inhibitory concentration (µg/ml).

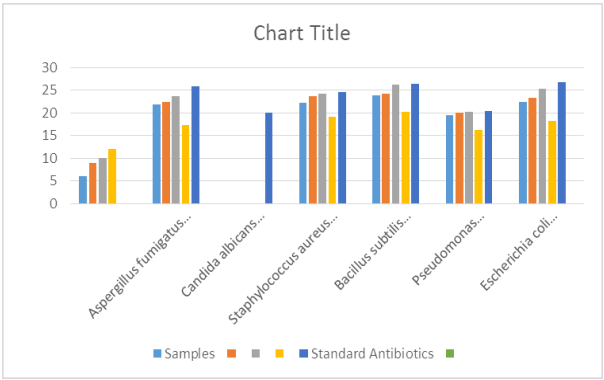



**Fig. 4: Antimicrobial activities of new synthesized compounds against the bacterial strains tested based on diffusion agar technique**

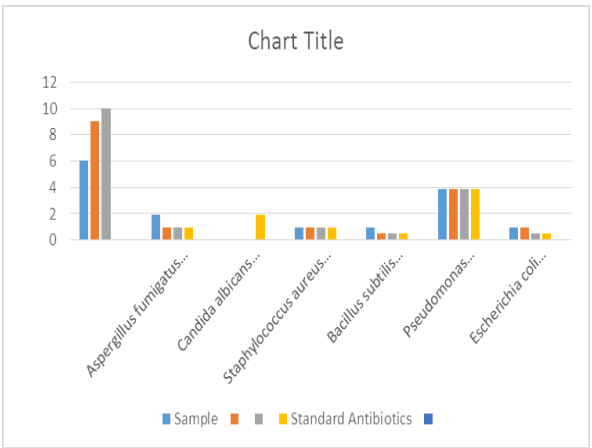



**Fig. 5: Antimicrobial activity as MICs (µg/ml) of tested compounds against tested microorganisms**

### 4.2. Tumor cell line screening

Among the synthesized triazole derivatives **(4-14)**, five of them **(4, 6, 9, 10, 12)** were tested at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University (Nasr city, Cairo, ARE) and tested initially at a single dose (10 µM) in the full RCMB cells panel derived from two clinically isolated cancer types (ovarian and breast)[21]. Compounds **(4, 9, 10)** resulted to be quite more active than the other derivatives **(6, 12)**. The $IC_{50}$ parameter is given for each cell line ($IC_{50}$ = compounds molar concentration inducing 50% net cell death) in Table 4.

**Table 4: In vitro antiproliferative activity against the RCMB cell lines panel derived from two clinically isolated human cancer types**

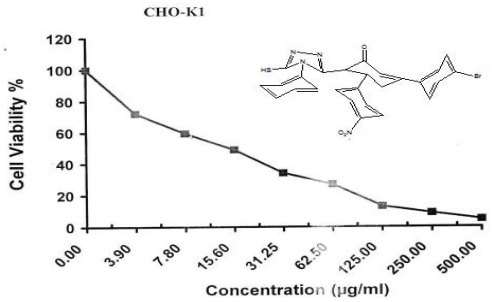
| Cancer types | Panel/cell line | $IC_{50}$ µg/ml | | | | |
|---|---|---|---|---|---|---|
| | | 4 | 6 | 9 | 10 | 12 |
| Ovarian | CHO-K1 | 14.8 | 220 | 62.1 | 57.1 | 366 |
| Breast | MCF-7 | 29.9 | 235 | 115 | 105 | 435 |

*$IC_{50}$ = compounds molar concentration inducing 50% net cell death.

