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Synthesis of new biological active derivatives and their polymers based on N-substituted Maleimide

Ammar Abdul-Hussein Awad [1]andMohanad Mousa Kareem [2]*

[1, 2] Department of Chemistry, College of Science, University of Babylon, P.O. Box 51002, Hilla, Iraq

[1] E-mail: amaarand6bs@gmail.com,

[2]*E-mail: sci.mohanad.mousa@uobabylon.edu.iq

Abstract. In this work a new homopolymers [P1-P5] were synthesized from five Maleimide derivatives [M3-M7] using Benzoyl peroxide BPO as an initiator under nitrogen. Five different drugs (Cefotaxime, Ciprofloxacin, Amoxicillin, Cephalexin, and Ceftriaxone) carried on Maleimide derivatives in presence of triethylamine. All these monomers and polymers were investigated by FT-IR, ^{1}H NMR, and GPC, controlled drug release and swelling % was studied at PH 2 and pH 8.0 for homopolymers [P1-P5]. Intrinsic viscosities were measured and applied the characteristic of solubility for these polymers. The physical properties, anti-bacterial activity for prepared compounds were investigated, as well as the Thiazolyl blue tetrazolium bromide (MTT)

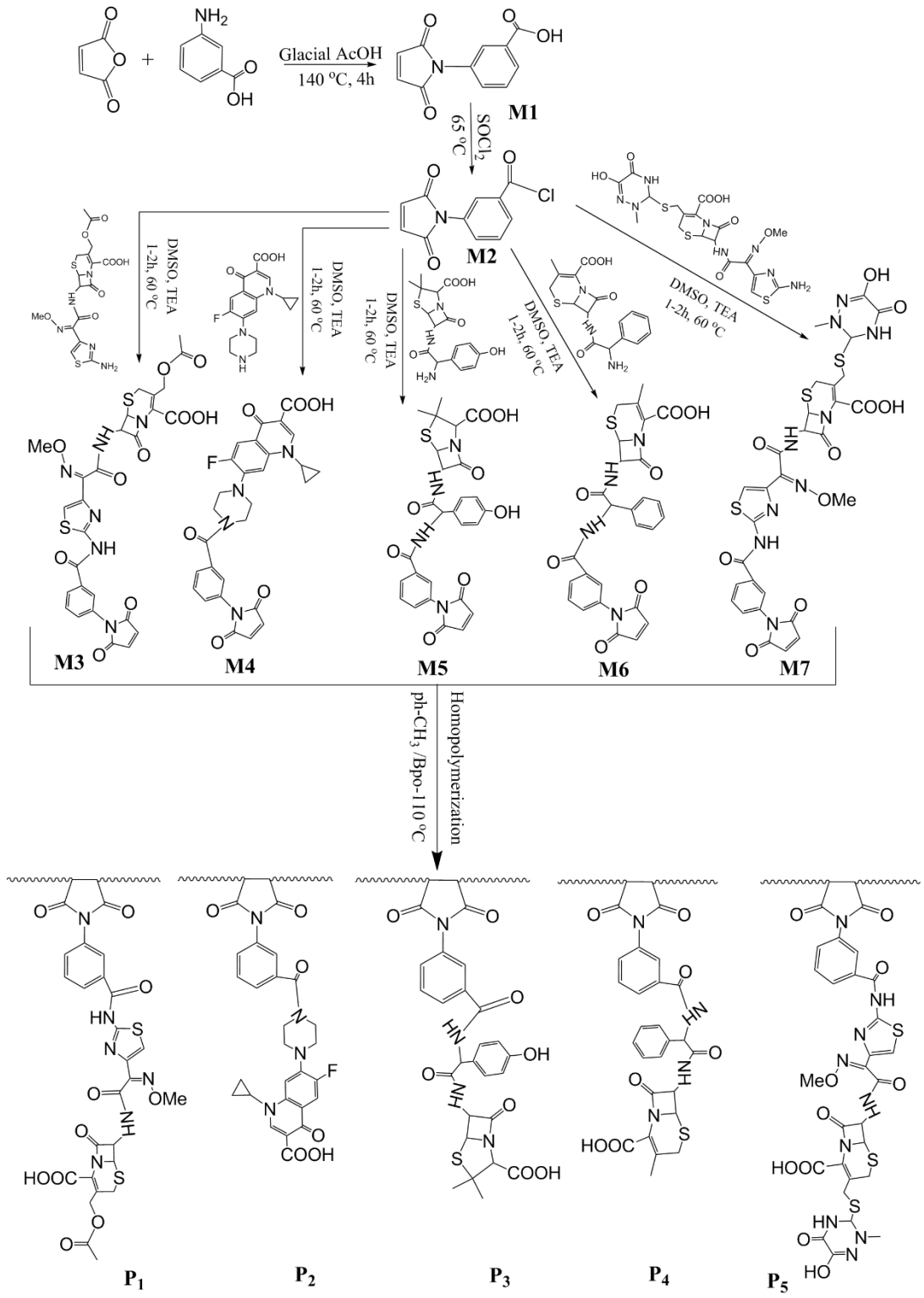
reduction assay in vitro in human breast cancer cell line Michigan Cancer Foundation-**7**(MCF7) were studied for the prepared polymers.

Keywords: MTT assay, MCF7, synergic effect**,** Anti-bacterial activity, Maleimide

Introduction

N-Substituted maleimide derivatives exhibit excellent electron withdrawing properties based on a rigid five-membered ring in the backbone, in addition to their thermal stability. Maleimide derivatives and polymeric drugs have attracted the attention of researchers in a wide variety of various fields such as air and water purification [1], wound dressings and protective bandages [2], antimicrobial agents [3], and anticancer [4]. Current studies have reported the use of conjugated polymers with drugs acting to inhibition of specific kinases [5], apoptotic routes and angiogenesis [6]. The entity of the polymer develops the solubility of the lipophilic drug [7] and improves its pharmacokinetic profile [8], rises plasma half-life and volume of distribution and reductions clearance by the kidneys or liver [9,27-44]. The polymer also protects the drug against degradation [10]. In the present study, new maleimide-drug

derivatives have been synthesized by condensation with different drugs, then polymerized and characterized. They are applied as a promising candidate for various biological applications.



