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Original Article

Antimicrobial, Sulphorhodamine (SRB) and Antiviral Evaluation of Cyanoacrylamide Derivatives

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ABSTRACT

Antitumor, antiviral, and antimicrobial evaluations were performed on a novel series of acrylamide incorporated benzo[*d*]thiazole compounds. The structures of these compounds were confirmed by different spectral tools. Most of these compounds except compounds **6** and **7** have high to moderate antimicrobial activity through binding to sterol components of cells or alteration in cell permeability and cell death [1]. Concerning the antitumor activity, the two molecules **5** and **6** exhibit strong cytotoxic effects against human breast cancer (MCF7), human liver carcinoma (HEPG2), and prostate cancer (PC3). It is believed that the cytotoxic effect of these compounds was attributed to different cellular pathways including tyrosine kinase inhibition [2], tubulin polymerization inhibition, and cell death via apoptosis. On the other hand, they have showed less toxic effect against the normal HBF4 (normal melanocytes) cells. Antiviral assay was shown for all synthesized compounds. Compounds **4, 5**, and **8** exhibit a promising antiviral activity against *vesicular stomatitis virus (VSV)*, while our lead compound **3** show the less viral inhibition in this series**.**

Keywords: 2-Cyanoacrylamide; Antiviral Activity; VSV; Cytotoxicity; MCF7; HEPG2; PC3; Antimicrobial test.
Running Title: SRB and Antiviral Evaluation of Cyanoacrylamide Derivatives.

INTRODUCTION

Several attempts to find the synthetic or naturally occurring derivatives which have significant biological activity or can be used as drug therapy still deserve current interests. Much attention has been focused on the 2-cyanoacrylamide derivatives due to their wide range of biological activities such as anti-cancer activity[3–7], anti-bacterial activity [8], anti-oxidation [9,10] and anti-viral activities [11].

Regarding the antitumor activity, cancer is amongst the three most common causes of death in most developed countries [12]. Recent treatment focused on the treatment of malignancy such as breast, colon, and lung cancer [13]. Previous studies, showed a number of biologically active compounds that incorporate an acrylamide group similar to those represented herein [13]. The acrylamide derivatives are biologically active across many cellular pathways included in the production of cytotoxic effects in different cell lines of cancer. These pathways include tubulin polymerization inhibition [14–17], cell death via apoptosis [18–20], and tyrosine kinase inhibition [2]. Compound **3** is the lead compound in our series and exhibited less biological activity herein. Therefore, we tried to modify the structure of compound **3** to obtain drug-like compound which has strong activity. Another study was performed on compound compatible to our lead showed that this compound sought to reduce the expression of signaling proteins Jak2/Stat3 pathway [21,22] or return them to their inactive state. These signaling proteins are target for cancer and our new derivatives inhibit the proliferation of tumor cells.

Concerning, the antimicrobial activity, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic, and synthetic. Another classification system is based on biological activity; in this classification, antibacterial agents are divided into two broad groups according to their biological effect on microorganisms; bactericidal agents kill

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bacteria, and bacteriostatic agents slow down or stall bacterial growth. In general, most of our derivatives exhibited broad spectrum antibacterial activity and could be classified as bactericidal agents. Compound **3** can be classified as bacteriostatic agent as it does not kill bacteria completely but it can slow down its growth or cause very little inhibition. On the other hand, compounds **6** and **7** have no antimicrobial activity. Several antimicrobial studies showed good activity on similar compounds [8], but our new derivatives as **4** and **5** exhibited stronger activity approximately equal to that of standard antibacterial drug tetracycline.

For antiviral evaluation, we used *Vesicular stomatitis virus VSV* (fig. 1 in the supplementary file) as an example to test the antiviral activity of our compounds. Serine protease is important for the life cycle of the virus and also for its replication pathway. Therefore inhibition of this enzyme is important to stop the virus replication. The nucleophilic oxygen in serine protease can be targeted by different kinds of electrophiles via reversible or irreversible covalent binding to the virus enzyme. This strategy of covalent inhibition of serine protease can be applied on peptidyle nitrile derivatives [23,24]. The nitrile group may act as an electrophilic trap for serine residue to produce a dipole-dipole interaction between our derivatives and protease.

Herein, our interest is focused on the synthesis of heterocycles containing acrylamide incorporating benzo[*d*]thiazole moiety, to identify new derivatives that may be important in designing new, potent, selective and less toxic antiviral, antitumor, antibacterial and antifungal agents.

RESULTS AND DISCUSSION

Synthetic chemistry: Firstly, *N*-(benzo[*d*]thiazol-2-yl)-2-cyanoacetamide **3**, is prepared via the direct cyanacetylation of 2-aminobenzo[*d*]thiazole **1** using the 2-cyano-*N*-(3,5-dimethyl-1*H*-pyrazol-1-yl)acetamide reagent **2** [25]. Then Knoevenagel condensation of **3** with different aldehyde derivatives occurred in the presence of piperidine affording the related *N*-(benzo[d]thiazol-2-yl)-2-cyano-3-arylylacrylamide derivatives **4**-**9**. The structures of the title compounds were characterized by inspection of their spectral data. For example, the mass spectrum of **9** revealed molecular ion peak as a base peak at m/z 311. IR indicated the appearance of NH band at 3241; CN band at 2224 and the CO band at 1671 cm^{-1}. ^{1}H NMR revealed singlet signal at 8.23 for the yildene-H3 proton. In addition it indicated the presence of broad singlet signal at 13.4 ppm corresponding to NH group. The signals of the benzo[*d*]thiazole and thiophene protons appeared in the area of 6.86-8.18 pp. Furthermore full assignment of ^{13}C NMR was found also to be in agreement with the proposed structure where it indicated the CN signal at 113.4 ppm. It also featured the carbonyl amide signal at 168.6 ppm. All other carbons appeared at their expected positions (a detailed analytical and spectral data of synthesized compounds are given in supplementary material to this paper).

