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Synthesis, Characterization of New Formazan Derivatives and Study of Biological and Anticancer Activity

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Abstract. The aim of the research is the synthesis of formazan derivatives and the study of its biological activity and anticancer (breast cancer).The first step: reaction of the amino derivative 4-Methoxy-2-nitroaniline with Chloroacetyl Chloride to prepare the derivative (1), the second step: reaction of the derivative (1) with Hydrazine hydrate to prepare the derivative (2),the third step: the reaction of the derivative (2) with the Vanillin to prepare the Schiff base derivative (3), and the last step: included the reaction of the following amines (2-Amino benzimidazole, 3-Bromoaniline, 1-Phenol-2,3-dimethyl-4-aminopyrazolon-(5), 2-Amino-6-methoxybwnzothiazole) with sodium nitrite and hydrochloric acid to form diazonium salt which is reacted with Schiff base derivative (3) to prepare formazan derivatives (4-7). All the prepared derivatives were studied spectroscopically by means of a spectrum (FT-IR,^{1}H-NMR,^{13}C-NMR).

INTRODUCTION

Formazan is one of the chemical organic compounds with the general formula (NH--N = N-C = N-), which is characterized by its semi-cyclic colored compounds, which are characterized by bright colors extending from red to dark black due to the occurrence of electronic transitions of the type π - n * and * π - π [1,2]. The stability of formazan derivatives comes from their structure, which is due to the presence of more than two atoms of nitrogen, so formazan compounds are used as clikands in the formation of complexes [3]. In general, formazan is a solid material with a relatively low melting point despite its large molecular size. It is soluble in chloroform, acetone, ethanol and it is slightly soluble in water [4]. Formazan compounds are an important class of organic compounds whose chemical applications have attracted the interest of many research groups due to their wide pharmaceutical, biological, medical, industrial and chemical applications in various fields as well as their usefulness in analytical chemistry [5,6]. Tetrazolium salts are classified as probiotics for formazan known for their medicinal activities such as antiviral, anticancer, antimicrobial, anti-inflammatory, anti-fungal, anti-HIV, anti-fertility and anti-malarial [7,8]. The photochemical properties of formazan allow it to be used as analytic agents and resistance dyes. Many formazan dyes contain transition elements, for example copper (II) to increase the stability and chromophore properties [9,10]. The color and density of formazan dyes strongly depend on the substituents, the aryl derivatives are darker in color in contrast to those containing the aliphatic substitutes. Moreover, the presence of the nitro group in the aryl molecule leads to a deepening of the color compared to the methyl group. Formazan dyes with heterocyclic compensators can also exhibit color-thermal properties [11-13].

MATERIAL

All chemicals compounds in this work were of a high purity,include: Chloroacetyl Chloride, 4-Methoxy-2-nitroaniline, 2-Aminobenzimidazole, Sodium Hydroxide (Sigma Aldrich,Germany), Benzene dry (Avonchem), Hydrazine hydrate (Solvochem), Vanillin (Thomas Baker,India), 2-Amino-6-methoxybwnzothiazole (Fluorochem,UK), 1-Phenol-2,3-dimethyl-4-aminopyrazolon-(5) (Riedel-De haen), Sodium Nitrite, 3-Bromoaniline (B.D.H, England), Hydrochloric acid (Himedia, India), Ethanol absolute (Scharlau,Spain).

INSTRUMENTS

FT-IR Spectra (4000-400cm^{-1}) were recorded on Shimadzu FT–IR 8400S Fourier Transform infrared spectrophotometer as KBr disc. ^{1}HNMR was recorded on Fourier transformation Bruker spectrometer operating at (500MHz) with (DMSO) measurements were made at Tehran University , Iran , and Melting points were measured using digital (Stuart,UK).

EXPERIMENTAL

Synthesis of Derivative (1) 2-chloro-N-(4-methoxy-2-nitrophenyl)acetamide

(0.0059 mol, 0.46 mL) of Chloroacetyl Chloride was added dropwise to (0.0059 mol, 1g) of 4-Methoxy-2-nitroaniline dissolved in (30 mL) dry Benzene with continuous stirring by a magnetic stirrer at room temperature for (3 hours) then it was filtered, the filtrate was taken, dried and recrystallized with absolute ethanol, then weighed and calculated the percentage and physical properties of the prepared derivative as shown in Table (1-1) [14].

Synthesis of Derivative (2) 2-hydrazineyl-N-(4-methoxy-2-nitrophenyl)acetamide

Mixed (0.0041 mol, 1g) of derivative No. (1) with (0.0082 mol, 0.26 mL) of 80% Hydrazine hydrate in (30 mL) dry benzene with continuous stirring with a magnetic stirrer at room temperature for (10 hours). Where the reaction process was followed up using TLC technique, then it was filtered, the filtrate was taken, dried and recrystallized with absolute ethanol, then it was weighed and the percentage and physical properties of the prepared derivative were calculated as shown in Table (1-1) [14].