Scheme 1: Synthesis route of compounds (M3-M7 and P1-P5)

Material and Methods:

Chemicals and reagents: All solvents and reagents were in analytical grade and obtained from Fluka, Sigma-Aldrich, CDH, and Riedel-de Haen. All of drugs were obtained from SDI-Samarra Company.

Instruments: Melting points were determined using SMP30 melting point apparatus. UV absorbance was recorded with double beam spectrophotometer PG CECIL- CE7200. The densities of polymer sample solutions were determined at 23 ºC using METTLER TOLEDO (Densito 30px Portable Density Meter). The viscosity (η) measurements of prepared polymers were carried out in acetone at 23 ºC using an Ostwald viscometer. The ^{1}HNMR spectra were carried out on a Varian INOVA 500 MHzNMR spectrometer in dimethyl sulfoxide (DMSO-d6), chemical shifts are in δ (ppm).The FT-IR spectra measurements were recorded using a Fourier Trans Infrared Spector Promoter AT-FT-IR Bruker Tensor II within range (400-4000) cm^{-1}.

Synthesis of compound [M1]: N-(3-Carboxyphenyl) maleimide (m-CPMI)

A solution of m-aminobenzoic acid (6.85 g, 0.5mol) and maleic anhydride (4.9 g, 0.5 mol) in 75 mL of glacial acetic acid was stirred continuously for 2 h at room temperature until yellow precipitate of m-maleamic benzoic acid was formed ($R_{f=}$ 0.4 / 1hexane : 3ethylacetate). Then the mixture was refluxed for 4 h, at 140 °C until it became a homogenous light brown liquid. The solution was concentrated using rotary evaporator and poured on ice crushed and left for two hours until a yellow precipitate was formed. The precipitate was filtered and washed with 1% aq. $NaHCO_3$ and large excess amount of distilled water then dried and then recrystallized from ethyl acetate obtaining the product in 6.5 g, 60% yield with mp 238-240 °C (lit. mp. 239-241 °C)[11]. Color: Yellow. FT-IR (cm^{-1}): ~ 3500-2551 (COOH), 3098 (=C-H, maleimide and aromatic), 1709 and 1693 (CO-N-CO, and C=O, carboxylic acid), 1607, 1517, 1429 (C=C, aromatic), 1314 (C-N)(9), 852 and 834 (=C-H).

Synthesis of compound [M2]: N-[3-(Chlorocarbonyl) phenyl] maleimide (m-CPMIC)

A solution of(4.29 g, 0.011 mol) m-CPMI 4 in chloroform (30 mL) and 1.55 mL thionyl chloride was refluxed at 65 °C for 3 h. The solvent was evaporated under reduced pressure and dried. The residual product was recrystallized from DCM to obtained pure light yellow crystals of acid chloride, 90% yield, mp 125-127 °C (Lit. mp = 126-128^{o}C) [11]. FT-IR (cm^{-1}): 3165 and 3088 (=C-H), 1774 (COCl), 1714, (CONCO), 1601, 1582 (Aromatic C=C), 1379 (C-N), 889 and 831 (=C-H).

General Procedure for the Synthesis of Maleimide-Drug Monomer Derivatives [M_3-M_7]

3-Maleimide benzoyl Chloride (4 mmol, 1 g) was added to stirred solution of 4 mmol of 5 different Drugs (Cefotaxime, Ciprofloxacin, Amoxicillin, Cephalexin and Ceftriaxone respectively) in 10 ml DMSO and 1 ml of TEA at room temperature for 1 h, and then heated for 1-2 h at 60 °C. The reaction was monitored by TLC until the reactant spot was vanished. The final solution was poured in crushed ice, left for (30min), then filtered and further crystallized from Ethanol: water (1: 3).

Compound [M3]: Chemical Formula: $C_{27}H_{22}N_6O_{10}S_2$, color: light brown, m.p. = 210- 212°C,

Yield 80%. FTIR (cm^{-1}): ~3500-2624 (COOH), 3308 and 3210 (-NH, amide). 3118 and 3052 (=CH, aromatic rings and maleimide), 2974- 2874 (C-H, methyl and methylene groups),1788 (C=O, beta-lactam) [12], 1752 (CO, ester)[13]. 1713 (CO-N-CO, imide), 1668 (C=O, amide), 1621 and 1591 (C=N-OMe and C=N thiazole ring)[13]. 1528-1438 (C=C, aromatic), 1420 (C-H bending), 1379 (C-N stretching), 1026 (C-C stretching of -COMe, ester) [14]. ^{1}HNMR (500 MHz, $DMSOd_6$, δ ppm): 2.480 (s, 3H, -CO-CH_3), 3.775 and 3.789 (d, 2H, aliphatic thiazine protons), 3.839 and 3.950 (d, 2H, methylene of ethanoic ester), 4.011 (s, 3H, =NO-CH3), 4.731 and 4.831 (d, 1H, beta lactam), 5.036 and 5.146 (d, 1H, beta lactam N-CH-), 5.916 (s, 1H, -NH-beta-lactam), 6.731 and 6.855 (d, 2H, CH=CH), 7.229-7.999 (m, 5H, phenyl and thiazole rings) 9.675 (s, 1H, -NHthiazole), 13.117 br (s, 1H, COOH).