Biology

***Antimicrobial activity*:** New series of acrylamide derivatives were synthesized and targeted to evaluate their *in vitro* antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, and Streptococcus faecalis* as examples of Gram positive bacteria and *Escherichia coli, Pseudomonas aeuroginosa, and Neisseria gonorrhoeae* as examples of Gram negative bacteria. They also were evaluated for their *in vitro* antifungal activity against *aspergillus flavus, candida albicans* and *Fusarium oxysporum* fungal strains. Agar-diffusion disc method was used for the evaluation of the preliminary antibacterial and antifungal activities. Tetracycline and amphotericin B (sigma- Aldrich) were used as reference antibacterial and antifungal drugs respectively. Resistance of microorganism to certain drug is often due to the acquisition of new genes or for a protein that protects bacterial ribosomes from the action of drugs. Many of these genes are associated with mobile plasmids or transposons. A limited number of bacteria acquire resistance by mutations, which alter the permeability of the outer membrane or alter the 16S rRNA [26]. On the other hand, some drugs act by binding to the sterol component of a cell membrane, leading to alterations in cell permeability and cell death [27]. Also it may have a higher affinity for the ergo-sterol component of the fungal cell membrane leading to cytotoxicity [27]. The results obtained were recorded for each derivative as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm based on 3 replicas. The inhibition zone diameter values are recorded in Table 1. The results obtained revealed that compound **3** which is the lead compound, is only active towards one gram positive and one gram negative bacteria. It also indicated that most of the tested compounds displayed highly inhibitory effects on the growth of the tested microorganisms except compounds **6** and **7** which show less activity compared to the lead compound **3**. This may be due to the presence of methoxy groups in **6** and **7**. These results are in agreement to that published by Alcaraz *et al* [28], who reported that methoxy groups cause drastically decrease in the antibacterial activity of flavonoids

[28]. In general, most of the tested compounds revealed better activity against the Gram negative rather than the Gram positive bacteria. Also, it is clear that compound **5** is the most active compound in these series where the presence of additional Cl atom in the para position of ylidene ring increase the activity compared to that of compound **4**. This is also similar to that reported in literature that the electron withdrawing groups increase the inhibitory potency [29].

On the other hand, substituting the benzene ring by heterocyclic rings, like thiophene and furan groups, increase the inhibitory effects toward the tested microorganism. Also we have found that, compounds **8** and **9** are more active towards gram negative bacteria than gram positive bacteria (table 1). In support of this viewpoint, it was reported by Behbehani *et al* [30] that thiophene containing compounds are bioactive molecules. In contrast to this result that was published by Darwish *et al* [31]and Abdelhamid *et al*[32] who reported that thiophene ring might decrease the antibacterial activity.

On the other hand, regarding antifungal agents, all tested derivatives gives moderate antifungal activity toward the fungal species *fusarium oxysporum* [8]. Compound **5** remains the most active compound as an antifungal agent. It is worth mentioning that, despite our compounds are strong antibacterial and antifungal agents, the two species *aspergillus flavus* and candida *albicans* showed strong resistance to them, this is compatible to that reported by Behbehani *et al* [30]. Finally we can say that the additional *p*-chlorobenzylidene, thiophen-2-yildene and furan-2-yildene to the lead compound **3**, would have increased the antimicrobial potency of **4**, **5**, **9**, and **8** respectively, while additional methoxy and trimethoxy groups in compounds **6** and **7** decrease the antimicrobial activity.

Cytotoxicity evaluation: Strong targeted therapies are needed to fight many kinds of human cancer, so we tried to prepare new derivatives in a trial to have high cytotoxic effect on tumor. All new compounds exhibited high to moderate activity towards human tumor cell lines as breast cancer (MCF7), liver carcinoma (HEPG2), and prostate cancer (PC3) as shown in (figure 2 in the supplementary material showed that compounds **4**-**9**). It was noted that the cell line PC3 is highly sensitive towards all compounds following to it in activity are HEPG2 and MCF7 respectively. In this study, Doxorubicin is used as a positive standard anticancer agent. Also we have reported that, the additional ylidene double bond to the skeleton of the lead compound **3** gives no observed change in the IC50 values of compound **4** with respect to MCF7 and HEPG2 cell lines, while in case of PC3 cell line, we noted that the addition of ylidene group into the lead compound with (IC50= 11.5 μg/ml) increase the activity of compound **4** (IC50 = 5.63 μg/ml).

Also, we have found that, derivative **6** is the most active antitumor compound in this series towards all tested cell lines (MCF7, HEPG2, and PC3), with IC50 values (3.68, 3.98, and 4.73 μg/ml), respectively. The enhancement in activity has been observed in case of **6** which is approximately equal to that of the standard anticancer drug doxorubicin with IC50 values (3.53, 3.9, and 4.2 μg/ml), on the same cell lines respectively (fig. 2).

