'Synthesis and Biological Assessment of Some New Acrylonitrile Derivatives as Potential Antitumor and Antimicrobial Agents'

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Abstract: A series of novel acrylonitrile derivatives **4-10** have been synthesized and characterized by spectral data. The *in-vitro* antitumor activity of all compounds was assessed in the MCF-7 human breast cancer cell line. The results showed that compound **8a** exhibited promising anticancer activity with IC_{50} = 9.92 µg/mL while, compounds **4b, 4c**, **7b**, **8c** and **10b** possessed moderate cytotoxic effect with IC_{50} ranging 15.64-20.76 µg/mL**.** The final targets were also tested for their antimicrobial activity. The results revealed that compounds **4a**, **5b, 8a** and **8c** showed remarkable broad spectrum antimicrobial activity, while compounds **5c**, **8a** and **8c** displayed high antifungal activity against *candida albicans* compared to amphotericin B reference drug with IC_{50} = 9.30, 6.25 and 2.30 µg/mL, respectively.
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1. Introduction

Cancer is a disease, in which the control of growth is lost in one or more cells, leading to a solid mass of cells known as a tumor. The initial tumor often becomes life-threatening by obstructing vessels or organs. However, death is most commonly caused by spread of the primary tumor to one or more other sites in the body. On the other hand, the most common cancer in women is breast cancer which remains the most frequent cause of malignancy-associated death among women [1]. Although the use of available chemotherapeutics is often limited due to undesirable side effects and the interest in novel anti-cancer agents is stimulated by growing incidence of drug resistance to cancer chemotherapeutic agents [2], more efforts should be developed in this field.

Literature survey, many investigators reported that cyanoacrylate and acrylamide derivatives [3-7] either substituted aromatic or heteroaromatic compounds were found to display potent anti-proliferative activity against MCF-7 cell lines [8]. They have attracted considerable attention on the part of synthetic chemists and pharmacists because some representatives exhibit anticancer activity [9-12].

The present work comprises the combination between 2-cyanoacrylate pharmacophore with either aminoguanidine moiety as in compounds (**4a-c**) or thiosemicarbazide as in compounds (**5a-c**) which possessed potent anticancer activity [13-16] (scheme1). Additionally, in light of anticancer activity observed by imidazole moiety [17-19] and 1,2,4-triazole moiety [20-24], it was interesting to synthesize conjugates of cyanoacrylamide hydrazide and each moiety (compounds **6-10**) (schemes 2 and 3), to study the potential additive effect of the combined molecule towards cytotoxic activities [25,26] hoping the new combination may enhance the potential anticancer profile.

From the view point of molecular design, the combination of two biologically active molecules or pharmacophores is a well-known approach for the build-up of drug-like molecules, which allows us to find more potent agents. It was thought that it would be of interest to synthesize a single molecule containing more than one pharmacophore conjugates.

On the other hand, the development of effective antibacterial is essential to avoid the major cause of death by bacterial infection. During the past decades, the human population had been affected with life-threatening infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria [27]. Moreover, the long term use of several drugs to treat microbial infections may cause serious health problems, especially in patients with impaired liver or kidney functions [28]. Therefore, there is an increasing need to design new antibacterial and antifungal agents with better activity and higher safety profile. Additionally, we are facing now with the major problem of increasing bacterial resistance to antibacterial drugs. Since, there is unmet need for the synthesis of new antibacterial agents to overcome this increase in bacterial resistance.

Furthermore, in light of the antimicrobial and antifungal importance of cyanoacrylamide, hydrazine moiety and /or aromatic, heterocyclic compounds, hybridization of the different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects. The biological relevance of these heteroaromatic moieties is due to their being good bioisosteres of biomolecules. Based on these prior observations, we postulated that compounds containing

both acrylamide hydrazide [29-32] and imidazole [33-35] or triazole [36-38] pharmacophores could be effective as antibacterial agents. These merged pharmacophores, may be addressing the active site of different targets for the purpose to overcome drug resistance, as well as reducing side effects. In the meanwhile, multi-targets drug strategies have emerged as a therapeutic approach to treat diseases that stem from a combination of factors and leading to the final pathology such as cancer. Using this strategy, a single molecule hits multiple targets, which participate in pathways implicated to a given disease, leading to more efficacious therapy and minimizing the emergence of resistance.

2. Experimental Protocols

2.1 General Remarks:

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England). Microanalysis was carried out at Micro-analytical Unit, the regional centre for microbiology and biotechnology, Al-Azhar University. Infrared Spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan), and expressed in wave number (cm^{-1}), using potassium bromide discs. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethylsulphoxide (DMSO-d_6). Chemical Shifts are quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were chloroform/methanol (9:1) or benzene/acetone (8:2) and the spots were visualized at 366 and 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Compound **4a** was reported with no data [39,40].

2.2. Chemistry

2-(2-Cyano-3-substitutedphenylacryloyl) hydrazinecarboximidamide (4a-c)

Method A: a mixture of aminoguanidine bicarbonate (1.36 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01 mol), anhydrous potassium carbonate (2.76 g, 0.02 mol) and the appropriate aromatic aldehyde (0.01mol) in absolute ethanol (25 mL) was heated under reflux for 12 hours. The formed precipitate was filtered while hot, washed twice with water (20 mL), dried and crystallized from ethanol.

Method B: a mixture of aminoguanidine bicarbonate (1.36 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01mol), and the appropriate aromatic aldehyde (0.01 mol) was added to sodium ethoxide solution (sodium metal 2.3 g in absolute ethanol (25 mL) with continuous stirring for 30 min. The reaction mixture was heated under reflux for 5 hours. The solvent was evaporated under vacuum and after cooling the residue was extracted twice with ethyl acetate (20 mL). The ethyl acetate layer was washed twice with 10% hydrochloric acid (15 mL), and then washed twice with 5% sodium hydroxide (15 mL). The organic layer dried over anhydrous sodium sulphate, filtered and the filtrate was evaporated under reduced pressure and cooled. The crystalline solid was separated, collected and recrystallized from methanol.

2-(2-Cyano-3-substitutedphenylacryloyl) hydrazinecarboximidamid (4a-c) and 2-(2-Cyano-3-substitutedphenylacryloyl) hydrazinecarboxthioamide (5a-c)

Method C: a mixture of ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01mol), and the appropriate aromatic aldehyde (0.01 mol) was added to potassium hydroxide solution (potassium hydroxide (0.56 g, 0.01 mol) in dry dimethylformamide (20 mL) with continuous stirring for 30 min. A solution of aminoguanidine bicarbonate (1.36 g, 0.01 mol) or thiosemicardazide (0.91 g, 0.01 mol) in dry dimethylformamide (10 mL) was added with continuous stirring for 24 hours at room temperature. The formed precipitate was filtered and suspended in a solution of acetic acid/water (15 mL)(1:1) and filtered. The precipitate was dried and crystallized from ethanol.

2-(2-Cyano-3-phenylacryloyl)hydrazine carboximidamide (4a)

Faint yellow cubic crystals; yield 21% [method A], yield 30% [method B], yield 81% [method C]; mp: 166-167˚C; IR (KBr, cm^{-1}): 3365, 3256 (NH_2, NH), 3171 (CH aromatic), 2214 (CN), 1686 (CO); ^{1}H-NMR (DMSO-*d6*): δ 3.61 (br, 2H, NH_2 exchangeable by D_2O), 4.64 (s, 1H, NH exchangeable by D_2O), 7.35-7.81 (m, 5H, ArH), 7.56 (br, 1H, NH exchangeable by D_2O), 8.05 (s, 1H, =CH-C_6H_5), 9.51 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 227 (M-2), 228 (M-1); Anal. Calcd for $C_{11}H_{11}N_5O$ (229.24): C, 57.63; H, 4.84; N, 30.55; Found: C, 57.49; H, 4.81; N, 30.56.