Compound [M_4]: Chemical Formula: $C_{28}H_{23}FN_4O_6$, color: brownish yellow, m.p. = 171-172°C, Yield 80%. FTIR (cm^{-1}): 3379 (COOH, Ciprofloxacin drug) , 3081 and 3016 (=C-H, maleimide and aromatic), 2970 and 2847 (C-H, methylene groups) , 1710 (CO-N-CO, maleimide), 1679 (C=O, carboxylic acid and amide groups), 1625 (C=C of Maleimide and C=C-C=O conjugated Ketone of Quinolinone)[15], 1550-1452 (C=C, aromatic), 1337 (C-N), 1260 (C-F). ^{1}HNMR (500

MHz, DMSO-d_6, δ ppm): 1.121 and 1.228 (d, 4H, 2-CH_2-, cyclopropyl ring), 2.768-3.131 (m, 1H, cyclopropyl ring), 3.795 (t, 4H, 2-CH_2- piprazine), 4.185 (t, 4H, 2-CH2- piprazine), 7.182 (s, 2H, CH=CH, maleimide), 7.448 (s, 1H, Ar-H, quinolinone), 7.990 (s, 1H, Ar-H, quinolinone), 7.544 and 7.629 (d, 2H, benzene), 7.833 and 7.894 (d, 1H, benzene), 8.235 (s, 1H, benzene), 8.628 (s, 1H, vinylic proton), 12.488 (br, s, 1H, COOH).

Compound [M_5]: Chemical Formula: $C_{27}H_{24}N_4O_8S$, color: yellow, m.p. = 181-183°C, Yield 85%. FTIR (cm^{-1}): br, 3500~2500 (–OH, Carboxylic acid), 3447 (-NH, amide) 3305 (-OH, phenol, 3117, 3068 (=C-H,

Compound [M7]: Chemical Formula: $C_{29}H_{25}N_9O_{10}S_3$, color: Light yellow, m.p.= 191-193 °C, yield 80% . FTIR (cm^{-1}): br ~3500- 2551 (COOH) , 3273 (-NH, amide), 3153 and 3013 (=C-H, ^{1} HNMR (500 MHz, DMSO, δ ppm): 2.074 (-CH-, polymaleimide proton), 2.289 (s, 3H, -$COCH_3$), 3.071 (H_2O), 3.374 (d, 2H, aliphatic thiazine protons), 3.836 (d, 2H, -CH2- methylene),

aromatic rings and maleimide), 2978 and 2940 (sp^3C-H) ,1768 (C=O of betalactam), 1712 (CO-N-CO, imide ring), 1676 (C=O, carboxylic acid and amide groups), 1611
(C=C of Maleimide)[16]. 1591-1451 (C=C, aromatic), 1379 and 1293 (C-N
). ^{1}HNMR (500 MHz, DMSO-d_6, δ ppm): 1.518 (s, 6H, 2-CH_3), 4.119 (s, 2H, beta-lactam) 4.385
(s, 1H, thiozolidine), 4.620 (s, 1H, -CH- p-phenol), 6.703 (s, 2H, CH=CH), 7.189-7.589 (q, 4H, Ar-H, phenol ring), 7.845-8.250 (m, 4H, Ar-H of benzene), 8.617 (br, s, 1H, ph-OH), 9.379 and 9.587 (br, s, 2 ArCO-NH-), 13.064 (br, s, 1H, -COOH).

Compound [M_6]: Chemical Formula: $C_{27}H_{22}N_4O_7S$, color: red, m.p. = 277-280 °C, Yield 75%. FTIR (cm^{-1}): br, ~3400-2500 (COOH), 3305 and 3219 (-NH, amide groups), 3077 and 3013 (=CH, aromatic rings and maleimide) 2970-2824 (C-H, methyl), 1764 (C=O, beta-lactam), 1714 (CO-N-CO, imide ring), 1676 (C=C and-COOH of Cephalexin drug), 1612 and 1590 (C=C-C=O), 1557-1449 (C=C, aromatic), 1383 (C-N stretching), 1185 (C-O). ^{1}HNMR (500 MHz, DMSO-d_6, δ ppm): 2.489 (DMSO signal) [17]. 1.955 (s, 3H, -CH_3),), 3.060 (s, 2H, Cephalosporin-CH_2-),
3.317 (H_2O) [17], 3.875 (s, ph-CH), 4.087 and 4.991 (s, 2H, beta-lactam), 7.167 (s, 2H, CH=CH),
7.323-7.513 (m, 5H, ph-H), 7.625-8.160 (m, 4H, benzene), 9.075 and 9.371 (s, s, 2H, 2-CONH-)
13.004 (br, s, 1H, -COOH).

Ar-H and HC=CH), 2944 (C-H, methyl) ,1767 (C=O, beta-lactam), 1713 (CO-N-CO), 1667 and 1632 (C=O and C=C), 1589 and 1553 (C=N-OMe and C=N thiazole Ring)[13]. 1539-1413 (C=C, aromatic), 1375 (C-N), 1039 (O-Me, stretching). ^{1}HNMR (500 MHz, DMSO-d_6, δ ppm): 2.453 2.572 (DMSO signal)[17]. 3.072 (s, 2H, S-CH_2-), 3.332 (H_2O), 3.579 (s, 3H, N-CH_3), 3.817 (s, 3H, NO-CH_3), 4.063 and 4.094 (d, 1H, beta-lactam), 4.354 and 4.383 (d, 1H, beta-lactam), 5.125 (s, 1H, triazin -CH-), 5.761 (s, 1H,triazine-NH), 6.724 (s, 1H, CH=CH), 7.201 (s, 1H, thiazole ring), 7.638-8.062 (m, 4H, benzene ring), 13.089 br (s, 1H, COOH).