The presence of methoxy substituent and its position has shown to play an important role on the observed growth inhibition. Acrylamides with methoxy group in the para position considerably improves the antitumor activity (compare **6** and **3**) which is compatible to that obtained by Peng *et al* [21] and Tarleton *et al* [13].

On the other hand, the introduction of additional two methoxy groups at ortho position in case of compound **7** resulted in the decrease in activity. Thus **7** is less active than **6** with IC50 values (11, 10.4, and 9.23 μg/ml), respectively on the same cell lines. This may be attributed to stearic hindrance effect of both methoxy groups in ortho positions.

It worth mentioning that, compound **5** is structurally related to the monomeric tyrphostins, which are known kinase inhibitors [14]. The presence of an electron withdrawing group in compound **5** is presumably important and imparted favorable growth inhibition of this compound [22]. Incorporating heterocyclic rings like thiophene into the lead structure **3**, gives **9** which is the least active compound in this series with IC50 values (14.6, 12 μg/ml) against MCF7 and HEPG2 respectively, while arylacrylamide incorporating furan derivative **8** gives moderate activities with IC50 values (9, 9.32 μg/ml) on the same lines. The two heterocyclic substituents **9** and **8** give moderate and approximately equal activities toward the cell line PC3 with IC50 value (8.48 μg/ml and 8.18 μg/ml) respectively.

Thus we can say that the antitumor activity of arylacrylamide incorporating furan is higher than that containing thiophene derivative against MCF7 and HEPG2 as demonstrated in IC50 values above. This study is similar to that reported by Zhenghong Peng *et al* [21]. Finally, concerning the effect of these derivatives on the normal cell line HFB4 (normal melanocytes), the prepared compounds

showed selective cytotoxicity to the malignant MCF7, HEPG2, and PC3 cell lines in comparison to the normal HFB4 cells as shown in Fig. 2 where, normal melanocytes cells (HFB4) were affected to a much lesser extent.

Antiviral evaluation: Antiviral activity was determined by subtracting the VSV mean titer in treated and non-treated cells. The difference between both titers refers to the antiviral activity [33]. Evaluation was based on cell treatment with test compounds for 24 h for expression of genes responsible for virus inhibition. The results of antiviral activity (table 2, Fig. 3) revealed that compound **3** which is the lead compound showed almost no effect against the tested virus recording 0.059 $\log_{10}$ / 0.1 ml (0.089%).

On the other hand, compound **8** showed the highest antiviral activity represents 0.865 $\log_{10}$ / 0.1 ml (12.9%) followed by compounds **4** and **5** recording 0.845 and 0.835 $\log_{10}$ / 0.1 ml (12.8% and 12.6 %) respectively. While Compounds **6** and **9** were in the medium category of antiviral activity categorization recording a depletion rate in the order of 0.447 $\log_{(10)}$ / 0.1 ml (6.777%). Finally IFN α-2a is used as a standard one showed the superior activity when used as 10 IU / ml recording a depletion rate; 3.595 $\log_{(10)}$ / 0.1 ml (54.5 %) [fig. 3]. In pharmacological term, the additional furan residue may be directly responsible for the highest antiviral activity of compound **8**. Replacement the furan residue with thiophene gives **9** with moderate activity, where oxygen atom increase the electrophilic character of nitrile group more than sulfur and, make it highly attracted to the nucleophilic cysteine or serine protease of the virus [34,35]. Also compound **5** and **4** are potent compounds but addition of electron withdrawing group (Cl) as in structure **5** slightly reduces its activity when compared to compound **4** [11]. On the other hand, addition of methoxy group in the para position as in **6** decreases the activity about 50 % in comparison with **4**. Finally we can say that addition of new groups to the skeleton of the lead **3** improve the antiviral characters of these compounds.

EXPERIMENTAL PART

Chemistry: Melting points were determined on a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded as KBr pellets using a Bruker-vector 22 spectrophotometer FTIR. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 as solvent at 400 MHz and 100 MHz respectively on Varian Gemini NMR spectrometer using TMS as internal standard. Chemical shifts are reported in δ units (ppm). Mass spectra were measured on a Shimadzu GMSS -QP-1000 EX mass spectrometer at 70 eV.

Synthesis of *N-(benzo[d]thiazol-2-yl)-2-cyanoacetamide (3):* A solution of 1-cyanoacetyl-3,5-dimethylpyrazole **2** (0.1 mol) was added to a solution of 2-aminobenzothiazole **1** (0.1 mol) in toluene (100 ml) and the mixture was refluxed for 4 h. After cooling, the deposited solid was collected and crystallized from ethanol-dioxane.

Yield: (85 %) as a solid, this was recrystallized from ethanol to give white crystals (m.p 244-246) ^{o}C. IR (KBr, cm^{-1}): 3296 (NH), 2261 (CN), 1690 (CO); ^{1}H NMR (400 MHz, DMSO-d6): *δ*, ppm: 4.12 (s, 2H, CH2), 7.31-7.47 (m, 2H, Ar-H), 7.76-8.01 (m, 2H, Ar-H), 12.68 (br. S, 1H, NH) ;^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 26.8, 115.6, 121.1, 122.3, 124.3, 126.7, 131.9, 148.7, 158.0, 163.4; MS (EI): m/z (%) = 217 (M^+).
Anal. Calcd. for $C_{10}H_7N_3OS$ (217.246): C, 55.29; H, 3.25; N, 19.34; S, 14.76. Found: C, 55.45; H, 3.18; N, 19.67; S, 14.52

***General procedure for the preparation of compounds* (4-9):** A mixture of substituted benzaldehyde (1 mmol), *N*-(2-benzothiazolyl)cyanoacetamide (**3**, 1 mmol) was refluxed in ethanol in the presence of piperidine as a catalyst for 3 h. The solid product so formed is recrystallized from ethanol.