2-(2-Cyano-3-(4-fluorophenyl)acryloyl) hydrazinecarboximidamide (4b)

yellow needle crystals; yield 20% [method A], yield 33% [method B], yield 76% [method C]; mp: 140-141˚C; IR (KBr, cm^{-1}): 3362, 3255 (NH_2, NH), 3144 (CH aromatic), 2218 (CN), 1682 (CO); ^{1}H-NMR (DMSO-*d6*): δ 3.20 (br, 2H, NH_2 exchangeable by D_2O), 4.25 (s, 1H, NH exchangeable by D_2O), 7.33, 7.96 (2d, 4H, ArH), 8.05 (br, 1H, NH exchangeable by D_2O), 8.19 (s, 1H, =CH-C_6H_5), 9.01 (s, 1H, NH exchangeable by D_2O); ^{13}C-NMR (DMSO): 168.66, 165.44, 161.57, 156.34, 144.32, 130.67, 130.15, 116.62, 116.37, 114.90, 114.61; MS (EI) m/z: 248 (M+1); Anal. Calcd for $C_{11}H_{10}FN_5O$ (247.23): C, 53.44; H, 4.08; N, 28.33; Found: C, 53.52; H, 4.15; N, 28.11.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl) hydrazinecarboximidamide (4c)

Dark yellow micro crystals; yield 19% [method A], yield 28% [method B], yield 74% [method C]; mp: 146-147˚C; IR (KBr, cm^{-1}): 3353, 3256 (NH_2, NH), 3144 (CH aromatic), 2214 (CN), 1682 (CO); ^{1}H-NMR (DMSO-*d6*): δ 3.42 (br, 2H, NH_2 exchangeable by D_2O), 5.25 (s, 1H, NH exchangeable by D_2O), 6.06 (br, 1H, NH exchangeable by D_2O), 7.28, 7.72 (2d, 4H, ArH), 7.61 (br, 1H, NH exchangeable by D_2O), 8.18 (s, 1H, =CH-C_6H_5); MS (EI) m/z: 305 (M-2), 307 (M+); Anal. Calcd for $C_{11}H_{10}BrN_5O$ (308.13): C, 42.88; H, 3.27; N, 22.73; Found: C, 42.59; H, 3.21; N, 22.93.

2-(2-Cyano-3-phenylacryloyl)hydrazine carbothioamide (5a)

Dark yellow crystals; yield 68% [method C]; mp: 158-160˚C; IR (KBr, cm^{-1}): 3387, 3264 (NH_2, NH), 3174 (CH aromatic), 2261 (CN), 1662 (CO), 1372 (CS); ^{1}H-NMR (DMSO-*d6*): δ 4.50 (s, 2H, NH_2 exchangeable by D_2O), 7.20 (s, 1H, NH exchangeable by D_2O), 7.37 (m, 3H, C_3, C_4, C_5 ArH), 7.78 (m, 2H, C_2, C_6 ArH), 8.21 (s, 1H, =CH-C_6H_5), 11.41 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 247 (M+H); Anal. Calcd for $C_{11}H_{10}N_4OS$ (246.29): C, 53.64; H, 4.09; N, 22.75; Found: C, 53.44; H, 4.12; N, 22.87.

2-(2-Cyano-3-(4-fluorophenyl)acryloyl)hydrazine carbothioamide (5b)

Faint brown crystals; yield 61% [method C]; mp: 125-127˚C; IR (KBr, cm^{-1}): 3379, 3260 (NH_2, NH), 3175 (CH aromatic), 2241 (CN), 1644 (CO) , 1365 (CS); ^{1}H-NMR (DMSO-*d6*): δ 4.51 (s, 2H, NH_2 exchangeable by D_2O), 7.21 (m, 2H, C_3, C_5 ArH), 7.57 (s, 1H, NH exchangeable by D_2O), 7.87 (m, 2H, C_2, C_6 ArH), 8.02 (s, 1H, =CH-C_6H_5), 11.44 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 262 (M-2), 263 (M-1); Anal. Calcd for $C_{11}H_9FN_4OS$ (264.28): C, 49.99; H, 3.43; N, 21.20; Found: C, 49.91; H, 3.21; N, 21.11.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl) hydrazinecarbothioamide (5c)

Brownish yellow crystals; yield 59% [method C]; mp: 230-231˚C; IR (KBr, cm^{-1}): 3433, 3287 (NH_2, NH), 3167 (CH aromatic), 2245 (CN), 1665 (CO) , 1388 (CS); ^{1}H-NMR (DMSO-*d6*): δ 4.52 (s br, 2H, NH_2 exchangeable by D_2O), 7.17 (m, 2H, C_3, C_5 ArH), 7.83 (m, 2H, C_2, C_6 ArH), 8.04 (s, 1H, =CH-C_6H_5), 8.61 (s, 1H, NH exchangeable by D_2O), 11.40 (s, 1H, NH exchangeable by D_2O); ^{13}C-NMR (DMSO): 181.19, 177.99, 164.59, 161.31, 141.13, 130.76, 130.73, 129.48, 129.37, 115.77, 115.48; Anal. Calcd for $C_{11}H_9BrN_4OS$ (325.18): C, 40.63; H, 2.79; N, 17.23; Found: C, 40.41; H, 2.32; N, 17.00.

General procedure for synthesis compounds 6a-c

A mixture of **4a-c** (0.01 mol) and ethyl chloroacetate (1.65 g, 1.38 mL, 0.01 mol) in absolute ethanol (25 mL) was heated under reflux for 15 hours with continuous stirring. The solvent was evaporated under reduced pressure and cooled. The formed precipitate was collected, dried and crystallized from ethanol.

2-Cyano-*N′*-(4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)-3-phenylacrylohydrazide (6a)

Buff micro crystals; yield 44%; mp: 266-267˚C; IR (KBr, cm^{-1}): 3402, 3236 (NH), 3170 (CH aromatic), 2224 (CN), 1701, 1662 (2CO); ^{1}H-NMR (DMSO-*d6*): δ 4.69 (s, 2H, CH_2CO), 4.64 (s, 1H, NH exchangeable by D_2O), 7.11-7.82 (m, 5H, ArH), 7.20 (br, 1H, NH exchangeable by D_2O), 8.90 (s, 1H, =CH-C_6H_5), 10.21 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 267 (M-2); Anal. Calcd for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; Found: C, 57.69; H, 4.41; N, 26.16.

2-Cyano-3-(4-fluorophenyl)-*N′*-(4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)acrylohydrazide (6b)

Faint yellow micro crystals; yield 44%; mp: >300˚C; IR (KBr, cm^{-1}): 3412, 3294 (NH), 3132 (CH aromatic), 2199 (CN), 1700, 1659 (2CO); ^{1}H-NMR (DMSO-*d6*): δ 3.53 (s, 2H, CH_2CO), 5.01 (s, 1H, NH exchangeable by D_2O), 7.29 (s, 1H, NH exchangeable by D_2O), 7.34 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.17 (s, 1H, =CH-C_6H_5); MS (EI) m/z: 285 (M-2), 286 (M-1), 287 (M+); Anal. Calcd for $C_{13}H_{10}FN_5O_2$ (287.25): C, 54.36; H, 3.51; N, 24.38; Found: C, 54.41; H, 3.54; N, 24.53.

3-(4-Bromophenyl)-2-cyano-*N′*-(4-oxo-4,5-dihydro-1*H*-imidazol-yl)acrylohydrazide (6c)

yellow micro crystals; yield 44%; mp: 266-267˚C; IR (KBr, cm^{-1}): 3452, 3325 (NH), 3150 (CH aromatic), 2227 (CN), 1710, 1667 (2CO); ^{1}H-NMR (DMSO-*d6*): δ 3.71 (s, 2H, CH_2CO), 4.61 (s, 1H, NH exchangeable by D_2O), 7.53-7.74 (m, 4H, ArH), 7.66 (s, 1H, NH exchangeable by D_2O), 8.09 (s, 1H, =CH-C_6H_5), 8.61 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 348 (M+H), 349 (M+2); Anal. Calcd for $C_{13}H_{10}BrN_5O_2$ (348.15): C, 44.85; H, 2.90; N, 20.12; Found: C, 44.69; H, 2.87; N, 20.16.

General procedure for synthesis compounds 7a-c

A mixture of **4a-c** (0.01 mol) and ethyl orthoformate (1.48 g, 1.66 mL, 0.01 mol) in acetic anhydride (10 mL) was heated under reflux with stirring for 8 hours. The solvent was removed under reduced pressure and the product was left overnight. The formed solid was collected, dried and crystallized from methanol.