General Procedure for the Synthesis of homo-polymers [P1-P5]

Monomer (M3-M7) (1 g) was mixed with 20 mL of toluene in a 50 mL two neck round bottom flask, which was tightly sealed and placed in an oil bath at 110 ºC and 0.05 g of Benzoyl peroxide (Bpo) was added to the reaction mixture as initiator. The reaction mixture was refluxed for 10 h under nitrogen

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4.183 and 4.284 (d, 1H, beta lactam), 4.826 (s, 3H, =N-OCH_3) 4.943 and 5.029 (d, 1H, beta lactam, 5.931 (s, 1H, -NH-beta-lactam amide), 7.072-7.997 (m, 5H, phenyl and thiazole rings) 9.672 (s, 1H, -NH-thiazole amide), 12.952 br (s, 1H, COOH).

Compound [P_2]: color: Red, yield 70%. FTIR (cm^{-1}): br~3500 (COOH), 3057 and 3024 (=C-H, aromatic rings), 2918 and 2849 (C-H, methyl and methylenegroups), 1716 (CO-N-CO, imide ring and C=O, carboxylic acid), 1627 (C=C-C=O, Quinolinone), 1549-1451 (C=C, aromatic), 1387 (C-N stretching), 1265 (C-F).

blanket and at the end of polymerization; the precipitate was filtered, washed using diethyl ether and finally dried in an oven at 45 ºC overnight.

Compound [P_1]: color: orange. FTIR (cm^{-1}): br ~3500-2723 (COOH), 3306 and 3212 (-NH, amide), 3063 (=C-H, aromatic rings), 2943 and 2989 (C-H, methyl), 1791 (C=O, beta-lactam), 1751 (C=O, ester), 1715 (CO-N-CO, imide), 1670 (C=O, amide), 1590 (C=N-OMe and C=N inside thiazole ring), 1527 and 1420 (C=C, aromatic), 1379 (C-N stretching), 1031 (O-Me, ester). ^{1}HNMR (500 MHz, DMSO, δ ppm): 1.181 and 1.306 (d, 4H, cyclopropyl ring), 2.491 (DMSO),

2.754-2.781(t, polymaleimide proton protons), 2.917 -2.985 (t, 1H, cyclopropyl ring) 3.359 (H_2O),

3.808 (t, 4H, piprazine), 4.170 (s, 4H, piprazine), 7.349 (s, 1H quinoline), 8.008 (s, 1H, quinoline),

7.478-7.939 (m, 4H, benzene), 8.650 (s, 1H, vinylic proton), 12.961 (br, s, 1H, COOH).

Compound [P_3]: color: yellow. FTIR (cm^{-1}): 3305 (OH, phenol), 3270 (-NH, amide), 3067 (=CH, aromatic rings), 2976 (C-H, methyl group) ,1775 (C=O, beta-lactam), 1710 (CO-N-CO, maleimide), 1649 (C=O, Carboxylic acid), 1611 (C=O of amide groups), 1591-1450 (C=C, aromatic), 1381 (C-N stretching)). ^{1}HNMR (500 MHz, DMSO, δ ppm): 1.529 (s, 6H, 2-CH_3), 2.284 (s, polymaleimide proton) , 2.515 (DMSO), 3.351 (H_2O), 4.107 , (s, 1H, thiozolidine proton), 4.258 (s, 1H, -CH-attached to p-phenol), 4.462 and 4.622 (s, 2H, beta-lactam), 7.0317.630 (m, 4H, Ar-H, phenol ring), 7.876-8.213 (m, 4H, Ar-H of benzene), 9.396 (br, s, 1H, phOH), 10.087 and 10.439 (br, s, 2 ArCO-NH-), 12.970 (br, s, 1H, -COOH).

Compound [P_4]: color: Brown, FTIR (cm^{-1}): 3270 (-NH, amide groups), 3064 and 3032 (=C-H, aromatic rings), 2922 (C-H, methyl), 1767 (C=O, beta-lactam), 1708 broad (CO-N-CO, in an imide and C=O, carboxylic acid group and C=O of Amide groups), 1589 (C=C-COOH, α,βconjugation), 1553-1449 (C=C, aromatic), 1378 (C-N stretching), 1177 (C-OH stretching).

1HNMR (500 MHz, DMSO, δ ppm): 2.149 (d, polymaleimide proton protons), 2.483 (DMSO), 2.551, (s, 3H, -CH3), 2.619 and 2.687 (s, 2H, Cephalosporin-CH2-), 3.076 (H2O), 4.214 (s, phCH), 4.278 (s, 2H, beta-lactam), 5.908 (s, 1H, beta-lactam-CO-NH), 7.351-7.598 (m, 5H, ph-H), 7.855-8.075 (m, 4H, benzene), 9.363 (s, 1H, -CONH-), 13.004 (br, s, 1H, -COOH).

Compound [P_5]: color: Light Brown. FTIR (cm^{-1}): br~ 3500-2530 (COOH), 3294 and 3213 (NH, Amide groups), 3035 (=C-H, aromatic rings), 2944 (C-H, methyl and methylenegroups) ,1770 (C=O, beta-lactam), 1711 (CO-N-CO, imide), 1630 (C=O, amide and C=C of the drug), 1589 (C=N-OMe and C=N inside thiazole Ring), 1542-1413 (C=C, aromatic), 1377-1180 (C-N stretching), 1039 (O-Me stretching).

^{1}HNMR (500 MHz, DMSO, δ ppm): 2.290 (s, polymaleimide proton) , 2.507 (DMSO), 3.318 (H_2O), 3.562 (s, 2H, S-CH_2-) 3.648 (s, 3H, N-CH_3), 3.807 (s, 3H, NO-CH_3), 4.053 and 4.111 (d, 1H, beta-lactam), 4.342

and 4.400 (d, 1H, beta-lactam), 5.136 (s, 1H, triazin -CH-), 5.764 (s, 1H,triazine-NH), 7.188 (s, 1H, thiazole ring), 7.477-8.055 (m, 4H, benzene ring), 13.010 br (s, 1H,
COOH).