## DISCUSSION

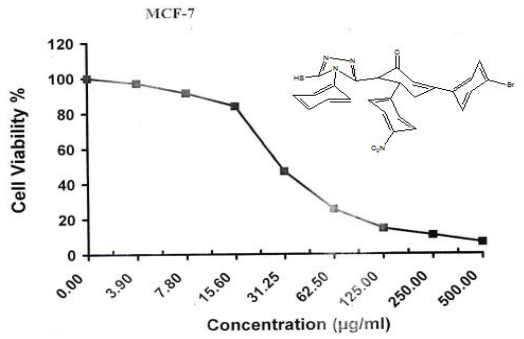
The pharmacological results of all the tested compounds showed that the 1,2,4-triazolyl derivative **(4)** produced the best antiproliferative profile. The remarkable difference of activity between **(4 and 6, 12)** is due only to the different nature of the substitution at position 5: thione⇌thiol for compound **(4)**, mercapto ethyl acetate for compound **(6)**, mercapto ethyl carbonitrile for compound **(9)**, hydrazide-amide linkage for compound

**(10)** and mercapto methyl oxadiazolo derivative **(12)**. As the 1,2,4-triazolyl derivative **(4)** is more lipophilic in character than derivative **(6, 9, 10 and 12)**, it seems that lipophilicity plays an important role in the antiproliferative activity. This trend was previously observed for the hydrazide derivative **(10)** and to a lesser extent for the carbonitrile derivative **(9)**. Compound **(4)** showed antiproliferative activity against the two types of cancer cell lines (ovarian and breast).




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 4.79 |
| 250 | 8.67 |
| 12.5 | 12.84 |
| 62.5 | 26.79 |
| 31.25 | 34.02 |
| 15.6 | 48.93 |
| 7.8 | 59.41 |
| 3.9 | 72.18 |
| 0 | 100 |

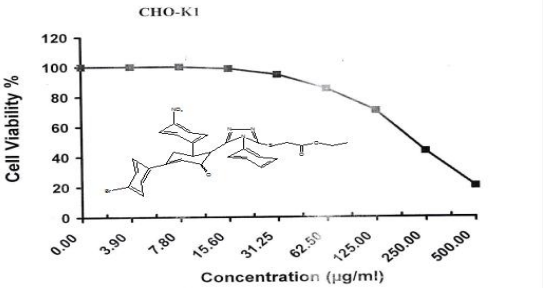
***Comment:***
*Inhibitory activity against Ovary carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 14.8 µg/ml.*




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 6.43 |
| 250 | 10.52 |
| 12.5 | 14.33 |
| 62.5 | 25.47 |
| 31.25 | 46.92 |
| 15.6 | 83.95 |
| 7.8 | 91.47 |
| 3.9 | 97.04 |
| 0 | 100 |

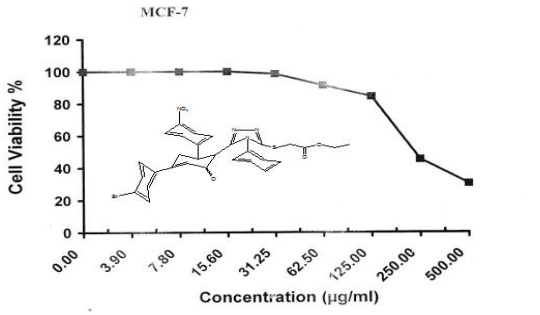
***Comment:***
*Inhibitory activity against Breast carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 29.9 µg/ml.*

**Fig. 6: Antitumor activities of compound (4) against ovary and breast carcinoma cell lines**




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 20.46 |
| 250 | 43.59 |
| 12.5 | 70.67 |
| 62.5 | 85.31 |
| 31.25 | 94.65 |
| 15.6 | 98.72 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

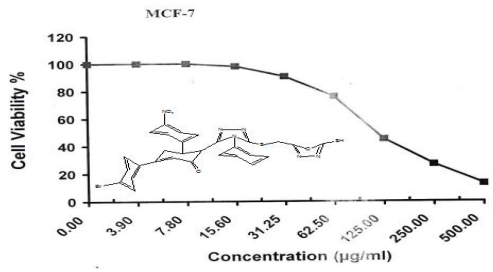
***Comment:***
*Inhibitory activity against Ovary carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 220 µg/ml.*