***N*-(1-(2-Cyano-3-phenylacryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7a)**

Brownish yellow micro crystals; yield 45%; mp: 302-303˚C; IR (KBr, cm^{-1}): 3433, 3267 (NH), 3179 (CH aromatic), 2936 (CH aliphatic), 2222 (CN), 1708, 1641 (2CO); ^{1}H-NMR (DMSO-*d6*): δ 2.05, 2.20 (2s, 3H, CH_3CO), 7.30-7.47 (m, 5H, ArH), 7.80 (s, 1H, triazole), 7.49 (s, 1H, NH exchangeable by D_2O), 8.01 (s, 1H, =CH-C_6H_5); ^{13}C-NMR (DMSO): 179.11, 175.07, 149.97, 140.87, 134.57, 128.12 (2C), 126.99 (2C), 111.62, 115.80, 24.73, 22.88; MS (EI) m/z: 282

(M+H); Anal. Calcd for $C_{14}H_{11}N_5O_2$ (281.27): C, 59.78; H, 3.94; N, 24.90; Found: C, 59.90; H, 3.74; N, 24.61.

***N*-(1-(2-Cyano-3-(4-flourophenyl)acryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7b)**

Brownish yellow micro crystals; yield 39%; mp: 217-218˚C; IR (KBr, cm^{-1}): 3437, 3244 (NH), 3082 (CH aromatic), 2890 (CH aliphatic), 2214 (CN), 1713, 1681 (CO); ^{1}H-NMR (DMSO-*d6*): δ 2.05, 2.22 (2s, 3H, $\underline{CH_3}CO$), 7.19 (m, 2H, Ar$\underline{H}$), 7.86 (m, 2H, Ar$\underline{H}$), 8.16 (s, 1H, triazole), 8.21 (s, 1H, =$\underline{CH}$-C_6H_5), 11.09 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 299 (M+), 300 (M+1); Anal. Calcd for $C_{14}H_{10}FN_5O_2$ (299.26): C, 56.19; H, 3.37; N, 23.40; Found: C, 56.22; H, 3.42; N, 23.68.

***N*-(1-(3-(4-Bromophenyl)-2-cyanoacryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7c)**

Brownish yellow micro crystals; yield 39%; mp: 217-218˚C; IR (KBr, cm^{-1}): 3431, 3228 (NH), 3075 (CH aromatic), 2936 (CH aliphatic), 2212 (CN), 1712, 1685 (CO); ^{1}H-NMR (DMSO-*d6*): δ δ 2.04, 2.17 (2s, 3H, $\underline{CH_3}CO$), 7 .62-7.77 (m, 4H, Ar$\underline{H}$), 7.80 (s, 1H, triazole), 7.91 (s, 1H, =$\underline{CH}$-C_6H_5), 11.35 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 357 (M-2), 359 (M+) ; Anal. Calcd for $C_{14}H_{10}BrN_5O_2$ (360.17): C, 46.69; H, 2.80; N, 19.44; Found: C, 46.77; H, 2.87; N, 19.61.

General procedure for synthesis compounds 8a-c

A suspension of equimolar amounts of **4a-c** and an appropriate aromatic aldehyde (0.01 mol each) in absolute ethanol (20 mL) and glacial acetic acid (2 mL) was heated under reflux for 11 hours. After cooling, the obtained product was filtered and recrystallized from ethanol.

***N*-(4-Chlorobenzylidene)-2-(2-cyano-3-phenylacryloyl)hydrazine carboximidamide (8a)**

Buff needle crystals; yield 66%; mp: 164-165˚C; IR (KBr, cm^{-1}): 3444, 3256 (NH), 3140 (CH aromatic), 2214 (CN), 1686 (CO); ^{1}H-NMR (DMSO-*d6*): δ 7.44 (m, 3H, C_3, C_4, C_5 Ar$\underline{H}$), 7.62 (d, 2H, p-Cl-C_6H_5), 7.85 (m, 2H, C_2, C_6 Ar$\underline{H}$), 7.87 (s, 1H, NH exchangeable by D_2O); 7.96 (d, 2H, p-Cl-C_6H_5), 8.12 (s, 1H, =$\underline{CH}$-C_6H_4-Cl), 8.18 (s, 1H, =$\underline{CH}$-C_6H_5), 12.62 (br.s, 2H, NH exchangeable by D_2O); Anal. Calcd for $C_{18}H_{14}ClN_5O$ (351.79): C, 61.46; H, 4.01; N, 19.91; Found: C, 61.29; H, 4.11; N, 19.88.

***N*-(4-Chlorobenzylidene)-2-(2-cyano-3-(4-fluorophenyl)acryloyl) hydrazinecarboximidamide (8b)**

Buff scales crystals; yield 60%; mp: 282-283˚C; IR (KBr, cm^{-1}): 3424, 3395 (NH), 3140 (CH aromatic), 2214 (CN), 1681 (CO); ^{1}H-NMR (DMSO-*d6*): δ 7.25 (d, 2H, Ar$\underline{H}$), 7.37 (m, 2H, ArH), 7.91 (d, 2H, Ar$\underline{H}$), 7.94 (s, 1H, NH exchangeable by D_2O); 7.95 (m, 2H, ArH), 8.13 (s, 1H, =$\underline{CH}$-C_6H_4-Cl), 8.17 (s, 1H, =$\underline{CH}$-C_6H_5), 12.55 (br.s, 2H, NH exchangeable by D_2O); MS (EI) m/z: 367 (M-2), 369 (M+), 370 (M+1); Anal. Calcd for $C_{18}H_{13}ClFN_5O$ (369.78): C, 58.47; H, 3.54; N, 18.94; Found: C, 58.29; H, 3.41; N, 18.82.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl)-*N*-(4-chlorobenzylidene) hydrazinecarboximidamide (8c)

Yellow micro crystals; yield 60%; mp: 298-299˚C; IR (KBr, cm^{-1}): 3472, 3352 (NH), 3163 (CH aromatic), 2218 (CN), 1678 (CO); ^{1}H-NMR (DMSO-*d6*): δ 7.15 (s, 1H, NH exchangeable by D_2O), 7.41 (d, 2H, Ar$\underline{H}$), 7.43 (d, 2H, ArH), 7.70 (d, 2H, Ar$\underline{H}$),; 7.75 (d, 2H, ArH), 7.78 (s, 1H, =$\underline{CH}$-C_6H_4-Cl), 8.03 (s, 1H, =$\underline{CH}$-C_6H_5), 12.52 (br.s, 2H, NH exchangeable by D_2O); MS (EI) m/z: 429 (M+), 430 (M+H); Anal. Calcd for $C_{18}H_{13}BrClN_5O$ (430.69): C, 50.20; H, 3.04; N, 16.26; Found: C, 50.29; H, 3.01; N, 16.01.

General procedure for synthesis compounds 9a,b

A mixture of **4b,c** (0.01 mol) and dry dimethylformamide (10 mL) was heated under reflux with stirring for 13 hours. The solvent was reduced to half its volume under vacuum and the product was poured into ice-cold water (20 mL). The formed solid was collected, dried and crystallized from methanol.

***N'*-(5-(1-cyano-2-(4-fluorophenyl)vinyl)-1*H*-1,2,4-triazol-3-yl)-*N,N*-dimethylformimidamide (9a)**

faint yellow crystals; yield 73%; mp: >300˚C; IR (KBr, cm^{-1}): 3367 (NH), 3086 (CH aromatic), 2939, 2831 (CH aliphatic), 2218 (CN), 1631 (C=N); ^{1}H-NMR (DMSO-*d6*): δ 2.72, 2.88 (2s, 6H, $2CH_3$), 6.02 (s, 1H, NH exchangeable by D_2O), 7.07 (d, 2H, Ar$\underline{H}$), 7.38 (s, 1H, N=C$\underline{H}$), 7.95 (d, 2H, ArH), 8.50 (s, 1H, =$\underline{CH}$-C_6H_4-), MS (EI) m/z: 284 (M+), 285 (M+1); Anal. Calcd for $C_{14}H_{13}FN_6$ (284.29): C, 59.15; H, 4.61; N, 29.56; Found: C, 59.04; H, 4.72; N, 29.47.

