Thermally Stable Antimicrobial PVC/Maleimido Phenyl Thiourea Composites

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ABSTRACT: Four novel antimicrobial maleimido phenyl thiourea derivatives were synthesized from *N*-[4-(chlorocarbonyl) phenyl] maleimide with phenyl thiourea and its derivatives (*p*-methyl, *o*-chloro, and *p*-carboxy). Their structures were characterized by FTIR, ^{1}H NMR, mass spectra, and elemental analyses. Their antimicrobial activities against three types of bacteria (*Bacillus subtilis*, *Streptococcus pneumoniae*, and *Escherichia coli*) and against three crop-threatening pathogenic fungi (*Aspergillus fumigatus*, *Geotricum candidum*, and *Syncephalastrum racemosum*) were investigated. The results revealed that these derivatives are effective in inhibiting the growth of the tested bacteria and fungi as indicated from the inhibition zone diameter and minimum inhibitory concentration. The antibacterial activities of these derivatives were more effective against Gram-positive bacteria than Gram-negative bacteria. These derivatives were investigated as thermal stabilizers for rigid poly(vinyl chloride) at 180°C in air by measuring the rate of dehydrochlorination and the extent of discoloration. The results reveal the greater stabilizing efficiency of the investigated derivatives as shown by their longer thermal stability periods (*Ts*) and lower dehydrochlorination rates in relation to dibasic lead carbonate, cadmium–barium–zinc stearate, and *n*-octyltin mercaptide industrial stabilizers. The stabilizing efficiency increases with the introduction of electron-donating substituent groups in the aromatic ring of the stabilizer molecules. Moreover, the investigated stabilizers impart better color stability for the degraded samples as compared with the reference stabilizers. Adv Polym Technol 2015, 00, 21537; View this article online at wileyonlinelibrary.com. DOI 10.1002/adv.21537

KEY WORDS: Antimicrobial activity, Dehydrochlorination rate, Discoloration degree, Maleimido phenyl thiourea derivatives, PVC

Introduction

Poly(vinyl chloride) (PVC) has been widely used in the fields of construction materials, food package, decoration, medicine (e.g., for the fabrication of indwelling catheters in the hospital care) and commodities such as construction tubing, films, toys, and wallpaper. These materials and products cannot avoid smirching with bacteria or microbes during their daily usage so that it is important to develop antibacterial PVC composites for such applications. Some attempts have been performed to prepare antibacterial PVC composites using different antibacterial nanoparticles. Zirconium phosphate containing nanosized silver particles[1] and TiO_2/Ag^+ nanoparticles[2] were used for these studies. Furthermore, isothiocyanate nucleophilically substituted PVC could also be used as antibacterial PVC.[3]

On the other hand, PVC is known to undergo extensive degradation, especially during its molding and applications at high temperatures. Its thermal degradation occurs by autocatalytic dehydrochlorination reaction with subsequent formation of conjugated double bonds.[4] This results in an unacceptable discoloration of the polymer and a loss of its physical and mechanical properties together with a decrease or an increase in molecular weight as a result of chain scission or cross-linking of the polymer molecules, respectively[5–7] It is assumed that various defect sites in the polymer chains are responsible for this instability. Possible defect structures in PVC are branching, chloroallyl groups, end groups, oxygen containing groups, head to-head structures, and the stereo order of the monomer units (tacticity).[8–13] Various kinds of stabilizers have been used to inhibit the thermal degradation of PVC either by reacting with the evolved hydrogen chloride gas, produced as a result of the degradation process, such as basic salts[14], or by displacing the labile chlorine in PVC chains by more stable ester or mercaptide groups such as with metallic soaps[15], and esters or mercaptides of dialkyltin.[16] Most of the mentioned stabilizers lead to the formation of heavy metal chlorides as by-products, which will act as strong catalysts for the subsequent dehydrochlorination of PVC and may present a serious environmental problem.[17,18] Various organic ligands have been proposed as costabilizers so as to react with these metal chlorides and provide more protection to the polymer.[19,20] Recently, metal-free and environmentally acceptable fully organic stabilizers have been established for the thermal stabilization of PVC.[21–23] Since the time of fabrication of PVC is relatively short, and determination of the amount of stabilizer consumed after various processing times indicates

that most of the stabilizer remains unreacted, therefore the final product contains large amount of heat stabilizer. For this, a new trend has been established based on the use of thermal stabilizers of antimicrobial nature to obtain thermally stable antimicrobial PVC composites.[22,23]