(E)-N-(benzo[d]thiazol-2-yl)-2-cyano-3-phenylacrylamide (4): Yield: (88 %) as a solid, this was recrystallized from ethanol to give yellow crystals crystals (m.p 221-222) ^{o}C. IR (KBr, cm^{-1}): 3260 (NH), 2221 (CN), 1610 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 7.33-8.0 (m, 9H, Ar-H), 8.49 (s, 1H, H3), 9.95 (br. S, 1H, NH) ;^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 88.6, 110.4, 117.0, 123.0, 124.4, 127.4, 128.4, 129.8, 130.9, 132.4, 133.2, 135.7, 152.7, 155.7, 157.4, 161.2; MS (EI): m/z (%) = 305 (M^+).
Anal. Calcd. for $C_{17}H_{11}N_3OS$ (305.35): C, 66.87; H, 3.63; N, 13.76; S, 10.50. Found: C, 66.93; H, 3.47; N, 13.65; S, 10.63.

(E)-N-(benzo[d]thiazol-2-yl)-3-(4-chlorophenyl)-2-cyanoacrylamide (5): Yield: (90 %) as a solid, this was recrystallized from ethanol to give yellow crystals m.p 244-246 ^{o}C. IR (KBr, cm^{-1}): 3272 (NH), 2210 (CN), 1680 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 7.35-7.52 (m, 2H, Ar-H), 7.71 (d, 2H, Ar-H, *J* = 8.48), 7.97 (d, 1H, Ar-H), 8.05 (d, 2H, Ar-H, *J* = 8.48), 8.48 (s, 1H, H3), 9.90 (br. S, 1H, NH); MS (EI): m/z (%) = 339 (M^+).
Anal. Calcd. for $C_{17}H_{10}ClN_3OS$ (339.023): C, 60.09; H, 2.97; Cl, 10.43; N, 12.37; S, 9.44. Found: C, 60.28; H, 3.12; Cl, 10.35; N, 12.51; S, 9.63.

(E)-N-(benzo[d]thiazol-2-yl)-2-cyano-3-(4-methoxyphenyl)acrylamide (6): Yield: (88 %) as a solid, this was recrystallized from ethanol to give yellow crystals (m.p 236-238) ºC. 3289 (NH), 2212 (CN), 1688 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 3.88 (s, 3H, OCH_3), 7.15-7.52 (d, 2H, Ar-H, *J* = 7.8 Hz), 7.31-7.97 (m, 4H, Ar-H), 8.05 (d, 2H, Ar-H, *J* = 7.8 Hz), 8.48 (s, 1H, H3), 8.43 (s, 1H, H3), 13.2 (br. S, 1H, NH);^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 56.3, 115.16, 121.0, 122.2, 124.4, 124.9, 126.7, 131.9, 133.6, 138.5, 143.2, 148.9, 152.2, 157.9, 163.5, 167.8; MS (EI): m/z (%) = 335 (M^+).
Anal. Calcd. for $C_{18}H_{13}N_3O_2S$ (335.072): C, 64.46; H, 3.91; N, 12.53; S, 9.56. Found: C, 64.58; H, 3.78; N, 12.72; S, 9.39.

(E)-N-(benzo[d]thiazol-2-yl)-2-cyano-3-(2,4,6-trimethoxy-phenyl)acrylamide (7): Yield: (87 %) as a solid, this was recrystallized from ethanol to give yellow crystals (m.p 150-152) ºC. 3211 (NH), 2217 (CN), 1726 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 7.19-7.97 (m, 6H, Ar-H), 8.43 (s, 1H, H3), 13.4 (br. S, 1H, NH);^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 56.5, 60.8, 100.9, 108.8, 109.3, 116.5, 118.1, 121.2, 121.3, 125.9, 126.9, 131.4, 141.9, 142.6, 153.4, 155.6, 163.0; MS (EI): m/z (%) = 395 (M^+).
Anal. Calcd. for $C_{20}H_{17}N_3O_4S$ (395.433): C, 60.75; H, 4.33; N, 10.63; S, 8.11. Found: C, 60.83; H, 4.51; N, 10.82; S, 8.01.

(E)-N-(benzo[d]thiazol-2-yl)-2-cyano-3-(furan-2-yl)acrylamide (8): Yield: (90 %) as a solid, this was recrystallized from ethanol to give greenish yellow crystals (m.p 244-246) ºC. 3263 (NH), 2209 (CN), 1679 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 7.32-7.37 (m, 2H, Ar-H), 7.46-7.50 (m, 1H, furan-H), 7.61 (d, 1H, furan-H), 7.95-7.97 (m, 2H, Ar-H), 8.18 (d, 1H, furan-H), 8.70 (s, 1H, H3), 13.2 (br. S, 1H, NH);^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 113.6, 117.2, 117.4, 123.0, 124.4, 127.4, 129.3, 136.5, 136.7, 139.1, 143.3, 143.6, 145.5, 154.6, 163.1; MS (EI): m/z (%) = 395 (M^+).
Anal. Calcd. for $C_{15}H_9N_3O_2S$ (295.041): C, 61.01; H, 3.07; N, 14.23; S, 10.86. Found: C, 61.23; H, 3.26; N, 14.51; S, 10.76.