Synthesis of Schiff Base Derivative (3) 2-(2-(4-hydroxy-3-methoxybenzylidene)hydrazineyl)-N-(4-methoxy-2-nitrophenyl)acetamide

In a circular flask, (0.0042 mol, 1g) of derivative No. 2 was mixed with (0.0042 mol, 0.6333g) of Vanillin in (30 mL) absolute ethanol in the presence of drops of glacial acetic acid, and the mixture was escalated at (70 °C)) for (10 hours), during which the process of following up the reaction process was carried out using TLC technology, then it was filtered, the filtrate was taken, dried and recrystallized with absolute ethanol, then it was weighed and the percentage and physical properties of the prepared derivative were calculated as shown in Table (1-1) [14].

Synthesis of Formazan Derivatives (4-7)

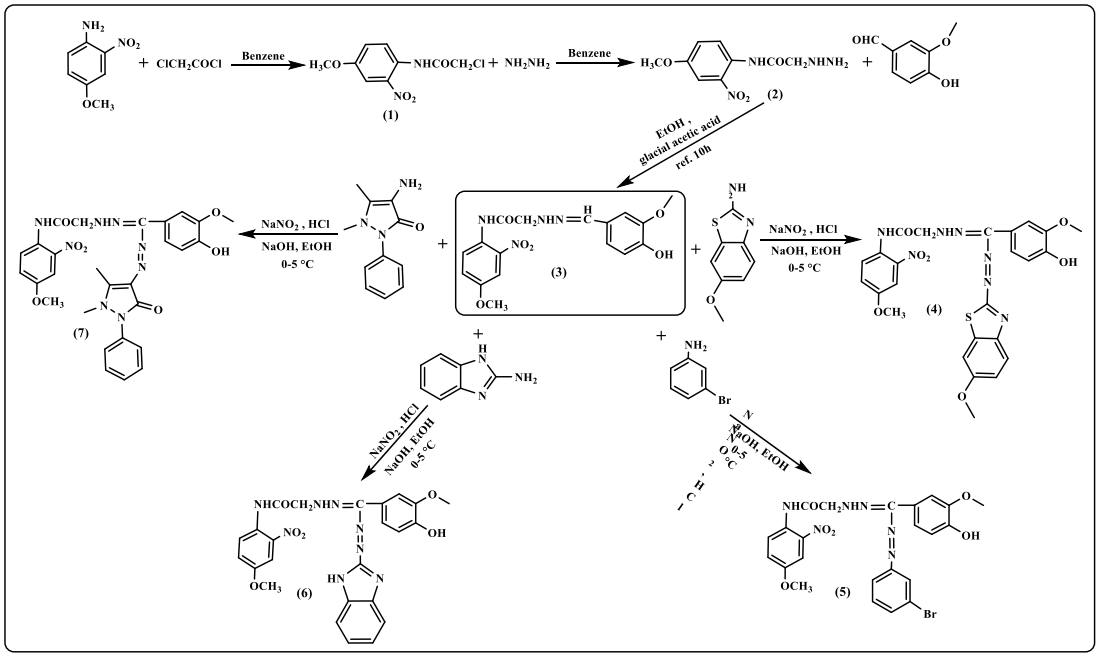
4:3-(4-hydroxy-3-methoxyphenyl)-5-(2-((4-methoxy-2-nitrophenyl)amino)-2-oxoethyl)-1-(6-methoxybenzo[d]thiazol-2-yl)formazan

5:1-(3-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)-5-(2-((4-methoxy-2-nitrophenyl)amino)-2-oxoethyl)formazan

6:1-(3-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)-5-(2-((4-methoxy-2-nitrophenyl)amino)-2-oxoethyl)formazan

7:1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-hydroxy-3-methoxyphenyl)-5-(2-((4-methoxy-2-nitrophenyl)amino)-2-oxoethyl)formazan

(0.0014 mol) of (2-Amino benzimidazole, 3-Bromoaniline, 1-Phenol-2,3-dimethyl-4-aminopyrazolon-(5), 2-Amino-6-methoxybwnzothiazole was dissolved) in (4 mL HCl + 30 mL distilled water) and the mixture was cooled to a temperature ranging between (0-5 °C) and then added to the whole mixture (0.0014 mol) of sodium nitrite Dissolved in (10 mL) of distilled water drop by drop with continuous stirring for (20 min). Then each solution of the prepared solutions was gradually added to the solution consisting of dissolving (0.0014 mol) of Schiff base derivative (3) to prepare derivatives from (4-7), respectively, and (1g) of sodium hydroxide dissolved in (30 mL) of cooled distilled water (0-5 °C) and (5 ml) of absolute ethanol. Then the precipitate was filtered, washed with distilled water, dried and recrystallized with absolute ethanol, then weighed and calculated the percentage and physical properties of the prepared derivatives as shown in Table (1-1) [14,15].