Phenyl thiourea derivatives represent a class of compounds possessing a wide range of biological activities.[24–30] Important biological properties concerning bactericidal, fungicidal, and anticancer properties were also reported for N-substituted maleimides.[31–36] It is known that maleimides are inhibitors of cysteine proteinase or other protein with an essential cysteine. Maleimides interact preferably with the hydrophobic domains of enzymes through the inactivation of sulfhydryl groups. They are usually considered as nonspecific toxic and inexpensive family of products and could be interesting candidates for the formation of new antimicrobial activity.[37]

N-Substituted maleimides[38] and phenyl thiourea derivatives[39] have been proven to be effective stabilizers for PVC against thermal degradation. The stabilizing efficiency of the N-substituted maleimides is strongly affected both by the nature and position of the substituents in the aryl ring being greater for substituents of electron-donating nature and lower for those having electron-withdrawing effect, whereas the nonsubstituted derivative is in the middle.[38] Also, the maleimides act as powerful radical traps and can exchange the labile chlorine in the PVC chains for a more stable maleimide moiety. On the other hand, the phenyl thiourea derivatives act mainly as hydrogen chloride gas absorbers, in addition to their ability to form metal complexes. This property enables them to react with various metal chlorides, which result as by-products from the soap or basic salt stabilizers, thus preventing or at least minimizing their catalytic degradation effect.

In view of the above, it would be expected that maleimido phenyl thiourea derivatives combine the characteristics of both of the maleimides and the phenyl thiourea, could greatly improve the thermal stability of PVC, and could inhibit effectively growth of bacteria and fungi. In the present study, we hereby report the synthesis, characterization, and evaluation of antibacterial and antifungal activities of some new maleimido phenyl thiourea derivatives containing substituent groups at their aromatic rings of phenyl thiourea parts. It is of our great interest to investigate these derivatives as a new type of antimicrobial agents for stabilization of rigid PVC against thermal degradation and to obtain thermally stable antimicrobial PVC composites. The effect of the substituent group on the inhibition of the thermal degradation of rigid PVC is also investigated.

Experimental

MATERIALS

The PVC (suspension) used in this study was additive free, with a *K* value of 70 and was supplied by Hüls (Frankfurt, Germany). The cadmium–barium–zinc (Cd–Ba–Zn) stearate complex obtained from G. Siegle (Stuttgart, Germany); *n*-octyltin mercaptide (*n*-OTM) obtained from America Company for PVC manufacturing (Alexandria, Egypt); dibasic lead carbonate (DBLC) obtained from the National Lead (Darmstadt, Germany); *p*-amino benzoic acid, aniline, and potassium thiocyanate obtained from Oxford (Mumbai, India); and *p*-toluidine and *o*-choloroaniline obtained from Schuchard (München, Germany) were also used. *N*-[4-(Chlorocarbonyl) phenyl] maleimide was synthesized according to the method described by Oishi and Fujimoto.[40]

PREPARATION OF MALEIMIDO PHENYL THIOUREA AND ITS DERIVATIVES

An equimolar amounts of dry potassium thiocyanate and *N*-[4-(chlorocarbonyl) phenyl] maleimide were dissolved separately in least amount of dry acetone and cooled in ice-salt bath at 0–5°C for 15 min. After complete dissolution, the *N*-[4-(chlorocarbonyl) phenyl] maleimide solution was added drop wisely to potassium thiocyanate solution with constant stirring for 1 h. The white precipitate of the formed ammonium chloride was removed by filtration and the filtrate was added gradually to the dry acetone solution of appropriate amount of the corresponding amine with stirring for 1 h. The reaction mixture was poured on crushed ice and the resulting crude was filtered, dried in oven at 80°C, and finally recrystallized from boiling water.[41]

PREPARATION OF PVC COMPOSITES

PVC composites for thermal degradation were prepared by thoroughly mixing 1 g of PVC powder with 2 mass% of the stabilizer in a mortar and 0.2 g of the resulting fine powder was used in the investigation.