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 30.27 |
| 250 | 45.28 |
| 12.5 | 84.36 |
| 62.5 | 91.28 |
| 31.25 | 98.47 |
| 15.6 | 100 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

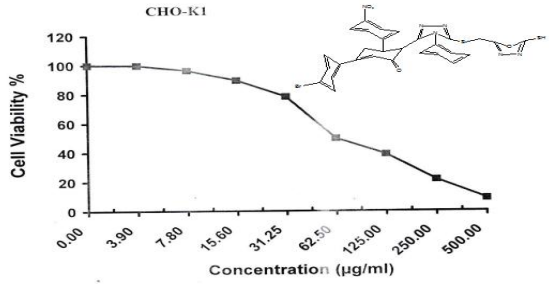
***Comment:***
*Inhibitory activity against Breast carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 235 µg/ml.*

**Fig. 7: Antitumor activities of compound (6) against ovary and breast carcinoma cell lines**




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 12.69 |
| 250 | 26.73 |
| 12.5 | 44.92 |
| 62.5 | 76.04 |
| 31.25 | 90.62 |
| 15.6 | 98.06 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

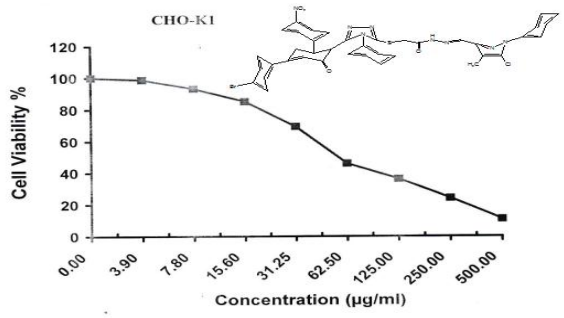
***Comment:***
*Inhibitory activity against Breast carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 115 µg/ml.*




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 8.54 |
| 250 | 21.36 |
| 12.5 | 38.75 |
| 62.5 | 49.64 |
| 31.25 | 78.19 |
| 15.6 | 89.45 |
| 7.8 | 96.24 |
| 3.9 | 99.72 |
| 0 | 100 |

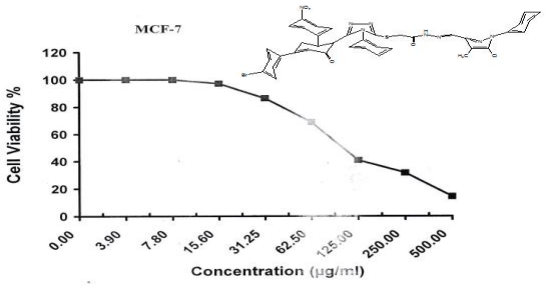
***Comment:***
*Inhibitory activity against Ovary carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 62.1 µg/ml.*

**Fig. 8: Antitumor activities of compound (9) against ovary and breast carcinoma cell lines**




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 10.78 |
| 250 | 24.02 |
| 12.5 | 36.15 |
| 62.5 | 45.93 |
| 31.25 | 69.41 |
| 15.6 | 85.13 |
| 7.8 | 93.22 |
| 3.9 | 98.75 |
| 0 | 100 |

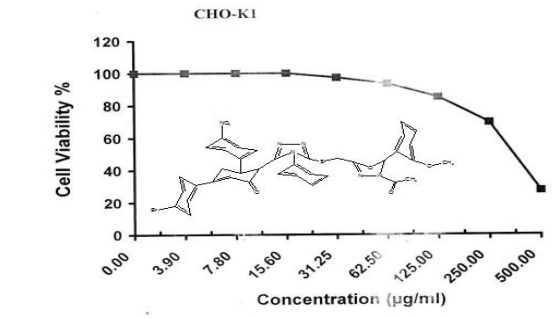
***Comment:***
*Inhibitory activity against Ovary carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 57.1 µg/ml.*




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 14.23 |
| 250 | 31.54 |
| 12.5 | 40.76 |
| 62.5 | 68.95 |
| 31.25 | 86.43 |
| 15.6 | 97.15 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