SCHEME 1. Synthesis of Formazan Derivatives (4-7)

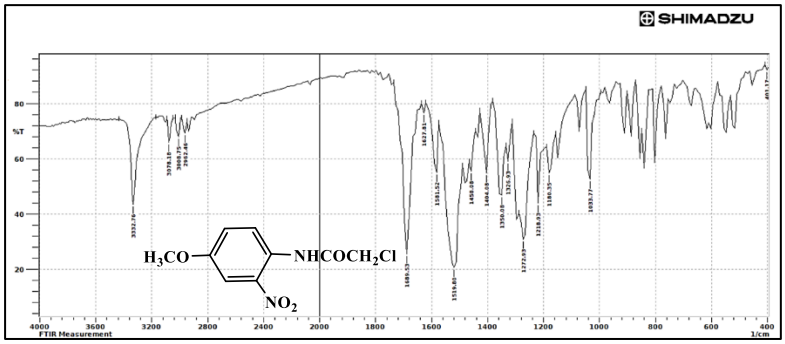
TABLE 1. Physical properties of the Derivatives (1-7)

Derivatives	Colour	M. P (ºC)	M,Wt (g/mol)	R.F	Yield %
1	Orange	105	244.63	0.48	71.4
2	Orange	107	240.22	0.52	76.3
3	Orange	93	374.35	0.51	72.5
4	Grey	106	565.56	0.6	73.9
5	Yellow	101-103	557.36	0.56	71.6
6	Yellow	105-103	518.49	0.61	70.7
7	Dark green	101-102	588.58	0.58	73.8

RESULTS AND DISCUSSION

1. Derivative (1)

FT-IR spectrum data showed band at 3332 $cm^{-1}$for (N-H), 3079 cm^{-1} for (C-H) Aromatic, 2962,2893 cm^{-1} for (C-H) Aliphatic, 1689 cm^{-1} for (C=O), 1519,11350 cm^{-1} for (NO_2), 1581,1458 cm^{-1} for (C=C) Aromatic, 1272 cm^{-1} for (C-N), 1218 cm^{-1} for (C-O), 680 cm^{-1} for (C-Cl). The ^{1}H-NMR spectrum data of derivative (1) showed δ : 10.4 (S,1H,NH), 7.3-7.6 (M,3H,Ar-H), 4.3 (S, 2H,CH_2), 3.8 (S, 3H,OCH_3). The ^{13}C-NMR spectrum data of derivative (1) showed δ : 165 for (C_9), 156 for (C_3), 109-143 for ($C_{arom.}$), 56 for (C_{10}), 43 for (C_1).



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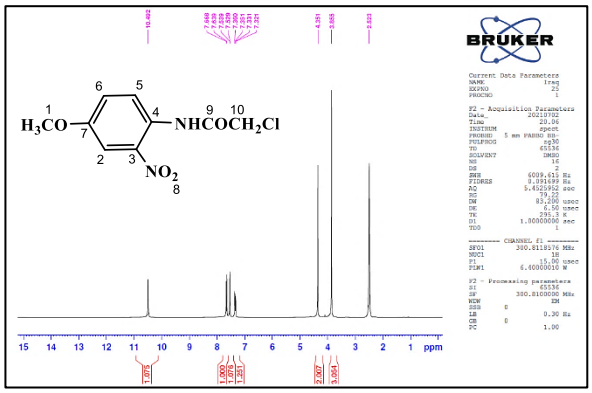

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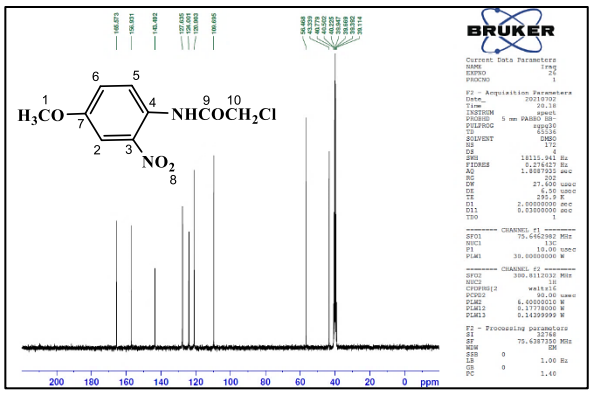
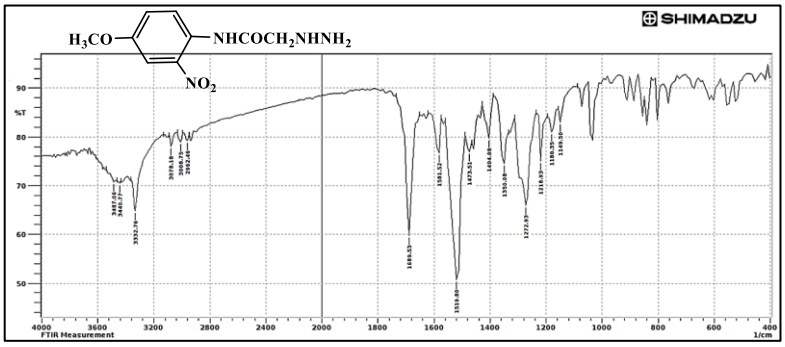


(c)

FIGURE 1. a-FT-IR Spectrum of the derivative (1), b-^{1}H-NMR Spectrum of the derivative (1), c-^{13}C-NMR Spectrum of the derivative (1)

2. Derivative (2)

FT-IR spectrum data showed band at 3487,3440 $cm^{-1}$for (NH_2), 3332 $cm^{-1}$for (N-H), 3078 cm^{-1} for (C-H) Aromatic, 2962,2939 cm^{-1} for (C-H) Aliphatic, 1689 cm^{-1} for (C=O),1519,1350 cm^{-1} for (NO_2),1581,1473 cm^{-1} for (C=C) Aromatic, 1272 cm^{-1} for (C-N), 1218 cm^{-1} for (C-O). The ^{1}H-NMR spectrum data of derivative (2) showed δ : 10.5 (S,1H,NH^9), 10 (S,1H,NH^{12}), 7.3-7.6 (M,3H,Ar-H), 4.3 (S, 2H,NH_2), 3.8 (S, 2H,CH_2), 3.4 (S, 3H,OCH_3). The ^{13}C-NMR spectrum data of derivative (2) showed δ : 165 for (C_{10}), 156 for (C_3), 109-143 for ($C_{arom.}$), 56 for (C_{11}), 43 for (C_1).