***N'*-(5-(2-(4-bromophenyl)-1-cyanovinyl)-1*H*-1,2,4-triazol-3-yl)-*N,N*-dimethylformimidamide (9b)**

Faint brownish yellow crystals; yield 67%; mp: 212˚C; IR (KBr, cm^{-1}): 3325 (NH), 3089 (CH aromatic), 2958, 2812 (CH aliphatic), 2210 (CN), 1632 (C=N); ^{1}H-NMR (DMSO-*d6*): δ 2.73, 2.91 (2s, 6H, $2CH_3$), 6.23 (s, 1H, NH exchangeable by D_2O), 7.13 (d, 2H, Ar$\underline{H}$), 7.34 (s, 1H, N=C$\underline{H}$), 7.93 (d, 2H, ArH), 8.51 (s, 1H, =$\underline{CH}$-C_6H_4-); Anal. Calcd for $C_{14}H_{13}BrN_6$ (345.2): C, 48.71; H, 3.80; N, 24.35; Found: C, 48.76; H, 3.78; N, 24.44.

General procedure for synthesis compounds 10a,b

A mixture of **4b,c** (0.01 mol) and ethyl orthoformate (4.44 g, 5 mL, 0.03 mol) in absolute ethanol (5 mL) was heated under reflux with stirring for 6 hours. The solvent was removed under reduced pressure and the product was left overnight. The formed solid was collected, dried and crystallized from methanol.

Ethyl *N*-1-(2-cyano-3-(4-fluorophenyl)acryloyl)-1*H*-1,2,4-triazol-3-ylformimidate (10a)

Yellow fine crystals; yield 63%; mp: 193-194˚C; IR (KBr, cm^{-1}): 3089 (CH aromatic), 2931, 2851 (CH aliphatic), 2212 (CN), 1690 (C=N); ^{1}H-NMR (DMSO-*d6*): δ 1.11 (t, 3H, CH_3), 3.53 (q, 2H, CH_2), 7.31 (d,

2H, ArH), 7.60 (s, 1H, N=CH-O), 7.91 (d, 2H, ArH), 7.96 (s, 1H, triazole), 8.52 (s, 1H, =CH-C_6H_4); MS (EI) m/z: 312 (M-1), 313 (M+); Anal. Calcd for $C_{15}H_{12}FN_5O_2$ (313.29): C, 57.51; H, 3.86; N, 22.35; Found: C, 57.58; H, 3.93; N, 22.43.

Ethyl *N*-1-(3-(4-bromophenyl)-2-cyanoacryloyl)-1*H*-1,2,4-triazol-3-ylformimidate (10b)

Dark yellow crystals; yield 56%; mp: >300˚C; IR (KBr, cm^{-1}): 3113 (CH aromatic), 2920, 2843 (CH aliphatic), 2210 (CN), 1694 (CO); ^{1}H-NMR (DMSO-*d6*): δ 1.07 (t, 3H, CH_3), 3.47 (q, 2H, CH_2), 7.29-7.62 (m, 4H, ArH), 7.58 (s, 1H, N=CH-O), 7.94 (s, 1H, triazole), 8.51 (s, 1H, =CH-C_6H_4); ^{13}C-NMR (DMSO): 158.83, 146.64, 138.88, 131.57, 131.38, 130.92, 130.66, 130.12, 129.69, 128.38, 121.08, 120.60, 118.37, 48.20, 22.52; Anal. Calcd for $C_{15}H_{12}BrN_5O_2$ (374.19): C, 48.15; H, 3.23; N, 18.72; Found: C, 48.23; H, 3.21; N, 18.89.

2.3. Biological Evaluation

The antitumor screening of the novel synthesized compounds was carried-out at the National Cancer Institute (NCI), Biology Department, Pharmacology Unit, Cairo, Egypt. However, the antimicrobial testing was carried-out at Biotechnology Center, Faculty of Pharmacy, Cairo, Egypt.

2.3.1. Preliminary *in-vitro* antitumor screening

The novel synthesized compounds were subjected to SulfoRhodamine-B (SRB) assay for cytotoxic activity against human breast adenocarcinoma cell line (MCF7), at concentrations between 1 and 10 mg/mL according to Shehan method [41]. The data was represented in Table 1 and Figs. 1 & 2.

2.3.1.1. Measurement of cytotoxicity by SRB assay

The cytotoxic activity of some of the newly synthesized compounds was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the method of Skehan. Cells were plated in 96-multiwell microtiter plate (10^4 cells\well) for 24 hours before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (0, 1, 2.5, 5 and 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 hours at 37^{o}C and in atmosphere of 5% CO_2 . After 48 hours, cells were fixed, washed and stained for 30 minutes with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated.

Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically.

Probity regression analysis procedure will be introduced to select the best model that describes the relationship among the Probity (IC) (as a dependent variable) in order to be used for prediction of the concentration of the drug that cause inhibition of 50% or 90% of cancer cells. The probity (P) = intercept + (regression coefficient x conc.).

The results are given in Table 1, and presented graphically in Figs. 1 & 2.

2.3.2. Antimicrobial activity screening

The newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans* ATCC 60193 *and Aspergillus niger* ATCC 1718109 using the microbroth dilution method [42].

The Gram-positive antibacterial agent, amoxacillin, the Gram-negative antibacterial agent, gentamycin, and the anti-fungal agent, amphotericin B, were used as controls. In addition to, MICs (minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC_{50} (the concentration which inhibits 50% of microorganisms) of all compounds were determined according to reported method [42,43]. The *in vitro* antimicrobial properties against a number of Gram-negative and Gram-positive bacteria, and yeasts are presented in Tables 2 & 3 and Fig. 3, respectively.

2.3.2.1. Determination of the Minimum Inhibitory Concentration (MIC)

The preliminary MICs were firstly determined by the microbroth dilution method [42]. Briefly, 100 µL of double strength DMSO (Sigma-Aldrich, Germany) were placed in each well of a 96-well microtiter plate. Aliquot of 100 µL of the solutions to be tested were added to the first column. Then two fold dilutions were carried out from one well to the next up to final well in each row for each tested compound.

MICs were then determined using agar streaking technique as per Clinical Laboratory Standard Institute guidelines [42]. A total of 15 mL molten (45^o C) Neutrient agar (Sigma-Aldrich, Germany) were supplemented with the required concentration then were added into sterilized Petri dishes, allowed to solidify. Then 10 µL of each bacterial or fungal suspension (10^5 CFU mL^{-1}) were streaked onto the surface. Finally all plates were incubated at 37 ^{o}C for 24 hours for bacterial strains and 25 ^{o}C for 48 hours fungal strains under aerobic conditions. MIC was

determined as the average between the last plate had growth and the first plate with no growth.

2.3.2.2. Determination of the MBC and IC_{50}

MBC and IC_{50} were determined in 96 well microtiter plate where a 100 µL of trypcase soya broth (Oxoid, USA) for bacterial isolates or sabaroud's dextrose broth for fungal strains were placed in each well. A proper amount of the stock solution of the tested compounds was added to reach the desired concentration. All columns were then inoculated with 20 µL of bacterial suspension (10^6 CFU mL^{-1}) and incubated for 5-6 hours. An aliquot of 100 µL from each well was transferred into another pre-supplemented with 100 µL f Dey-engly broth medium (Fluka, USA) and allowed to stand for 10-20 minutes to neutralized any antimicrobial activities. Then these neutralized solutions were subjected to proper dilutions and streaked onto trypcase soya agar or sabaroud's dextrose agar plates to determine the viable count [43]. Controls were done for sterility and growth and subjected to the same regimen of treatment. MBC was determined as the lowest concentration which decreased the number of viable bacteria by 3 log units. IC_{50} was determined as the lowest concentration reduced the viable count by about 50 %.

3. Results and Discussion

3.1. Chemistry

The synthetic approaches adopted to obtain the target compounds **4-10** are depicted in Schemes 1-3. The structures of the newly synthesized compounds were established on the basis of their elemental analyses and spectral data.