Swelling ratio: The swelling ratio was calculated by immersing 0.05 g of homopolymers xerogel in 50 mL distilled water. It was left to soak for different period of time at (24 ^{o}C). The hydrogel was removed from the water after 1 hour and 24 hours, blotted with filter paper to remove surface water, weighedand the swelling ratio was computed using the equation below.

Swelling ratio (%)= (Ws - Wd)/Wd × 100

[Wd= Weight of polymer; Ws= weight of swollen polymer]

Release of drugs: By using UV-visible spectrophotometer, the release of drug from the prepared polymers was determined in two different buffer solutions (pH=2and 8.0) at constant temperature 24 ^{o}C. By immersing xerogel (0.005 g) from homopolymers in 50 mL of different buffer solutions, it was allowed to soak for different time at constant temperature 24 ^{o}C (9). The hydrogel was removed from the buffer solution at the stipulated time and measure the absorbance of buffer solution in order to determine the amount of drug release.

Cytotoxic assay [18]

In vitro MTT reduction assay in human breast cancer cell line (MCF7) were studied for all prepared polymer. The cells were incubated at 37° C with 5 percent CO_2 and 96 percent moisture in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS). After numerous subcultures, cells were distributed in 96-well plates at 1,000 cells per 100 µL of culture medium and incubated for 24 hours at the same temperature to allow cells to adhere to the bottom of the wells. The culture medium was then removed, and 100 µL of the same medium containing the drugs at different concentrations (500, 400, 300, 200, 100, 50, 25 µg/ml) were added in triplicate to each well. Plates were then incubated in the same conditions for another 3 days. The control was the last column of the plate, which contained 1000 cells in 100 µL of culture medium. The drug-containing medium was discharged after 3 days of incubation [19]. 25 mL of MTT solution (4 mg/mL in PBS) was added to each well for cell survival testing, and the plates were incubated for 3 hrs (in same condition).

After that, 100 µL of DMSO was added to each well, and the plates were lightly shaken to dissolve the formazan crystals that had formed. Using an ELISA plate reader, the absorbance of each well was measured at 540 nm %Growth Inhibition = 100 − (ODtest − ODcontrol) × 100, where ODtest is the mean

absorbance of treated cells and $OD_{control}$ is the mean absorbance of a negative control. Control cell survival was presumed to be 100%, and IC_{50} values were calculated using doseresponse curves for each cell line.

Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6 [20]. The values were presented as the mean ± SD of triplicate measurements [21].

Result and discussion

By combining 3-maleimido benzoyl chloride with various drugs (Cefotaxime, Ciprofloxacin, Amoxicillin, Cephalexin, and Ceftriaxone) in the presence of triethyl amine as a catalyst, new maleimide-drug monomers have been synthesized in high yield. Some of physical properties of prepared monomers [M1-M7] are listed in (Table 1).

Table 1: Physical properties of prepared monomers [M1-M7].

Comp.	Color	Yield (%)	m.p. °C	Rf
M1	Yellow	70%	238-240 °C	0.55 1hexane:3ethylacetate
M2	Light Yellow	90%	126-128 °C	0.75 1hexane:3ethylacetate
M3	light Brown	80 %	210 – 212°C	0.85 1hexane : 3acetone
M4	Brownish yellow	80%	171-172°C	0.8 1hexane : 3acetone
M5	yellow	85%	181- 183°C	0.7 1hexane : 3acetone
M6	Red	75%	277 - 280°C	0.62 1hexane : 3acetone
M7	Light Yellow	80 %	191-193 °C	0.44 1hexane : 3acetone

These prepared monomers were homopolymerized in toluene at 110 ˚C for 10 h under nitrogen flow with traces of benzoylperoxid as an initiator afford [P1-P5] homopolymers, the physical properties listed in (Table 2):

Table 2: physical properties of Homopolymers [P1-P5]

polymer	color	T °C	t S D.W	t unkS	dunk	dD.W	ŋD.w poise	ŋunk poise
P1	Orange	23	131.39	119.5	0.792	0.990	0.960	0.699
P2	Red			114.7	0.788			0.667
P3	yellow			115.2	0.793			0.675
P4	Brown			115.1	0.786			0.668
P5	Light Brown			115.5	0.789			0. 673

Spectral analysis:

Compound M1 FT-IR spectrum (fig.1) show no N-H absorption peak and the appearance of two characteristic absorptions at 1709 cm^{-1} and 3098 cm^{-1}, respectively, due to stretching of (CO-NCO) and (H-C=C-H). The spectrum also shows broad absorption from (COOH) of benzoic acid in the range 3500-2551 cm^{-1}, indicating that cyclodehydration of maleimide was effective. In FT-IR spectrum of M2 compound the broad absorption of hydroxyl group was disappeared and appearance of new band at higher frequency 1774 cm^{-1} belong to (COCl).

The FT-IR spectra of all prepared monomers show disappearance of (COCl) peak and appearance of other characteristic (CO-N-H-) amide absorption within the range of (3308-3200) cm^{-1} and Maleimide carbonyl groups at 1714–1709 cm^{-1} , characteristic stretching bands for compound (M3, M5, M6 and M7) at 1764-1788 cm^{-1} due to beta lactam carbonyl and broad absorption band of carboxyl groups in drug structures around 3500~2500 cm^{-1}.

The ^{1}HNMR spectra for all prepared monomers show characteristic signals for maleimide (CH=CH) protons at 6.718-7.182 ppm and for beta lactam protons at 4.063 – 5.146 ppm (M2, M4, M5 and M6), also broad and weak signal at 12.488-13.117 ppm for carboxylic acid group of drugs. The ^{1}HNMR spectra of all prepared polymers demonstrate the absence of (H-C=C-H) protons, as well as signals from the polymaleimide proton backbone protons at 2.074-2.781 ppm, beta lactam protons at 4.063–5.146 ppm, and carboxylic acid protons of drugs at 12.488-13.117 ppm.