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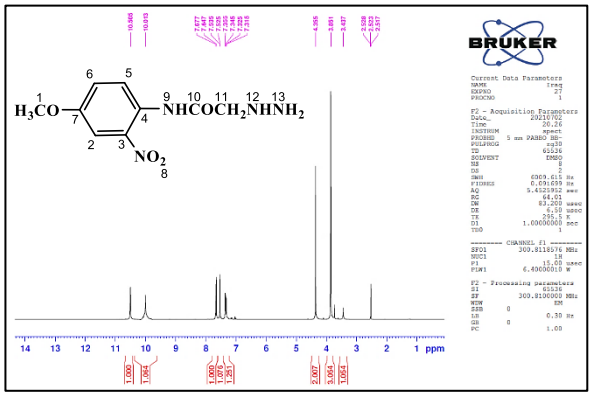

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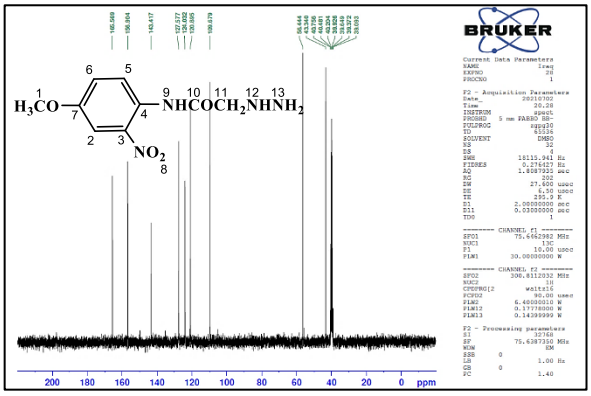
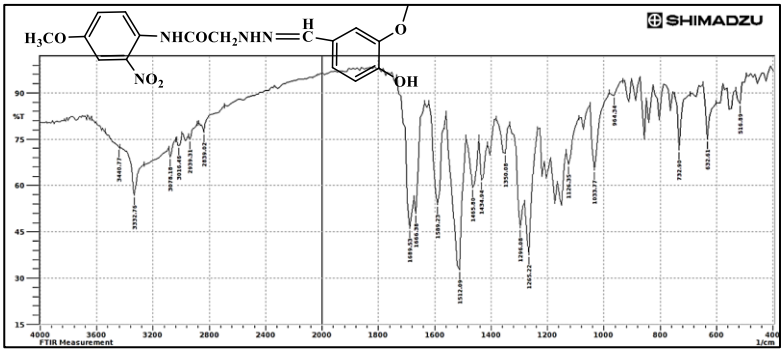


(c)

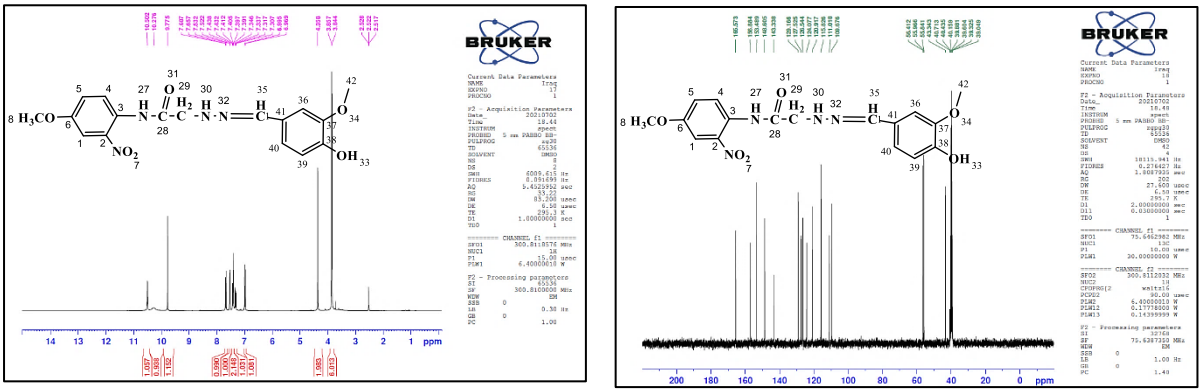
FIGURE 2. a-FT-IR Spectrum of the derivative (2), b-^{1}H-NMR Spectrum of the derivative (2), c-^{13}C-NMR Spectrum of the derivative (2)