The starting compounds **4a-c** was synthesized by three methods varying in the yield percentage. Firstly, it was prepared in 19-21% yield through heating ternary component (ethyl cyanoacetate, appropriate aldehyde and aminoguanidine) in boiling ethanol according to the reported method [44]. The second procedure is one pot reaction by refluxing the reactants in strong alkaline medium for five hours to give compounds **4a-c** in yield 28-33% following Fadda procedure [45]. Method C was adopted by stirring the reactants in alkaline polar aprotic solvent DMF (dimethyl formamide) to afford **4a-c** in yield 74-81%. On the other hand, the target compounds **5a-c** was prepared in 59-61% following method C by using thiosemicarbazide instead of aminguanidine. However, the three procedures failed to obtain cyclic aminotriazepine compound **3**. IR spectra of compounds **4a-c** & **5a-c** showed absorption bands in the range 3433-3365 cm^{-1} of (NH_2, NH) and bands at 1645–1683 cm^{-1} which confirmed the presence of carbonyl function, additionally characteristic band at 1365, 1388 due to C=S (compounds **5a-c**) . Furthermore, ^{1}H-NMR spectra of **4a-c** and **5a-c** showed sharp singlet signal for CH protons at 8.22 ppm [open structure]. ^{13}C-NMR depicted spectra at 168.66 (C=NH) in **4b** and 181.19 (C=S) in **5c**, respectively.

In scheme 2, the reaction of ethyl chloroacetate and compounds **4a-c** was adopted according to the literature method [46] to obtain imidazoacrylohydrazide **6a-c**. The structures of all synthesized compounds were determined by spectral and microanalytical analyses. The ^{1}H-NMR spectra of **6a-c** have shown new singlet signals around δ 3.53 and 4.69 ppm corresponding to the CH_2CO protons. All the other aromatic protons were observed in the expected regions. The title compounds were further confirmed by mass spectral data which showed the molecular ion peak. Moreover, compounds **7a-c** was achieved by cyclization of 2-(2-Cyano-3-substitutedphenyl acryloyl)hydrazinecarboximidamide (**4a-c**) using ethyl orthoformate [47] followed by acetylation of amino (imino) group by acetic anhydride. IR spectra exhibited very similar features and showed the expected bands for the characteristic groups which are present in the compounds such as NH stretching vibrations, amide C=O stretching, and another aliphatic band for CH_3 vibrations. ^{1}H-NMR spectra were consistent with the proposed structures which showed two singlet peaks around δ 2.04 and 2.22 ppm corresponding to the acetyl group, in addition to the aromatic protons observed in the expected region. ^{13}C-NMR depicted spectra at 24.73 (CH_3) of acetyl amino and at 22.88 (CH_3) of acetyl imino, respectively. 4-Chlorobenzylidene derivatives **8a-c** was obtained by refluxing of **4a-c** with an equimolar amount of 4-chlrobenzaldehyde in acidified absolute ethanol [48]. The structures of new compounds were elucidated by analytical and spectroscopic measurements, ^{1}H-NMR spectra showed benzylidene CH around 7.78 and 8.18 ppm.

Scheme 3 deals with the preparation of the target 1,2,4-triazole derivatives **9a,b** and **10a,b**. Refluxing of **4b,c** in dimethylformamide (DMF) yielded the corresponding *N,N*-dimethylformimidamide derivatives **9a,b**. The reaction proceeds through cyclodehydration followed by condensation with DMF, the experimental procedure was similar to the literature [49,50]. IR spectra showed appearance of absorption bands for methyl group in the range of 2958-2830 cm^{-1} and disappearance of carbonyl group. ^{1}H-NMR spectra revealed two equivalent peaks in the region 2.72-2.91 ppm due to methyl group and additional sharp peak at 7.34 and 7.38 for CH=N proton corresponding to **9a** and **9b**, respectively. In addition to, the mass spectrum data of compound **9a** showed the molecular ion peak. On the other hand, compounds **10a,b** obtained in 56-63% yield through heating **4b,c** in excess ethyl orthoformate [51]. ^{1}H-NMR spectra showed the characteristic triplet and quartet peaks which were informative to the ethyl group and singlet signal of triazole at 7.96 and 7.94 ppm corresponding to **10a** and

10b, respectively. Moreover, ^{13}C-NMR depicted spectra at 48.20 and 22.52 pointed to ethyl group in compound **10b** (c.f. experimental part).

Table 1: *In vitro* cytotoxic activity of some of the synthesized compounds against the human breast cancer cell line (MCF-7).

Compds.no.	Cell line	
	IC_{50}	**IC_{90}**
4a	46.62^{ik}±2.11	89.29^{i}±0.73
4b	**19.52**^{c}±1.29	65.41^{b}±1.38
4c	**16.45**^{b}±1.45	71.79^{f}±2.59
5a	26.59^{de}±0.03	71.04^{f}±2.59
5b	47.93^{k}±0.57	82.55^{h}±0.85
5c	26.76^{de}±1.39	71.36^{f}±0.57
6a	41.39^{h}±1.53	78.13^{g}±2.46
6b	28.05^{f}±2.38	74.11^{f}±1.53
6c	44.78^{ij}±1.57	82.96^{h}±1.79
7a	27.5f±1.19	80.36^{g}±1.24
7b	**16.19**^{b}±2.57	64.33^{a}±0.99
7c	**23.77**^{d}±0.52	69.23^{cd}±2.28
8a	**9.92**^{a}±2.62	68.49^{bc}±0.95
8b	30.56^{fg}±2.25	72.94^{f}±0.72
8c	**20.76**^{c}±1.61	67.71^{bc}±2.7
9a	26.36^{de}±2.05	66.80^{bc}±1.86
9b	42.27^{i}±1.58	79.18^{g}±0.62
10a	32.92^{g}±0.57	68.32^{cd}±2.73
10b	**15.64**^{b}±2.78	69.67^{cd}±0.64
F-value	126.30	47.46
p-value	0.000*	0.000*

All values are represented as Mean ± S.D

*= There is a significant difference between the com. no. by using One Way ANOVA at $p< 0.05$

The different letters means that there is a significant difference between the two compounds by using Duncun multiple comparison test at $p<0.05$

Values were calculated from dose-response curves done in triplicate for each compound.

IC_{50}µg/mL : dose of the compound which caused 50% reduction of survival.

IC_{90}µg/mL: dose of the compound which caused 90% reduction of survival.

3. 2. Results of *in-vitro* antitumor screening

All compounds were evaluated for their *in-vitro* antitumor activity against human breast adenocarcinoma cell line (MCF-7). The IC_{50} and IC_{90} (the concentration required for 50% and/or 90% inhibition of cell viability) were calculated for each compound and the results are given in Table 1 and Figs. 1 & 2.

All the newly tested compounds were found to possess moderate to weak antitumor activities against breast adenocarcinoma cell line (MCF-7) with IC_{50} range from 9.92 to 47.93 µg/mL). Nevertheless, compound **8a** displayed the highest anti-breast cancer activity with IC_{50}=9.92 µg/mL. and IC_{90}=68.49 µg/mL (the concentration required for 90% inhibition of cell viability). On the other hand, compounds **4b,c, 7b, 7c, 8c** and **10b** possessed moderate activity with IC_{50} ranged from 15.64 to 23.77 µg/mL and IC_{90}=64.33-71.79 µg/mL.

Regarding the structure activity relationship of 2-(2-Cyano-3-substituted phenylacryloyl) hydrazine carboximidamide (**4a-c**) toward MCF-7 breast cancer cell lines**:** Compounds **4b** and **4c** showed significant activity more than the unsubstituted congener **4a.** This may attributed to the lipophilicity of halogen substitution which enhances the cytotoxic activity [52]. On the other hand, the thioxo analogues **5a-c** found to be moderate to weak anticancer effect with IC_{50}= 26.59-47.93 µg/mL. Whereas, compound **4b** displayed promising activity against MCF-7 cell panel compared to **5b**, this could be assigned to the presence of the amino group which favors the potency than that thioxo moiety [53]. The cyclized imidazolidinone acrylohydrazide derivatives **6a-c** led to compounds that had slightly inhibitory efficacy compared to the precursor hydrazine carboximidamide **4a-c**. For instance, the analogue p- Fluoro **6b** exhibited the best member in this series with IC_{50}=28.05 µg/mL. All acetamidotriazole derivatives **7a-c** showed moderate anticancer activity [54] with IC_{50} around 16.19 to 27.50 µg/mL. Interestingly, within Shiff's bases derivatives **8a-c** displayed potent to moderate antitumor activity against MCF-7 breast cancer, which is well known to have potential anticancer activity [55,56]. It was envisioned that compound **8a** possessed remarkable cytotoxic activity than other congeners **8b** and **8c**. On the other hand, triazole derivatives compound **9a** recorded fair antitumor activity with IC_{50}=26.36 µg/mL, while compound **9b** showed weak activity with IC_{50} value 42.27 µg/mL although the *N,N*-dimethylformimidamide derivatives proved recently to be have potential antitumor activity [57]. Moreover, compounds **10a** and **10b** displayed moderate to weak anticancer activity with IC_{50}=15.64 and 32.92 µg/mL, respectively.