(E)-N-(benzo[d]thiazol-2-yl)-2-cyano-3-(thiophen-2-yl)acrylamide (9): Yield: (93 %) as a solid, this was recrystallized from ethanol to give greenish yellow crystals (m.p 215-217) ºC. 3241 (NH), 2224 (CN), 1671 (CO); ^{1}H NMR (400 MHz, DMSO-d6): δ, ppm: 6.86-6.88 (m, 1H, thiophene-H), 7.30-7.48 (m, 3H, Ar-H), 7.58 (d, 1H, thiophene-H), 7.94 (d, 1H, thiophene-H), 8.18 (m, 1H, Ar-H), 8.23 (s, 1H, H3), 13.4 (br. S, 1H, NH);^{13}C NMR (100 MHz, DMSO-d6): δ, ppm: 113.4, 114.6, 116.9, 122.5, 122.8, 124.1, 127.2, 137.1, 143.3, 143.5, 144.4, 149.0, 149.6, 153.1, 168.6; MS (EI): m/z (%) = 311 (M^+).
Anal. Calcd. for $C_{15}H_9N_3OS_2$ (311.018): C, 57.86; H, 2.91; N, 13.50; S, 20.59. Found: C, 57.92; H, 2.79; N, 13.69; S, 20.42.

Antimicrobial Assay: Antimicrobial test of all new derivatives was determined according to Mohamed *et al* [32], using a modified Kirby–Bauer disk diffusion method [36]. Our compounds were investigated using three gram positive bacterial strains as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, and three game negative strains as *Escherichia coli*, *Pseudomonas aeuroginosa*, *and Neisseria gonorrhoeae*. Also we tested these derivatives on three fungal species as *aspergillus flavus*, *candida albicans* and *Fusarium oxysporum*, 100 µl of all human pathogenic bacteria and fungi were grown in 10 ml of fresh media until they reached a count of approximately, 108 cells / ml for bacteria or 10^5 cells/ml for fungi [37]. One hundred microliters of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Blank filter paper disks (Whatman) with a diameter of 11.0 mm were impregnated with 100 µl of tested compound dissolved in 1 % DMSO of the stock solutions (50 mg/ml). Disk diffusion method for bacteria and filamentous fungi were tested by using approved standard method developed by the by National Committee for Clinical Laboratory Standards [38]. Bacterial strains were incubated at 37 °C for 24 h. While, *A. flavus*, *C. albicans* and *Fusarium oxysporum* were incubated at 30 °C for 48 h. After incubation, the antimicrobial activity was measured in terms of the zone of inhibition in mm [36]. Standard disks of tetracycline (standard Antibacterial agent), Amphotericin B (standard antifungal agent) served as positive controls for antimicrobial assay and filter disks impregnated with 100 µl of solvent (DMSO) were used as a negative control.

SRB Analysis: Anticancer test of acrylamide derivatives was performed according to Mohamed *et al* [32], [39] using three different tumor cell lines MCF7, HEPG2, and PC3. Cells were seeded at a density of 3×10^3 per well in 96-well microtiter plates. Cells were left for 24 h before incubation with various concentrations of the tested compounds (0, 5, 15, 25, and 50 µg/ml) for time interval 48 h. Cytotoxicity was determined using SRB assay according to previously explained by Skehan *et al* [40]. Fixation of cells was performed by the addition of 10 % cold trichloroacetic acid. After 1 h incubation at 4 °C, cells were washed five times with deionized water. The cells were then

stained with 0.4 % SRB dissolved in 1 % acetic acid for at least 30 min and subsequently washed four times with 1 % acetic acid to remove unbound stain. The plates were left to dry at room temperature and bound protein stain was solubilized with 100 µl/well Tris base (10 mM, pH10.5) and the optical density of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan reader, Germany) and the mean value of each drug concentration was calculated. Data from cell viability analysis were analyzed using Prism Software program (Graphpad Software incorporated, version 3) to determine IC50 values of the tested compounds in the three different cancer cell lines after 48 h. In addition, the compound derivatives were tested against the normal cell line HFB4 (normal melanocytes) using the same above SRB method to test the toxic effect of these compounds on HBF4 line as an example of normal cell.