3. Derivative (3)

FT-IR spectrum data showed band at 3440 $cm^{-1}$for (OH), 3332 $cm^{-1}$for (N-H), 3078 cm^{-1} for (C-H) Aromatic, 2939,2839 cm^{-1} for (C-H) Aliphatic, 1689 cm^{-1} for (C=O), 1666 cm^{-1} for (C=N), 1512,1350 cm^{-1} for (NO_2), 1589,1434 cm^{-1} for (C=C) Aromatic, 1265 cm^{-1} for (C-N), 1218 cm^{-1} for (C-O). The ^{1}H-NMR spectrum data of derivative (3) showed δ : 10.5 (S,1H,OH), 10.2 (S,1H,NH^{27}), 9.6 (S,1H,NH^{30}), 6.9-7.6 (M,6H,Ar-H), 4.3 (S, 2H,CH_2), 3.85,3.84 (S, 6H,OCH_3). The ^{13}C-NMR spectrum data of derivative (3) showed δ : 165 for (C_{28}), 156 for (C_{38}), 153 for (C_2), 148 for (C_{35}), 109-143 for ($C_{arom.}$), 56 for (C_{37}), 55 for (C_8), 43 for (C_{29}).



(a)

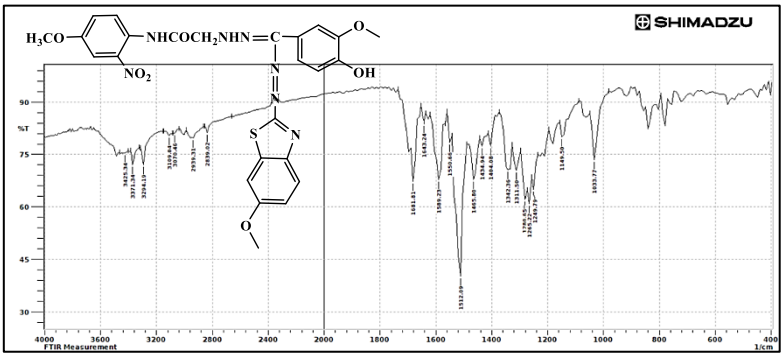


(b) (c)

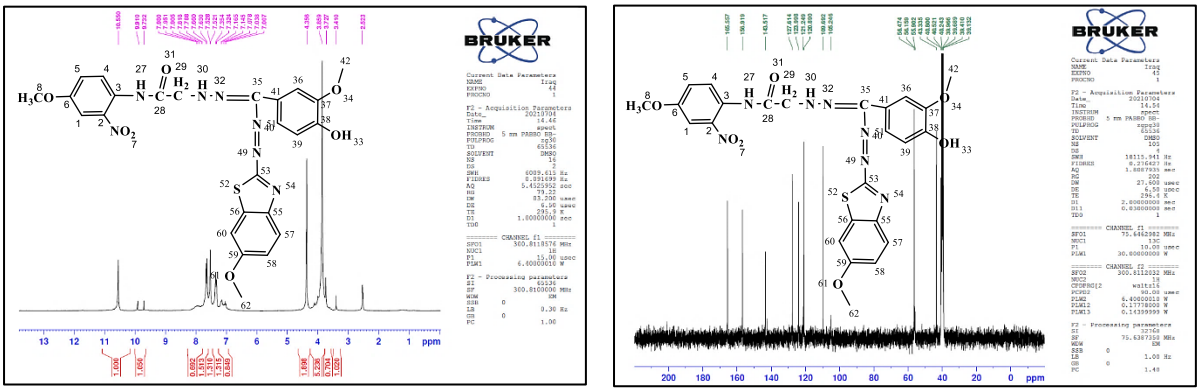
FIGURE 3. a-FT-IR Spectrum of the derivative (3), b-^{1}H-NMR Spectrum of the derivative (3), c-^{13}C-NMR Spectrum of the derivative (3)

4. Derivative (4)

FT-IR spectrum data showed band at 3371,3294 cm^{-1}for different environments (N-H), 3425 cm^{-1}for (OH), 3109 cm^{-1} for (C-H) Aromatic, 2939,2839 cm^{-1} for (C-H) Aliphatic, 1681 cm^{-1} for (C=O), 1643 cm^{-1} for (C=N), 1512,1334 cm^{-1} for (NO_2), 1589,1434 cm^{-1} for (C=C) Aromatic, 1465 cm^{-1} for (N=N), 1280 cm^{-1} for (C-N), 1249 cm^{-1} for (C-O). The ^{1}H-NMR spectrum data of derivative (4) showed δ : 10.4 (S,1H,OH), 9.9 (S,1H,NH^{27}), 9.7 (S,1H,NH^{30}), 7-7.9 (M,9H,Ar-H), 4.3 (S, 2H,CH_2), 3.7,3.8,3.4 (S, 9H,OCH_3). The ^{13}C-NMR spectrum data of derivative (4) showed δ : 165 for (C_{28}), 156 for (C_{38}), 105-143 for ($C_{arom.}$), 56.4 for (C_{29}), 56.1 for (C_{42}), 43 for (C_{29}), 55.9 for (C_{62}), 43.3 for (C_8).