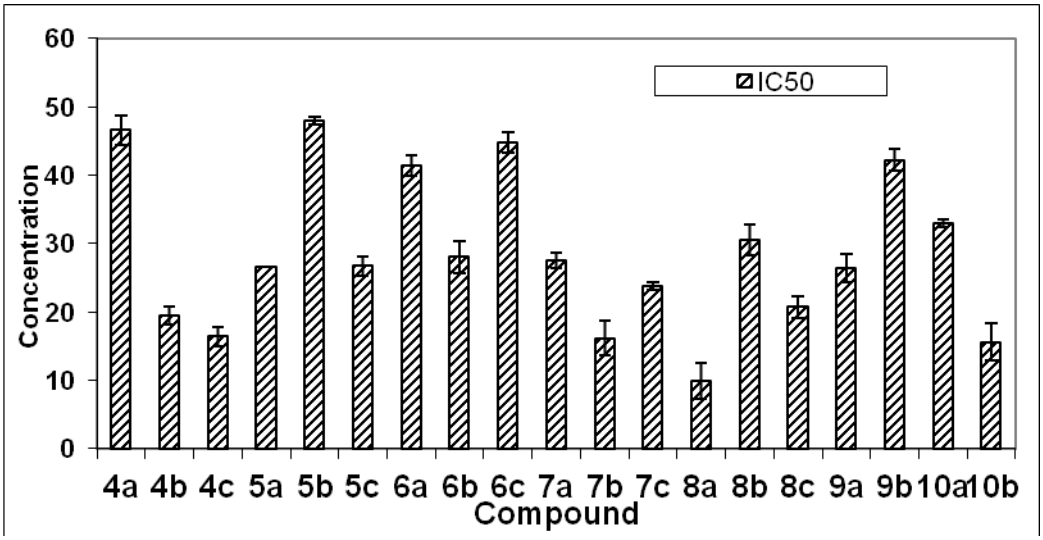


Fig. (1): IC_{50} values of the new synthesized compounds against the human breast cancer cell line (MCF-7).

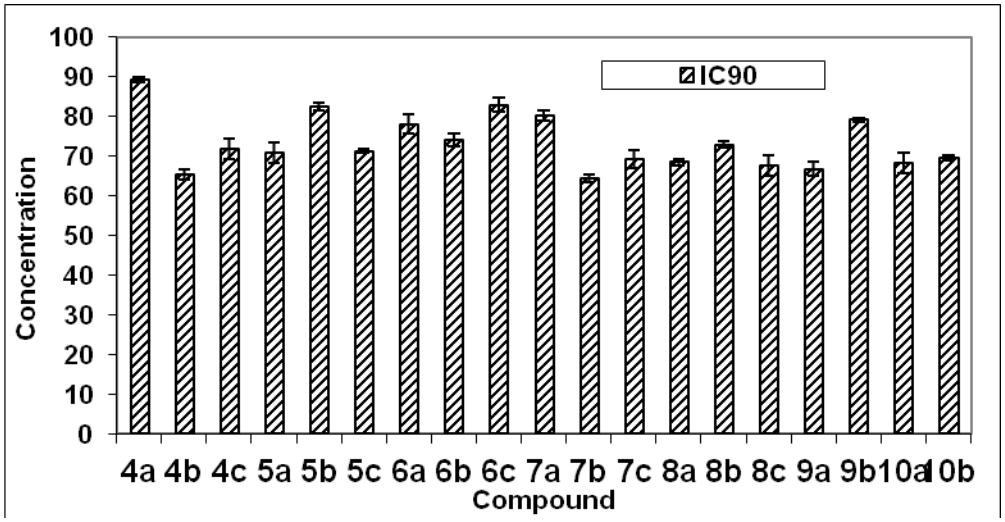


Fig. (2): IC_{90} values of the new synthesized compounds against the human breast cancer cell line (MCF-7).

3.3. Results of antimicrobial activity

The newly synthesized compounds were subjected for evaluation their antimicrobial activities using microbroth dilution method [42]. The data presented in table 2 which revealed that compounds **4a, 4c, 5b** and **8a** and **8c** showed broad spectrum antibacterial and antifungal activities, while compounds **7a** and **9a** were only active against Gram negative strains. Moreover, compounds **7b** and **10b** showed antibacterial activity against Gram positive bacteria. The remaining compounds **4b, 6a-c, 7c, 8b** and **9b** had no significant activity against any of the tested strains at concentration up to 50 µg/mL. (Fig. 3).

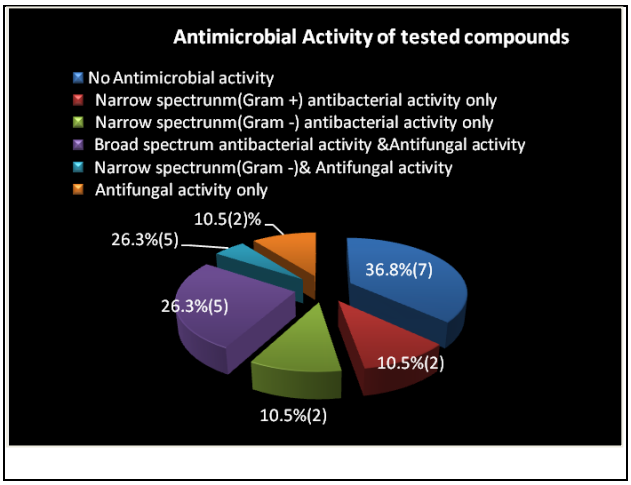


Fig. 3: Pie Chart of the % antimicrobial activity of tested compounds

Table 2: Antimicrobial activity of the synthesized compounds expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and concentration that inhibit 50% of microorganisms (IC_{50}) in µg /mL against the pathological strains based on two fold serial dilution technique.

Compds. no.		*S. aureus*	*B.subtilis*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*
4a	MIC	**18.75**	**18.75**	**18.75**	**18.75**	**18.75**	**37.50**
	MBC	**18.37**	**18.75**	**18.75**	**18.75**	**18.75**	**37.50**
	IC_{50}	**12.50**	**12.50**	**9.30**	**12.50**	**12.50**	**12.50**
4b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
4c	MIC	**37.50**	**37.50**	**37.50**	**37.50**	**37.50**	>50
	MBC	**37.50**	**37.50**	**37.50**	**37.50**	**37.50**	>50
	IC_{50}	**12.50**	**25**	**25**	**25**	**12.50**	>50
5a	MIC	>50	>50	**18.75**	**37.50**	**37.50**	>50
	MBC	>50	>50	**18.75**	**50**	**37.50**	>50
	IC_{50}	>50	>50	**9.30**	**37.50**	**25**	>50
5b	MIC	**18.75**	**18.75**	**9.38**	**9.38**	**18.75**	**18.75**
	MBC	**18.75**	**18.75**	**9.30**	**9.30**	**18.75**	**18.75**
	IC_{50}	**9.30**	**12.50**	**6.25**	**6.25**	**9.30**	**12.50**
5c	MIC	>50	>50	>50	>50	**9.38**	**18.75**
	MBC	>50	>50	>50	>50	**12.50**	**18.75**
	IC_{50}	>50	>50	>50	>50	**9.30**	**12.50**
6a	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6c	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
7a	MIC	>50	>50	**18.75**	**18.75**	>50	>50
	MBC	>50	>50	**18.75**	**25**	>50	>50
	IC_{50}	>50	>50	**9.30**	**12.50**	>50	>50
7b	MIC	**37.50**	**37.50**	>50	>50	>50	>50
	MBC	**37.50**	**42.50**	>50	>50	>50	>50
	IC_{50}	**12.50**	**28.75**	>50	>50	>50	>50
7c	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
8a	MIC	**4.69**	**2.34**	**4.69**	**2.34**	**9.38**	**18.75**
	MBC	**4.70**	**2.30**	**4.70**	**2.30**	**12.50**	**25**
	IC_{50}	**3.13**	**0.78**	**1.17**	**0.78**	**6.25**	**9.30**
8b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
8c	MIC	**4.69**	**4.69**	**9.38**	**9.38**	**4.69**	**18.75**
	MBC	**4.70**	**4.70**	**9.30**	**9.30**	**4.70**	**18.75**
	IC_{50}	**2.30**	**2.30**	**3.13**	**3.13**	**2.30**	**9.30**
9a	MIC	>50	>50	>50	**18.75**	**18.75**	>50
	MBC	>50	>50	>50	**25**	**18.75**	>50
	IC_{50}	>50	>50	>50	**18.75**	**9.30**	>50