Antiviral evaluation: Vero clone CCL-81 was kindly supplied from Cell Culture (CC) Department, VACSERA –Egypt. Cells were grown in 199 E-Hepes buffer (GIBCO-UK) growth medium was supplemented with 10% inactivated fetal calf serum (FCS), 5mM HEPES buffer and antibiotics (100 IU) of penicillin/ ml, 100 gm of streptomycin/ml) at 37 ºC and incubated in 5% CO_2 atmosphere. *Vesicular stomatitis virus (VSV)*, Indiana strain- 156, was kindly supplied by Applied Research Sector, VACSERA-Egypt. Virus infectivity titer was determined. Cytotoxicity (IC_{50}) of the selected synthesized compounds to Vero clone CCL-81 cell line was evaluated using MTT assay. Optical density was measured at 570 nm using ELISA reader X-800 (Biotech –USA). Antiviral activity of the new synthetic compounds and interferon against *Vesicular Stomatitis virus (VSV) Indiana strain -156* was determined according to the method reported by Shinji [41]. Test organic compounds (1 mg) were dissolved in DMSO in the presence of 0.02 µL tween 20 as a surfactant, test materials were 0.22 µm membrane filtrated using Millipore disposable filters (Millipore- USA) according **to** Emad *et al* [42]. Also, antiviral activity was determined, whereby nontoxic concentrations of test chemical compounds and rh-IFN (10 IU/ 0.1 cm^3) as a positive control were used for the treatment of 24 hrs pre cell culturing of Vero cells as 0.1 cm^3/well. One plate was maintained and left untreated for viral control titration. Virus infectivity titer was determined according to the method reported by Steven *et al* [43]VSV was tenfold serially diluted in 199 E-Hepes buffer (10^{-1}–10^{-8}). Each dilution was dispensed as 100 µl/well onto pre-culture treated Vero cells. Plates were inoculated using 10 fold serially diluted VSV. Plates were incubated at 37 ºC. Seven days post infection the 50 % end point (CCID50) was determined. Antiviral activity was determined by subtracting the VSV mean titer in the treated and non-treated cells. The difference between both titers refers to the antiviral activity. Test organic compounds (1 mg) were dissolved in DMSO in the presence of 0.02 µL tween 20 as a surfactant, test materials were 0.22 µm membranes filtrated using Millipore disposable filters (Millipore- USA).

CONCLUSION

In conclusion, in the present study we managed to prepare and investigate the antimicrobial, antitumor, and antiviral activities of new heterocycles incorporating acrylamide moiety. We also managed to prepare the lead compound **3** and improving its biological characters by incorporation of new different groups to its skeleton through synthesizing compounds **4**, **5**, **6**, **9**, **8**, and **7**. The structural activity relationship (SAR) indicated that, all compounds exhibited strong antimicrobial activity except the two derivatives **6** and **7**. Also it revealed that, the antitumor activity, **6** is the mostly active compound in this series while **5** shows moderate inhibition action against human breast cancer (MCF7), human liver carcinoma (HEPG2), and prostate cancer (PC3). Most of compounds in these series show less toxic effect against the normal HBF4 cells. Compounds **8** (12.9%), **4** (12.8%) and, **5** (12.6%) are the most potent antiviral derivatives here, while lead compound **3** is the less active derivative in this series as antiviral agent (0.089%).

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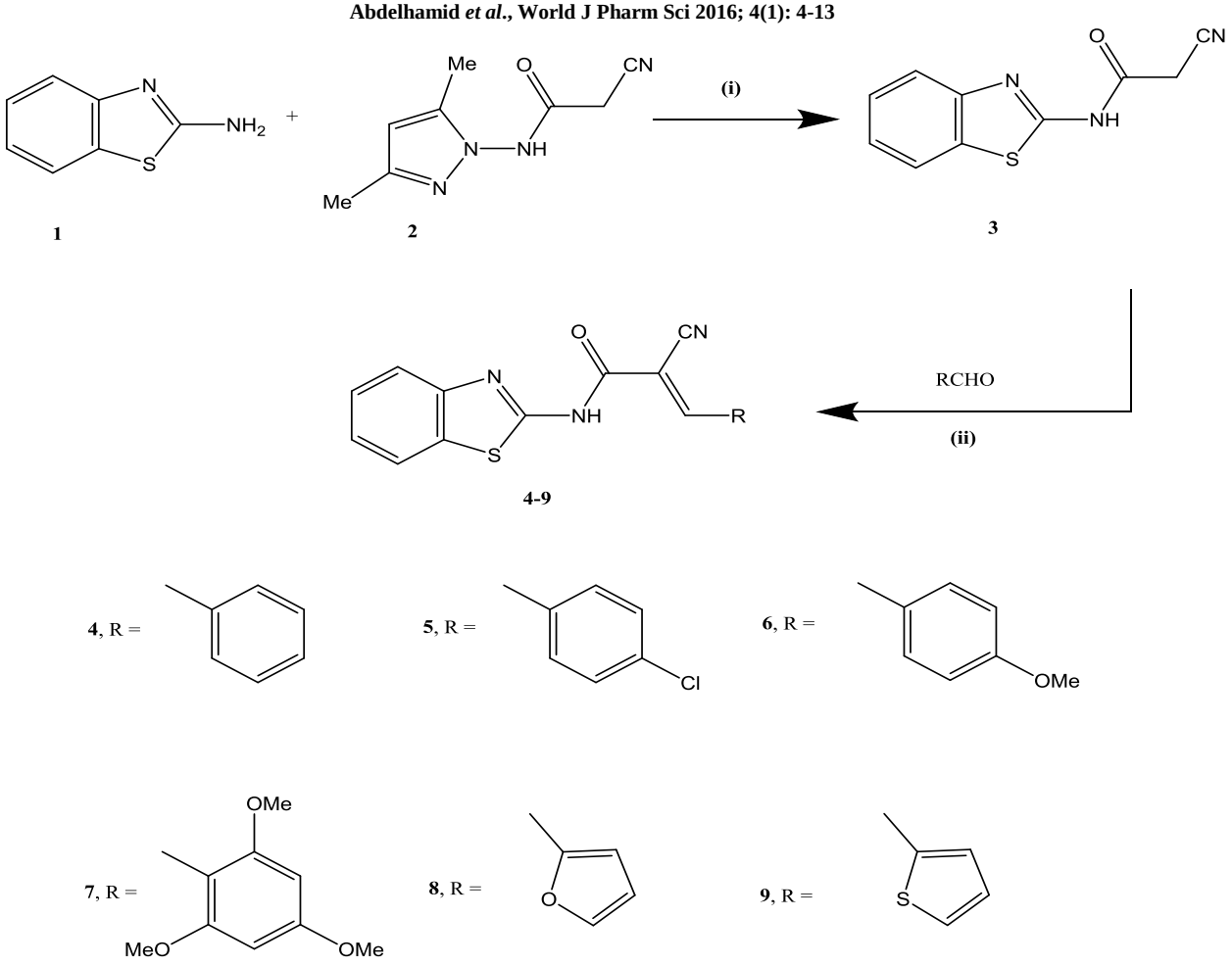