(a)



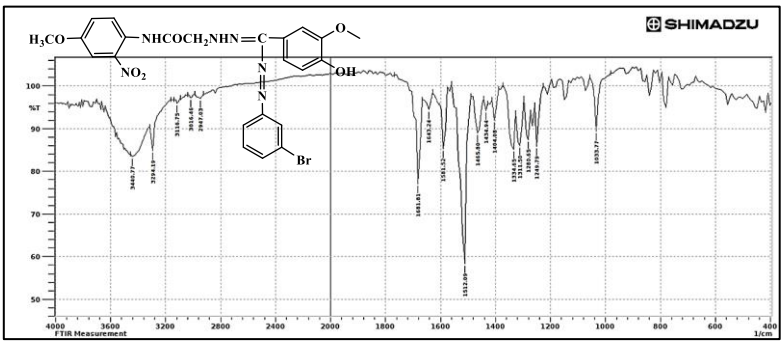
(b) (c)

FIGURE 4. a-FT-IR Spectrum of the derivative (4), b-^{1}H-NMR Spectrum of the derivative (4), c-^{13}C-NMR Spectrum of the derivative (4)

5. Derivative (5)

FT-IR spectrum data showed band at 3440 cm^{-1}for (OH), 3294 cm^{-1}for (N-H), 3116 cm^{-1} for (C-H) Aromatic, 2947,2839 cm^{-1} for (C-H) Aliphatic, 1681 cm^{-1} for (C=O), 1643 cm^{-1} for (C=N), 1512,1334 cm^{-1} for (NO_2), 1589,1434 cm^{-1} for (C=C) Aromatic, 1465 cm^{-1} for (N=N), 1280 cm^{-1} for (C-N), 1249 cm^{-1} for (C-O). The ^{1}H-

NMR spectrum data of derivative (5) showed δ : 10.4 (S,1H,OH), 9.9 (S,1H,NH^{27}), 9.7 (S,1H,NH^{30}), 7.3-7.6 (M,10H,Ar-H), 4.3 (S, 2H,CH_2), 3.8,3.4 (S, 6H,OCH_3). The ^{13}C-NMR spectrum data of derivative (5) showed δ : 165 for (C_{28}), 156 for (C_{38}), 109-143 for ($C_{arom.}$), 56 for (C_{29}), 43 for ($C_{8,42}$).



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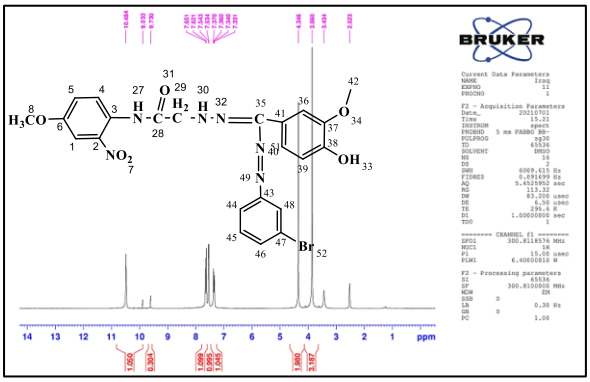
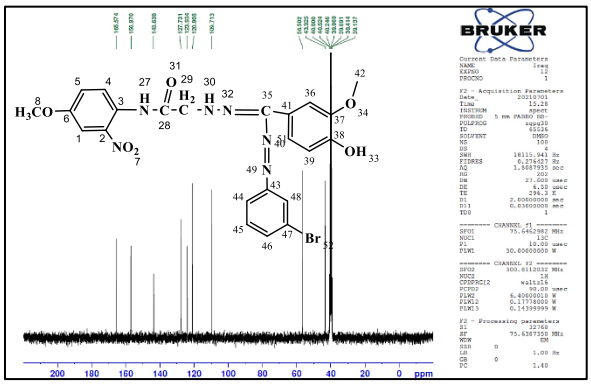


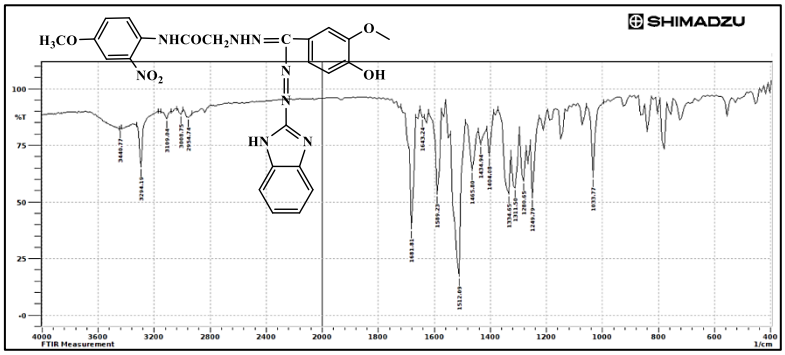


(b) (c)

FIGURE 5. a-FT-IR Spectrum of the derivative (5), b-^{1}H-NMR Spectrum of the derivative (5), c-^{13}C-NMR Spectrum of the derivative (5)