The Antibacterial Activity of Monomers and Homopolymers

The anti-bacterial activity for the all synthesized compounds and their loaded drugs were listed in (Table 3):

Table 3: Antibacterial activity of compound M3-M7 and P1-P5 at 0.5 mg/mL concentration.

Inhibition zone (mm(for tested microorganisms		
Samples	S. aurous	E. coli
Monomer		
M3	30	32
M4	30	42
M5	22	22
M6	32	30
M7	30	40
P1	25	30
P2	40	40
P3	30	26
P4	12	20
P5	30	32
Drug		
Cefotaxime	20	38
Ciprofloxacin	35	22
Amoxicillin	34	35
Cephalexin	28	15
Ceftriaxone	35	20

Antibacterial activity against pathogenic strains of Escherichia coli (ATCC 8739) and Staphylococcus aurous (ATCC25923) using solution of 0.5 mg from each compound and each loaded drug for comparison in 1 mL of DMSO was carried out using disk-diffusion method [22]. Also, the activity of the DMSO was also screened as a negative control which does not show any inhibition for bacterial growth.

The results of antibacterial activity against Gram-positive Staphylococcus aurous shows antagonist effect for M5, M7, P3 and P5 compounds inhibition zone were (22, 30, 30 and 30 mm respectively) in comparison to amoxicillin and Ceftriaxone, inhibition zone were (34 and 35 mm respectively). While, synergic effect for M3, M6, P1, P2 and, inhibition zone was (30, 32, 25 and 40mm respectively), in comparison to Cefotaxime and Cephalexin, inhibition zone were (20 and 28 mm respectively).

The prepared derivatives M4, P2, M6, M7 and P5 are showing efficient antibacterial activity against Gram negative (E. coli), they higher synergic effect was found as they show highest diameter of inhibition zone (42, 40, 30 40 and 32 mm) than Ciprofloxacin, Cephalexin and ceftriaxone (22, 15 and 20 mm). While, M3, P1, M5 and P3 show an antagonistic effect as result of decreases the diameter of inhibition. The lipophilic and neutral nature of the maleimide moiety allows it to seamlessly pass through biological membranes [23]. Gram negative bacteria's cell walls are made up of one or a few layers of peptidoglycan and a lipid-rich outer membrane [24], which explains why they have greater antibacterial activity against E. coli.

Cytotoxicity Assay

The cytotoxic effects of polymers were evaluated using the MTT (3-[4, 5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide) assay on human breast cancer cell line (MCF7), and the results were compared to the untreated control[18].

The MTT colorimetric assay was used to evaluate the cytotoxicity of synthesized polymers against MCF-7 cancer cells, as shown in figures (23-27). The results show that all polymers inhibited cell growth in a concentration-dependent manner when compared to the drugs.

Homo-polymer P4 at a concentration of (500 µg/ mL)l showed the highest degree of inhibition, with 75% of treated cells dying, while at a concentration of 100 µg/ml, 51% of cells died, and the Inhibitory Concentration value (IC_{50}) was 200.07 µg/ml (Fig. 26). The degree of cell inhibition at highest concentration (500 µg/ml) for P1 (68%), P2 (66 %), P3 (72 %) and P5 (70%), while at a concentration of 100 µg/ml from P2, 43 % of cells died, and the inhibitory concentration value (IC_{50}) was 220.19 (Fig. 24). The IC50 values for P1, P3 and P5 were (311.13, 280.12 and 260.78 µg/ml respectively). The results reveal that all of the prepared polymers were successful in reducing cancer cell proliferation, suggesting that they could be a useful and promising method for developing an effective drug delivery system for clinical use against breast cancer[25,26].