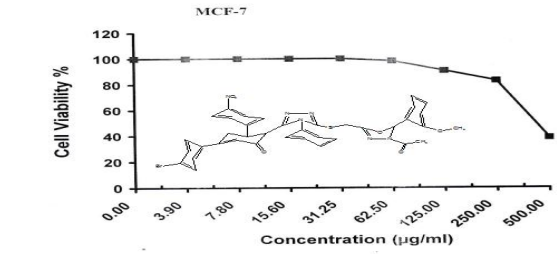
***Comment:***
*Inhibitory activity against Breast carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 105 µg/ml.*

**Fig. 9: Antitumor activities of compound (10) against ovary and breast carcinoma cell lines**




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 27.38 |
| 250 | 69.46 |
| 12.5 | 85.12 |
| 62.5 | 93.47 |
| 31.25 | 97.15 |
| 15.6 | 100 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

***Comment:***
*Inhibitory activity against Ovary carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 366 µg/ml.*




| Sample conc. (µg/ml) | Viability % |
|---|---|
| 500 | 38.42 |
| 250 | 82.93 |
| 12.5 | 90.67 |
| 62.5 | 98.15 |
| 31.25 | 100 |
| 15.6 | 100 |
| 7.8 | 100 |
| 3.9 | 100 |
| 0 | 100 |

***Comment:***
*Inhibitory activity against Breast carcinoma cells was detected under these experimental conditions with $IC_{50}$ = 435 µg/ml.*

**Fig. 10: Antitumor activities of compound (12) against ovary and breast carcinoma cell lines**

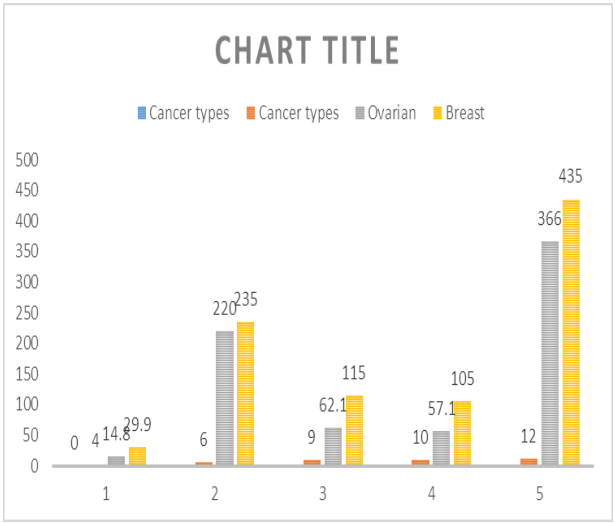



**Fig. 11: In vitro antiproliferative activity against the RCMB cell lines panel derived from two clinically isolated human cancer types**

## DISCUSSION

The antimicrobial results of the tested compounds **(6, 9, 10, 12)** showed that pyrazolyl derivative **(10)** produced the best antimicrobial profile, that is, nearly equal in activity against S.aureus and B.subtilis as the antibiotic Penicillin G, very close in activity to the antibiotic Amphotericin B against A.fumigatus and the antibiotic Streptomycin against P.aeruginosa and E.coli. Compound **(9)** (mercapto methyl oxadiazolyl) also, showed high activity, close to the standard antibiotics used but to a lesser extent except for P.aeruginosa, its effect was nearly as good as Streptomycin. The 1,2,4-triazolyl derivative showed weaker activity towards the tested microorganisms while the oxadiazolyl derivative **(12)** -although it showed results against the tested organisms- it was the least in its activity.

## CONCLUSION

New derivatives of cyclohexen-3,5,6-substituted-1,2,4-triazole **(6, 9, 10, 12)** were prepared and screened for their antimicrobial activity. The biological data obtained for these derivatives have demonstrated that the presence of the pyrazolyl nucleus, the 1,2,4-triazolyl nucleus and the cyclohexene nucleus all together in one component system increased the biological activity nearly to be close to the antibiotics Amphotericin B, Penicillin G and Streptomycin, accomplishing in this way the aim of our research.

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