6. Derivative (6)

FT-IR spectrum data showed band at 3440 $cm^{-1}$for (OH), 3294 $cm^{-1}$for (N-H), 3109 cm^{-1} for (C-H) Aromatic, 2954,2839 cm^{-1} for (C-H) Aliphatic, 1681 cm^{-1} for (C=O), 1643 cm^{-1} for (C=N), 1512,1334 cm^{-1} for (NO_2), 1589,1434 cm^{-1} for (C=C) Aromatic, 1465 cm^{-1} for (N=N), 1280 cm^{-1} for (C-N), 1249 cm^{-1} for (C-O). The ^{1}H-NMR spectrum data of derivative (6) showed δ : 10.4 (S,1H,OH), 9.8 (S,1H,NH^{27}), 9.6 (S,1H,NH^{30}), 9.5 (S,1H,NH^{56}), 7.3-7.6 (M,10H,Ar-H), 4.3 (S, 2H,CH_2), 3.8,3.4 (S, 6H,OCH_3). The ^{13}C-NMR spectrum data of derivative (6) showed δ : 165 for (C_{28}), 156 for (C_{52}), 109-143 for ($C_{arom.}$), 56 for (C_{29}), 43 for ($C_{8,42}$).



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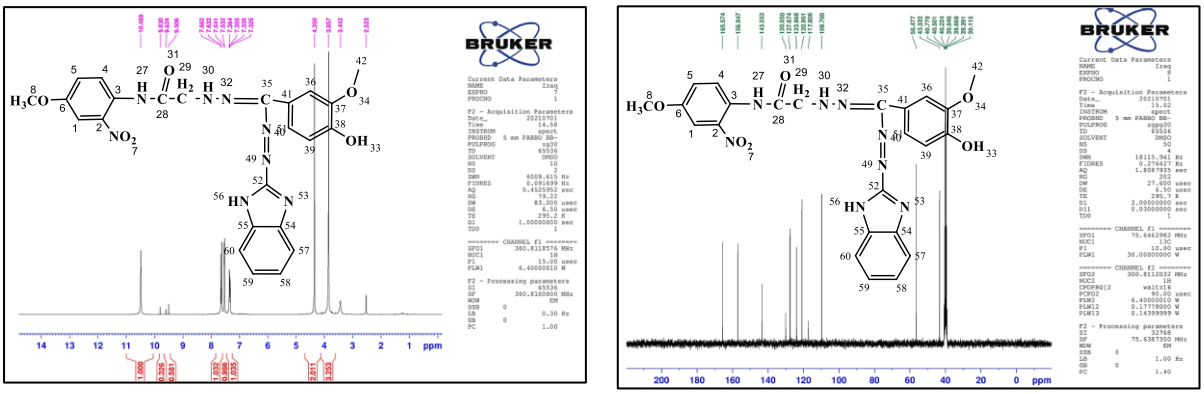
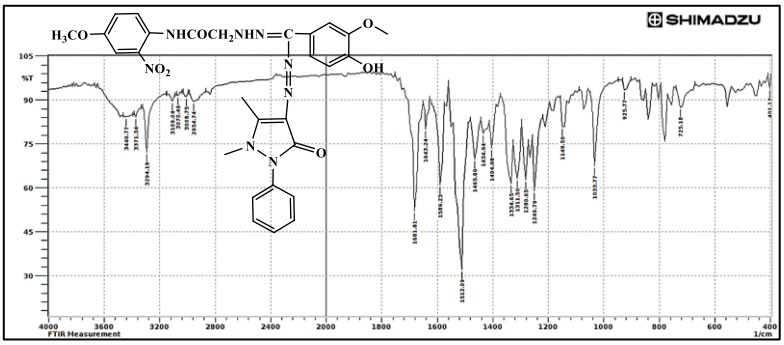


(b) (c)

FIGURE 6. a-FT-IR Spectrum of the derivative (6), b-^{1}H-NMR Spectrum of the derivative (6), c-^{13}C-NMR Spectrum of the derivative (6)

7. Derivative (7)

FT-IR spectrum data showed band at 3440 $cm^{-1}$for (OH), 3371,3294 $cm^{-1}$for different environments (N-H), 3109 cm^{-1} for (C-H) Aromatic, 2954,2839 cm^{-1} for (C-H) Aliphatic, 1681 cm^{-1} for (C=O), 1643 cm^{-1} for (C=N), 1512,1334 cm^{-1} for (NO_2), 1589,1434 cm^{-1} for (C=C) Aromatic, 1465 cm^{-1} for (N=N), 1280 cm^{-1} for (C-N), 1249 cm^{-1} for (C-O). The ^{1}H-NMR spectrum data of derivative (7) showed δ : 10.5 (S,1H,OH), 9.9 (S,1H,NH^{27}), 9.7 (S,1H,NH^{30}), 7.3-7.6 (M,11H,Ar-H), 4.3 (S, 2H,CH_2), 3.8,3.7 (S, 6H,OCH_3), 3.4,3.1 (S, 6H, CH_3). The ^{13}C-NMR spectrum data of derivative (7) showed δ : 165 for (C_{28}), 156 for (C_{38}), 109-143 for ($C_{arom.}$), 56 for (C_{29}), 43 for ($C_{8,42}$), 33 for (C_{57}), 28 for (C_{59}).