Scheme 1. Reagents and conditions: (i) Toluene, 100 ^{o}C, 60 min; (ii) RCHO , piperidine (cat), EtOH refux 4 h.

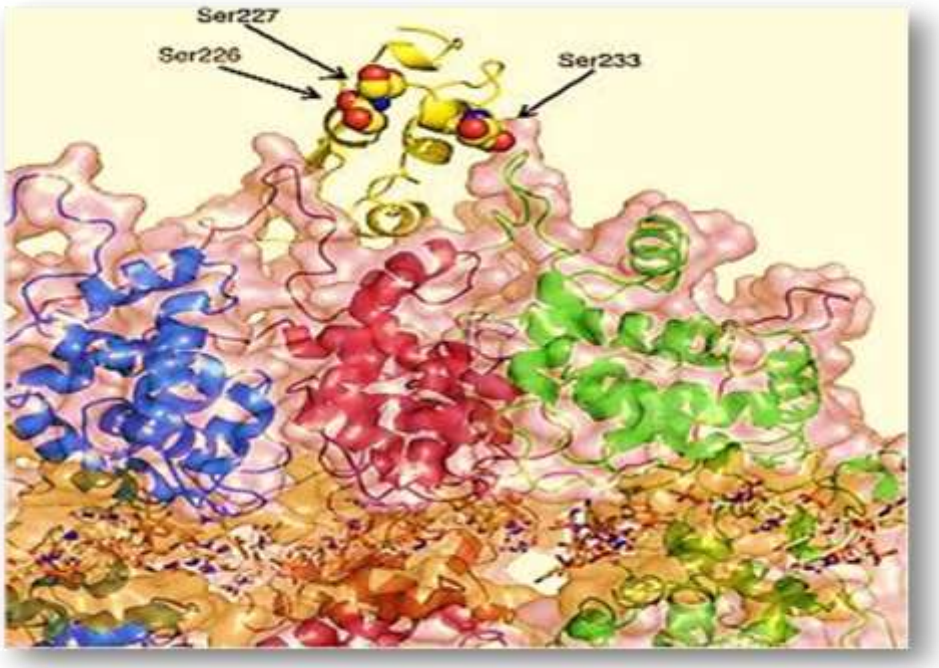

Figure: 1. 3D dimension structure of *Vesicular stomatitis virus (VSV).*
(http://www.pnas.org/content/106/28/11713/F4.expansion.html)