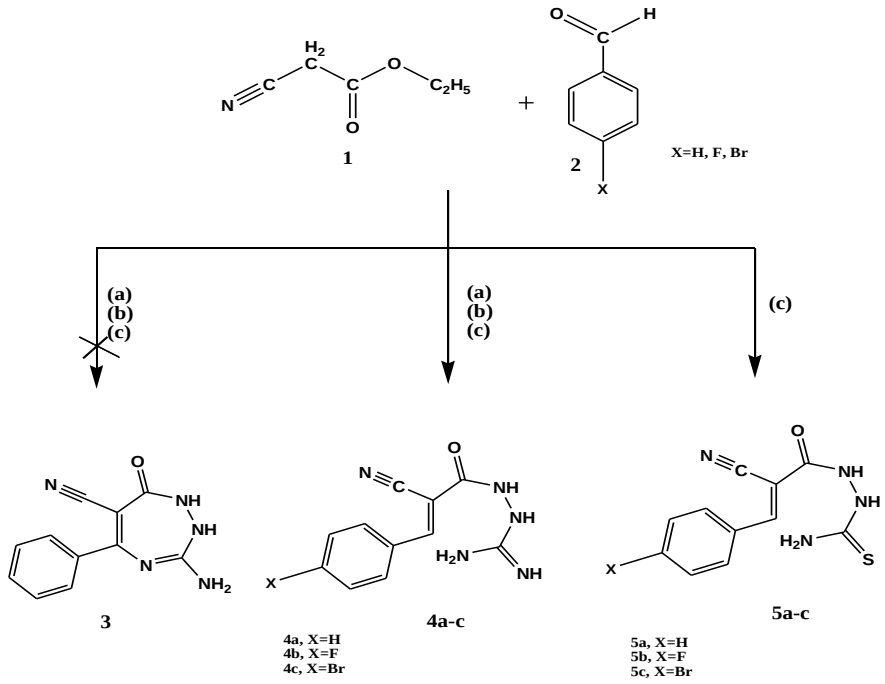
9b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
10a	MIC	>50	>50	>50	>50	>50	**37.50**
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
10b	MIC	**9.38**	**2.34**	>50	>50	>50	>50
	MBC	**12.50**	**3.13**	>50	>50	>50	>50
	IC_{50}	**6.25**	**2.30**	>50	>50	>50	>50
Amoxicillin	MIC	10	100	NA	NA	NA	NA
Gentamicin	MIC	NA	NA	10	25	NA	NA
Amphotericin B	MIC	NA	NA	NA	NA	10	15

NA: no action.

Table 3: Relative potency (%) of tested compounds against six microorganisms at concentration 50 µg/mL

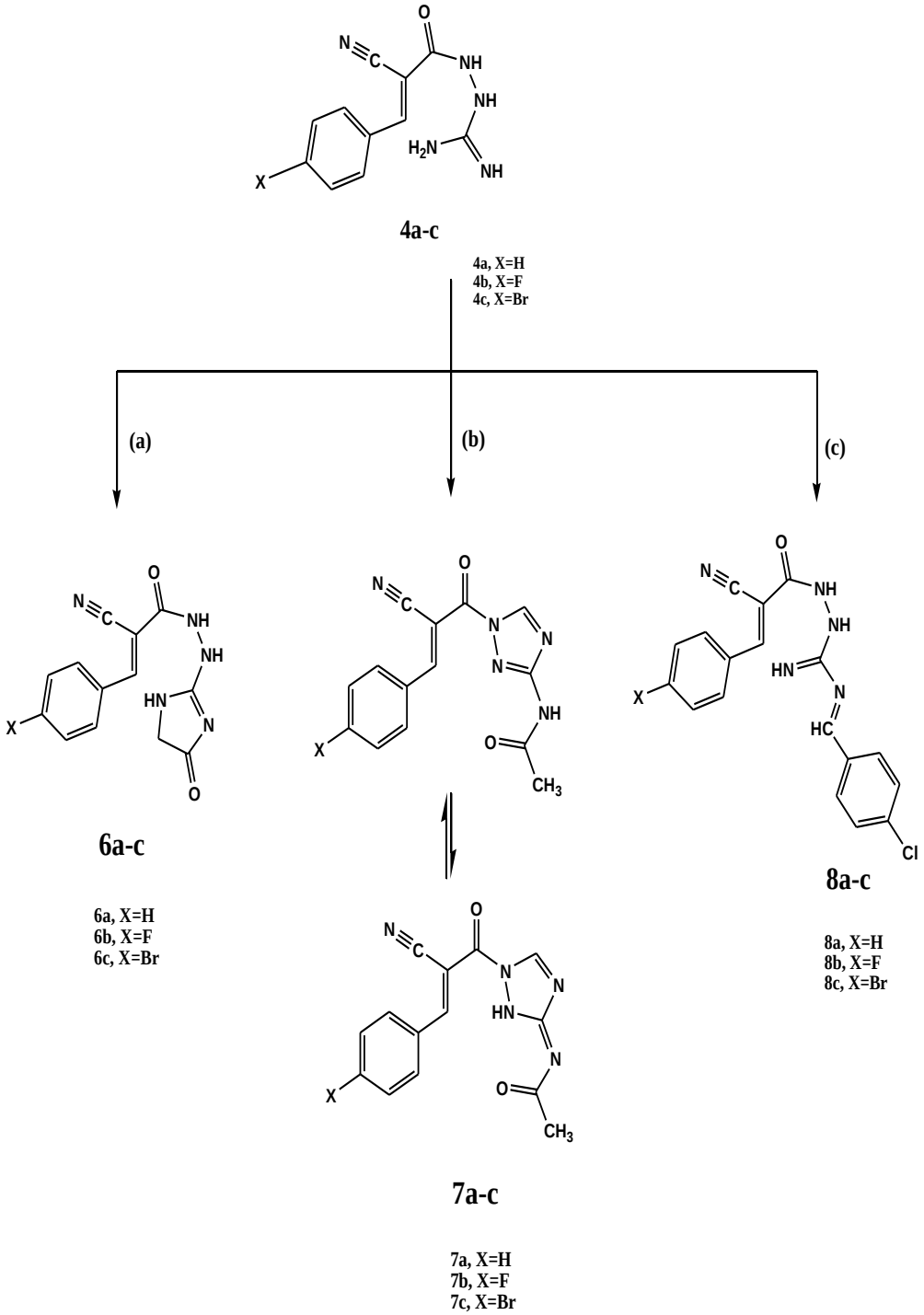
M. O.	**4a**	**4c**	**5a**	**5b**	**5c**	**7a**	**7b**	**8a**	**8c**	**9a**	**10a**	**10b**	Reference
S. aureu	54.45	40.9	0	45.45	0	0	40.91	**131.82**	**100**	0	0	86.36	Amoxicillin
B. subtilis	57.89	36.84	0	47.37	0	0	31.58	**200**	**147**	0	0	89.47	Amoxicillin
E. coli	50	40.9	68.81	81.81	0	63.63	0	**109.09**	**111.11**	50	0	0	Gentamicin
P.aeruginosa	72.22	44.44	83.33	100	0	83.33	0	**172.22**	**111.11**	55.56	0	0	Gentamicin
C. albicans	**133**	38.88	0	**138.88**	**122**	0	0	**127.77**	86	0	0	0	Amphotericin B
A. niger	62.5	0	0	77.71	**119**	0	0	93.38	75	0	56.25	0	Amphotericin B