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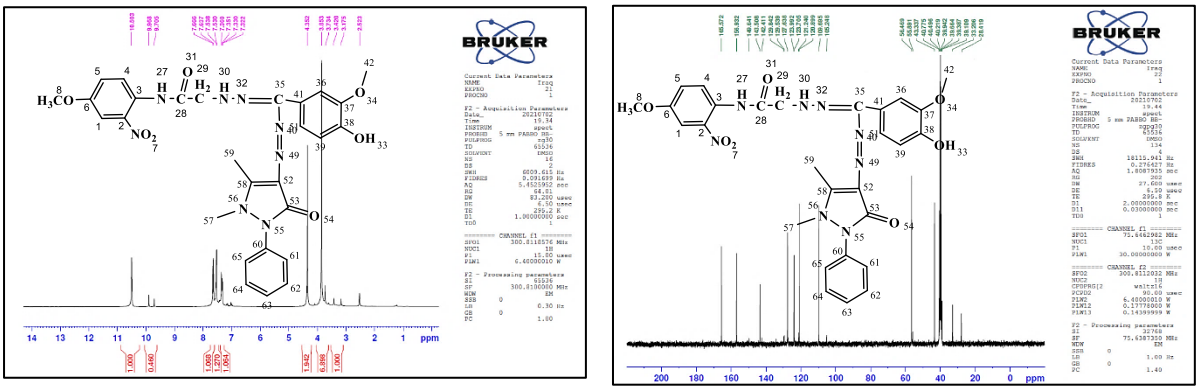

(b) (c)

FIGURE 7. a-FT-IR Spectrum of the derivative (7), b-^{1}H-NMR Spectrum of the derivative (7), c-^{13}C-NMR Spectrum of the derivative (7)

BIOLOGICAL STUDY

The biological study of synthesized derivatives (1-7) was studied and performed according to the agar plate method . The synthesized derivatives were evaluated against represented Gram Positive (*Staphylococcus aureus*)

and Gram negative (*E-Coli*), and (*Aspergillus Nige*r) fungicide. All derivatives were dissolved in Dimethyl Sulfoxide (DMSO) to give concentration 0.003M. Table (2) showed the zone of inhibition of the derivatives (1-7), we noted that derivatives (1,3,4) have Antibacterial activity against (*Staphylococcus aureus*), and that derivatives (2,3.5,6,7) have Antibacterial activity against (*E-Coli*), while were that derivatives (1-7) have higher Antifungal activity against (*Aspergillus Niger*) [16,17] .

TABLE 2. The antibacterial and antifungal activities of derivatives (1-7)

	Antibacterial Activity		Antifungal Activity
Derivatives	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Aspergillus Niger* (mm)
1	15	24	34
2	20	0	43
3	35	20	68
4	0	35	55
5	20	19	65
6	30	0	23
7	20	0	30

STUDT OF BIOLOGICAL ACTIVITY ON CANCER CELL

In this study, the breast cancer cell line MCF-7 and the healthy cell line WRL-68 were used for comparison and for the purpose of demonstrating their effectiveness on human body cells and the extent to which they can be used as cancer drugs. The (MTT) test was used for the biological examination of all cells, and the results showed that the type and concentration of the prepared compound are of great importance in determining the percentage of cytostatics.When studying the effect of the derivative (4) on the growth process of breast cancer cell lines MCF-7 and normal healthy cells WRL-68, it was noted that the highest percentage of growth inhibition of cells of breast cancer cell lines and normal cells was at 400µg/mL, which is the best inhibitory concentration compared to concentrations (6.25, 12.5, 25, 50, 100, 200) µg/mL. The half inhibitory concentration (IC_{50}) of the derivative (4) was equal to 96.5 µg/mL, and the least effect on healthy cells was 135.5 µg/mL to kill half of the healthy cells [18,19], as shown in Table 3 and Figure 8 .

TABLE 3. The effect of the derivative (4) on the cells of the breast cancer cell line MCF-7 and its comparison with the normal cell line WRL-68 for the same concentration using MTT test

	Cancer line cells of MCF-7		**Normal line cells WRL-68**	
Con.(µg/mL)	**Mean**	**SD**	**Mean**	**SD**
400.00	65.11	4.031	86.33	1.611
200.00	79.20	1.631	88.01	1.99
100.00	85.44	3.201	93.70	0.971
50.00	90.78	0.486	94.40	0.989
25.00	94.61	1.498	94.34	0.757
12.50	94.88	1.509	98.56	0.491
6.25	96.19	0.236	99.67	0.643

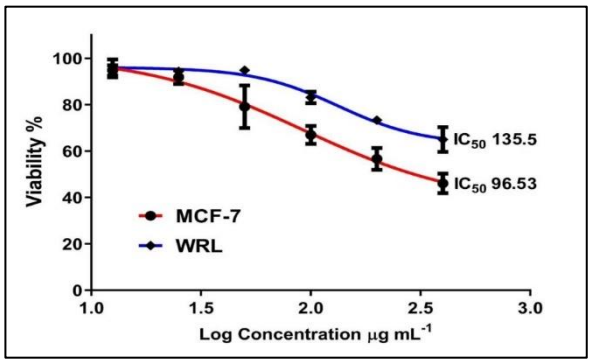

FIGURE 8. The half-inhibitory concentration (IC_{50}) of the breast cancer cell line MCF-7 cells and normal WRL-68 cells to the derivative (4)

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