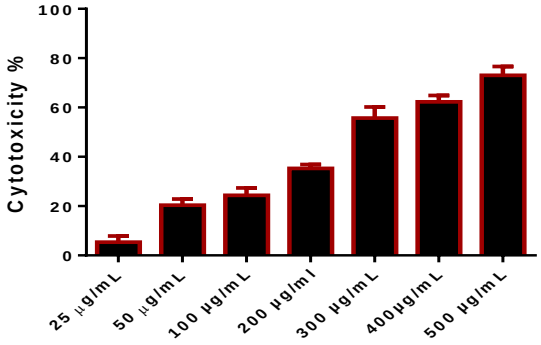

Figure 23: Cytotoxic effect of P1. IC50=311.13 µg/ml

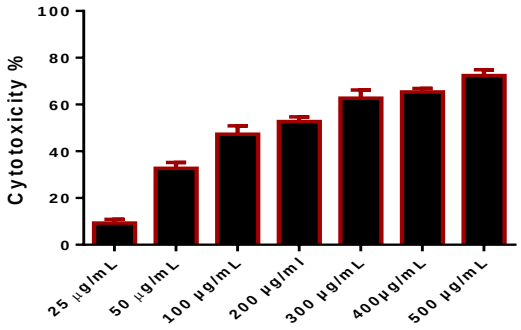

Figure 24: Cytotoxic effect of P2 in MCF-7 cells. IC50= 220.19 µg/ml

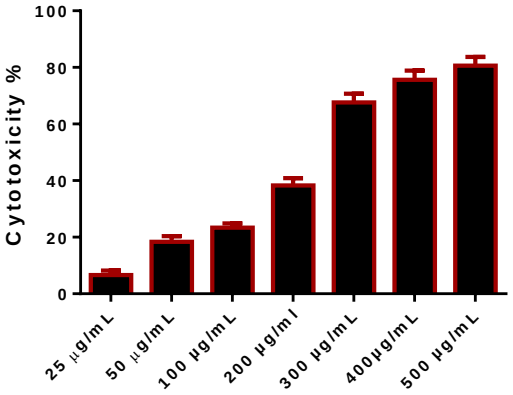

Figure 25: Cytotoxic effect of P3 in MCF-7 cells. IC50=280.12 µg/ml

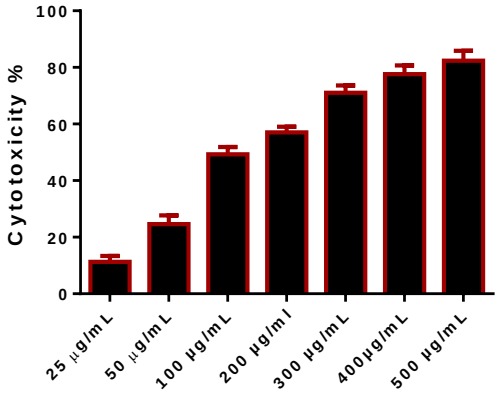

Figure 26: Cytotoxic effect of P4 in MCF-7 cells. IC50=200.07 µg/ml

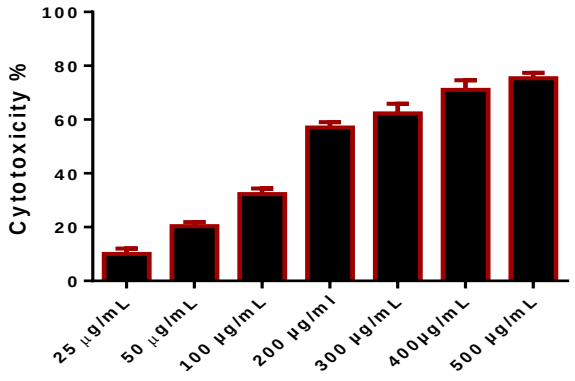

Figure 27: Cytotoxic effect of P5 in MCF-7 cells. IC50=260.78 µg/ml

Solubility: The synthesized monomers and polymers were insoluble in water and acidic media but having a good solubility in basic aqueous solution (pH=7.5-8). Solubility properties of prepared polymers in different solvents (H_2O, ethanol, $CHCl_3$, ether, toluene, DMSO, hexane, DMF and acetone) are listed in Table 4.

Table 4: The solubility of synthesized Homo-polymers

sample	H_2O	EtOH	$CHCl_3$	Ether	Toluene	DMSO	Hexane	DMF	Acetone
P1	-	+	partial	-	partial	+	-	+	+
P2	-	partial	-	-	-	+	-	+	partial
P3	-	+	-	-	partial	+	-	+	+
P4	-	partial	-	-	partial	+	-	+	partial
P5	-	+	-	-	partial	+	-	+	+

Swelling ratio: The swelling ratio was determined by immersing 0.05 g each polymer in 50 mL distilled water and allowed to soak for different period of time at 24 ˚C. Table 5 represents the swelling ratio of homo and hetero polymers in different period of time.

Table 5: Swelling ratio (%) of Homopolymers [P1-P5] at 24 °C

Time	Swelling Ratio %				
	Types of polymers				
Hour	P1	P2	P3	P4	P5
1	2	6	3.4	1	0.4
2	3.6	7.4	4.2	3.4	6.2
3	5	8.3	6	4.3	7.4
4	6.6	9.3	8.4	10.2	8
5	8.1	10	8.7	10.6	9.9
Day					
1	9.4	12.2	9.6	12.2	11.4
2	10.87	12.78	9.8	12.27	12.78

3	12.19	13.03	10.61	12.45	12.8
4	12.62	12.79	10.83	13.06	12.14
5	12.38	12.77	10.61	13.02	12.01

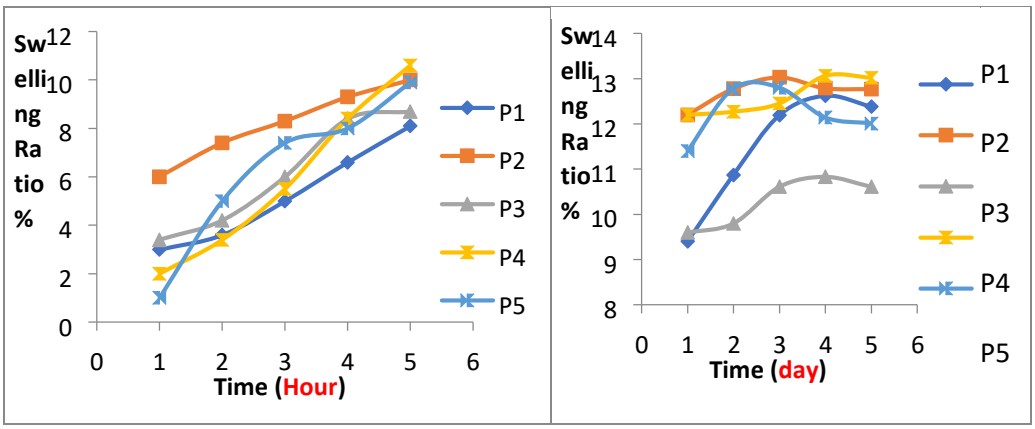

Figure 28: The Swelling diagram of Homo-polymers [P1-P5].

Release of drug: By using UV-visible spectrophotometer, drug release from the prepared polymers was determined in two different buffer solutions (pH 2 and 8.0) at 24 ˚C. Table 6 and 7: represents the drug release from the prepared polymers.