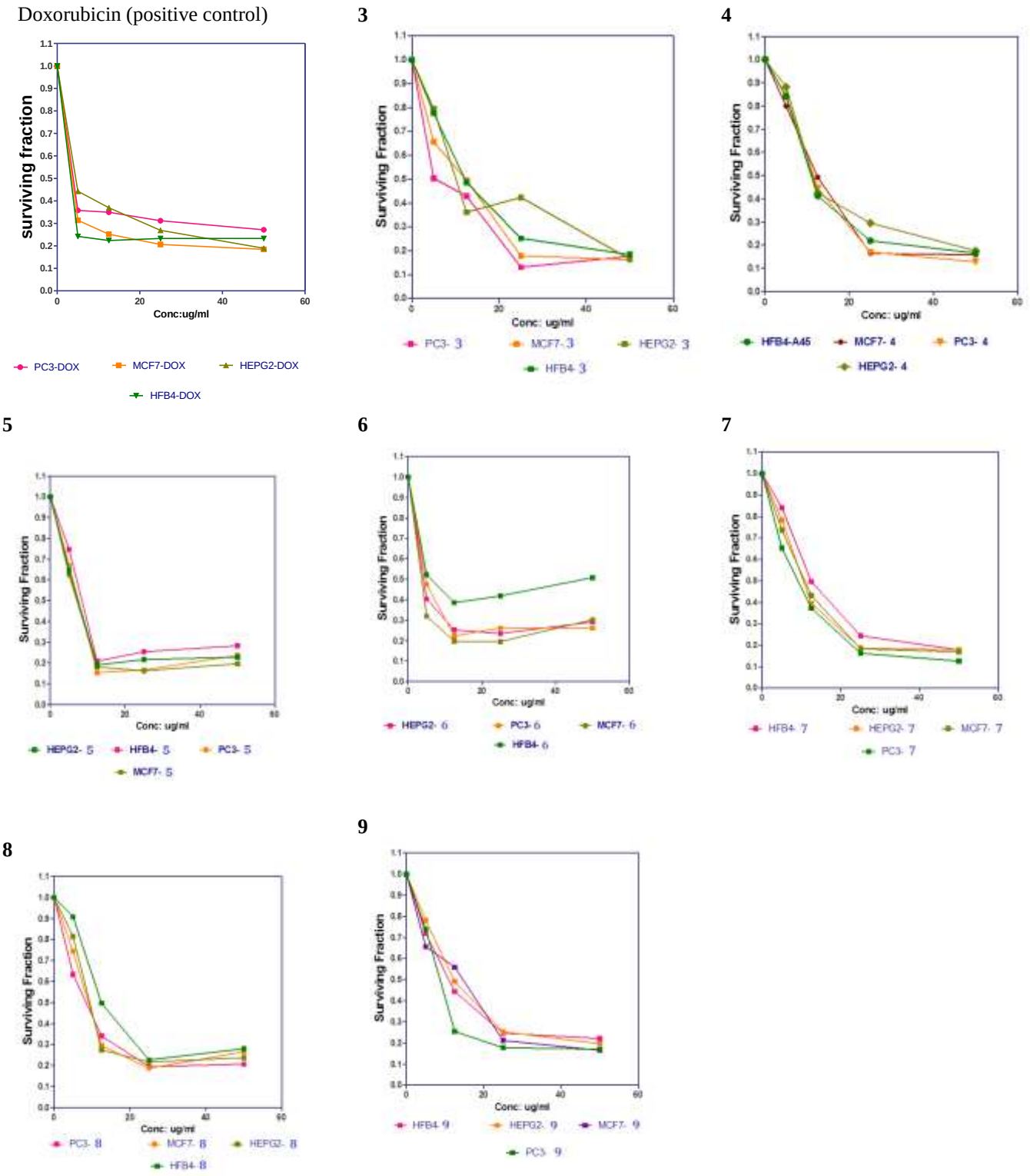

Fig. 2: cytotoxic activity of the new derivatives (**3**, **4**, **5**, **6**, **7**, **8** and **9**) against the MCF7, HEPG2, PC3 and the normal melanocytes HBF4 after 48 h exposure. Doxorubicin is used as standard agent against the same lines. Each point is the mean ± SD (standard deviation) of three independent experiments performed in triplicate, using prism software program (Graphpad software incorporated, version 3). [32]

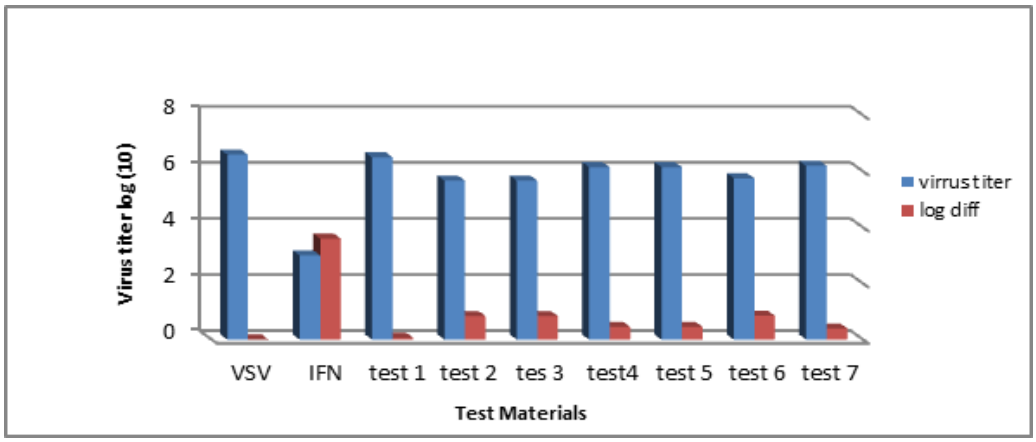

Fig 3: Evaluation of antiviral activity of test materials against *Vesicular Stomatitis Virus (VSV)* compared to Interferon α-2a (IFN α-2a).

	Inhibition Zone Diameter (mm / mg sample)									
	(-ve control)	**Tetracycline (+ve control)**	**Amphotericin B (+ve control)**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bacillus subtilis (G^+)	0.0	30	-	18	24	22	0.0	0.0	20	18
Staphylococcus aureus (G^+)	0.0	30	-	0.0	20	20	0.0	0.0	20	18
Streptococcus faecalis (G^+)	0.0	30	-	0.0	24	24	0.0	0.0	18	18
Escherichia coli (G−)	0.0	33	-	18	22	24	0.0	0.0	24	20
Pseudomonas aeuroginosa (G−)	0.0	31	-	0.0	24	26	0.0	0.0	20	20
Neisseria gonorrhoeae (G−)	0.0	33	-	0.0	24	24	0.0	0.0	20	20
Aspergillus flavus (fungus)	0.0	-	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fusarium oxysporum fungus)	0.0	-	14	10	13	14	0.0	0.0	13	12
Candida albicans (fungus)	0.0	-	14	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 1: Antimicrobial assay of new series of acrylamide derivatives (zone of inhibition in mm)
(– : no reaction**:** 0.0, no activity)

No. of Tests	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**	**Test 7**
Test compounds	**3**	**4**	**5**	**6**	**9**	**8**	**7**
IC50 (µgm/ ml)	**17.84**	**15.54**	**11.81**	**22.53**	**14.76**	**21.26**	**9.6**

Table 2 Cytotoxicity (IC50 µgm / ml) values of the selected synthesized compounds were evaluated using MTT assay.

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