Scheme 1



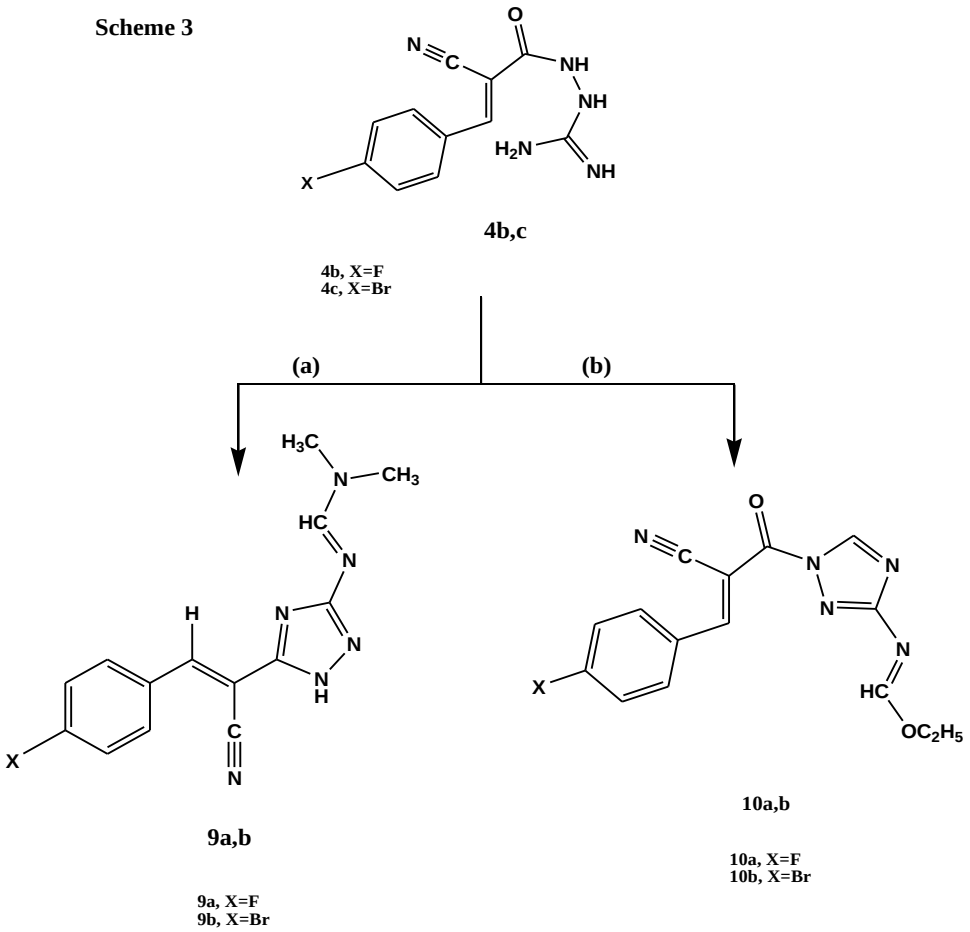
Scheme1: Reagents and condition; (a) Aminoguanidine bicarb/reflux in absolute ethanol/anhyd. /12 hrs. (b) Aminoguanidine bicarb/reflux in sod.ethoxide/ 5hrs.; (c) Aminoguanidine bicarb. or thiosemicarbazide/stirring in dry DMF/KOH/at R.T/24hrs.

Scheme 2



Scheme 2: Reagents and condition; (a) Ethyl chloroacetate/reflux in absolute ethanol/anhyd. K_2CO_3 /15 hrs. (b) Ethyl orthoformate/AC_2O/ 8hrs. (c) p-Chlorobenzaldehyde/reflux in absolute ethanol/HAC/12hrs.

Scheme 3



Scheme 3: Reagents and condition; (a) Reflux in dry DMF/13 hrs. (b) Reflux in ethyl orthoformate excess/ 7hrs.

The structures activity correlation of the tested compounds showed that the starting compound **4a** possessed broad antibacterial spectrum against *S. aureus* , *B. subtilis* (G+) and *E. coli, P. aeruginosa* (G^-) bacteria. Also, it recorded superior antifungal activity against *C. albicans* than amphotericin B reference drug at concentration 18.75-50 µg/mL. The percentage of relative potency of compound **4c** against (G+) bacteria ranged 40.9-36.8% compared to amoxicillin reference drug at concentration 37.50-50 µg/mL. However, it exhibited an inhibitory activity against (G^-) bacteria around 44.44 - 40.90% compared to gentamicin standard drug and it showed antifungal activity against *Candida albicans* with relative potency 38.88% at the same concentration. Compound **5a** displayed decent an inhibitory activity against *E. coli* and *P. aeruginosa* (G^-) bacteria with % of potency 68.81 and 83.33, respectively at concentration 18.75-50 µg/mL. Moreover, compound **5b** showed promising broad spectrum antimicrobial activities, this may be attributed to the combination of p-fluorophenyl and thiosemicarbazide enhances the antimicrobial activity [58]. It exhibited comparable activity against *P.aeruginosa* as gentamicin reference drug at concentration 9.38 µg/mL, while it showed higher antifungal activity against *C. albicans* compared to the standard at concentration 18.75 µg/mL . Additionally, compound **5c** showed better antifungal activity more than reference drug against *C. albicans* in concentration 9.38-50 µg/mL and against *A. niger* in concentration 18.75-50 µg/mL, respectively. The percentage of relative potency of compound **7a** against *E. coli* and *P. aeruginosa* (G^-) bacteria is 63.63 and 83.33%, respectively at concentration 18.75 µg/mL for

each. On the other hand, compound **7b** showed moderate activity against Gram positive bacteria only at concentration 37.50 µg/mL. Compound **8a** recorded the best antimicrobial activities derivative in this work. It displayed about two folded antibacterial activity against *B. subtilis* (G+) and *P. aeruginosa* (G⁻) bacteria compared to the reference drug in concentration 2.34 µg/mL. In additions to, it exhibited superior antimicrobial activity against *S. aureus* (G+), *E. coli* (G⁻) and *C. albicans* compared to the amphotericin B standard drug. This is may be referred to the combined factors of the unsubstited phenyl residue and shiff's base with two atom spacer may be potentiate the activity [59].

Moreover, compound **8c** registered an excellent antibacterial activities against both (G+ and G⁻) bacteria more than the reference drugs at concentration 4.69 and 9.38 µg/mL, respectively, while it exhibited good antifungal activity with percentage relative potency ranged 86 to 75% at concentration 4.69 and 18.75 µg/mL. On the other hand, compound **9a** showed narrow moderate inhibitory activity against *E. coli* and *P. aeruginosa* (G⁻) bacteria with percentage relative potency 50 and 55.56 %, respectively. Furthermore, compound **10a** showed moderate antifungal activity against *A. niger* at concentration 37.50-50 µg/mL. On the other hand, compound **10b** displayed promising antibacterial activity against *S. aureus* , *B. subtilis* (G+) bacteria with percentage relative potency values 86.36 and 89.47%, respectively.

4. Conclusion

This study reports the synthesis of acrylonitrile based compounds **4-10** as potential antitumor and antimicrobial agents. According to the results of bioactivity; the *in vitro* cytotoxic screening of novel derivatives revealed that most of the compounds had moderate to limited anticancer activity against MCF-7 human breast cancer cell line. However, compound **8a** exhibited potent inhibitory effect against human breast adenocarcinoma cell line with IC50 = 9.92 µg/mL. Considering the imidazolone derivatives **6a-c**, they proved to have negative impact on the anticancer activity. On the other hand, several of the newly compounds displayed promising antimicrobial activity compared to the reference drugs amoxicillin, gentamicin and amphotericin B. It can stated that, the starting amino derivatives **4a, 4c**, thioxo analogue **5b** and shiff's bases compounds **8a,c** were found to be broad spectrum than the remaining compounds. These findings demonstrated a new potential for acrylonitrile derivatives which could be useful templates for further derivatives to obtain more potent antitumor and antimicrobial agent(s).

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