Table 6: Release of drug of the homo-polymers at pH 2 at 24 ˚C

Time	Absorbance of polymers / λmax				
Hour	P1/ 314nm	P2/ 331nm	P3/ 308 nm	P4/ 306 nm	P5/ 314 nm
1	0.95	0.189	0.125	0.118	0.535
2	1.025	0.211	0.421	0.131	0.871
3	1.153	0.315	0.543	0.253	0.902
4	1.254	0.347	0.643	0.547	1.103
5	1.305	0.362	0.669	0.654	1.184
Day					
1	1.362	0.561	1.19	0.702	1.197
2	1.43	0.753	1.423	0.887	1.271

3	1.524	0.904	1.76	1.214	1.324
4	1.871	1.54	1.897	1.142	1.723
5	1.957	1.772	2.023	1.251	1.876
6	2.021	1.771	2.113	1.26	1.901

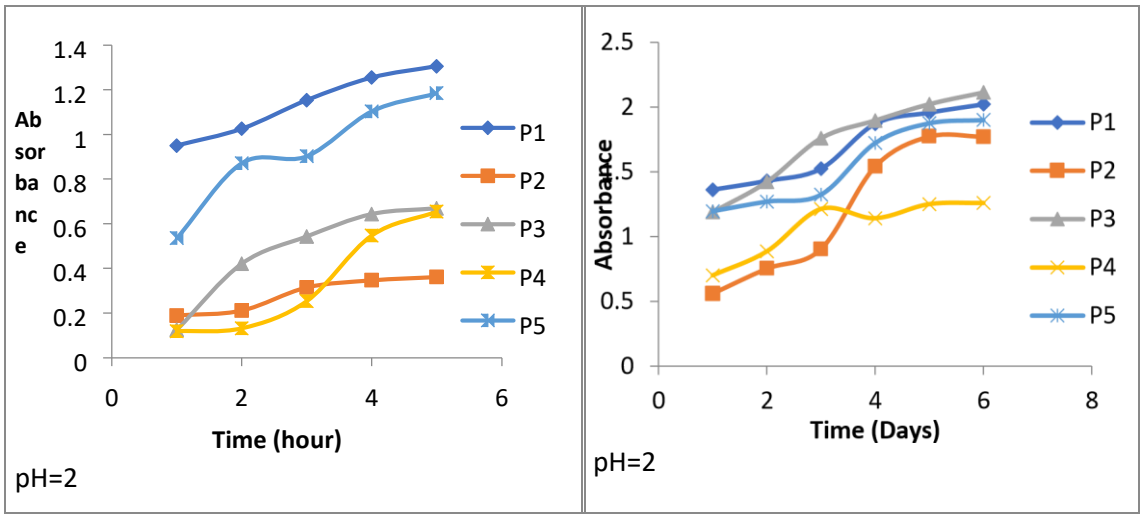

Figure 29: The Drug release diagram of homopolymers (P1-P5) at PH 2.

Table 7: Release of drug of the homo-polymers at pH 8.0 at 24 ˚C

Time	Absorbance of polymers / λmax				
Hour	P1/314nm	P2/ 331nm	PM3/308 nm	P4/ 306 nm	P5/314nm
1	0.311	0.237	0.131	0.119	0.424
2	0.51	0.412	0.339	0.47	0.558
3	0.671	0.59	0.495	0.631	0.65
4	0.774	0.817	0.616	0.803	0.78
5	0.912	0.974	0.903	0.964	0.9
Day					
1	1.153	1.401	1.275	1.21	1.153

2	1.325	1.632	1.414	1.425	1.455
3	1.545	1.911	1.841	1.64	1.874
4	2.021	2.124	2.011	1.959	2.16
5	2.355	2.701	2.181	2.511	2.419
6	2.36	2.692	2.177	2.501	2.42

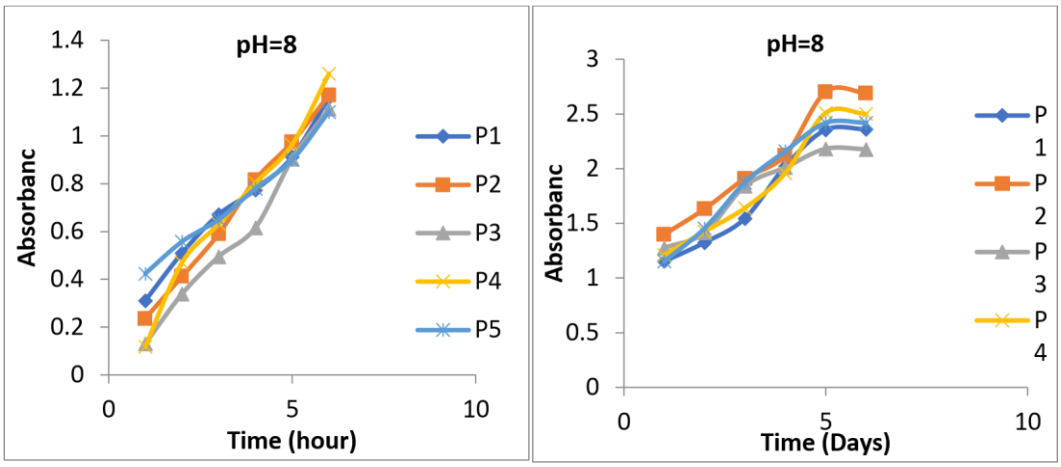

Figure 30: The drug release diagram of homopolymers (P1-P5) at PH 8

Conclusion

Five new Prodrug polymers based on maleimide-drug monomers were successfully prepared via a free radical polymerization reaction. Their structures have been confirmed using FTIR and ^{1}HNMR techniques. Most of synthesized compounds, having a high antibacterial activity as proved by their higher inhibition zone diameters. All polymers show good drug release results in the basic medium (fig. 30). The results show that polymers have a good effect on the MCF-7 a breast cancer cell line. Based on cytotoxicity tests, the prepared polymers could be an acceptable and promising strategy for developing effective drugs to clinical application against breast cancers.

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