ANTIMICROBIAL ACTIVITY

All tested microorganisms belong to the culture collection unit at the Regional Center for Mycology and Biotechnology. Antibacterial activities were investigated using the agar well diffusion method.[42] The activity of tested samples was studied against *Bacillus subtilis* (RCMB 010069) and *Streptococcus pneumoniae* (RCMB 010019) as Gram-positive bacteria and against *Escherichia coli* (RCMB 010055) as Gram-negative bacteria. Centrifuged pellets of bacteria from a 24-h-old culture containing approximately 104–106 colony forming unit (CFU) per milliliter were spread on the surface of nutrient agar (typetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0), which was autoclaved under 121°C for at least 20 min. Wells were created in medium with the help of sterile metallic bores and then cooled down to 45°C. The activity was determined by measuring the diameter of the inhibition zone (in mm). One hundred microliters of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds were prepared in Dimethyl sulphoxide (DMSO), and DMSO was loaded as control. The plates were kept for incubation at 37°C for 24 h, and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture: Ampicillin and gentamicin were used as antibacterial standard drugs (10 mg/mL).

Antifungal activities were investigated by screening the tested samples separately in vitro against *Aspergillus fumigatus*

(RCMB 02569), *Syncephalastrum racemosum* (RCMB 05925), and *Geotricum candidum* (RCMB 05098) fungi on sabouraud dextrose agar plates. The culture of fungi was purified by the single spore isolation technique. The antifungal activity was investigated by the agar well diffusion method[43] as follows: *Sabourad dextrose agar plates*: A homogeneous mixture of glucose–peptone–agar (40:10:15) was sterilized by autoclaving at 121°C for 20 min. The sterilized solution (25 mL) was poured into each sterilized Petri dish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30°C in an incubator to remove the moisture and to check for any contamination. *Antifungal assay*: A fungal strain was grown in 5 mL sabourad dextrose broth (glucose–peptone, 40:10) for 3–4 days to achieve 105 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min so that the culture was properly adsorbed on the surface of sabourad dextrosenagar plates. Small wells (4 mm × 2 mm) were cut into the plates with the help of a well cutter and the bottoms of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. One hundred microliters of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds were prepared in DMSO, and DMSO was loaded as control. The plates were examined for the formation of zone of inhibition. Each inhibition zone was performed three times for each fungus. Amphotericin B was used as an antifungal standard drug (10 mg/mL). To determine the minimum inhibition concentration (MIC) of tested samples, the agar plate method was used; twofold serial dilutions of each sample were added to nutrient broth for bacteria (beef extract 5 g, peptone 10 g added to 1000 mL distilled water, pH 7.0) and to sabouraud dextrose broth for fungi, DMSO was used as the control. Then, they were heated in an autoclave at 121°C for 25 min. The culture of each organism was diluted by sterile distilled water to 105–106 CFU/mL, a loop of each suspension was inoculation. The plates were incubated at 37°C for 24 h for bacteria, and at 30°C for 3–4 days for fungi. The colonies were counted and the MIC values were obtained. Determination of MIC value was performed once time for each microorganism. The MIC is considered to be the lowest concentration that completely inhibits against inoculums comparing with the control, disregarding a single colony or a faint haze caused by the inoculums.

MEASUREMENTS

FTIR spectra were recorded on a Shimadzu FTIR 8201 PC spectrophotometer (Tokyo, Japan) using KBr pellets. ^{1}H NMR spectra were recorded with a JEOL 270 MHz spectrophotometer (Tokyo, Japan) in DMSO-d_6 as a solvent and the chemical shifts were recorded in ppm relative to Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Shimadzu GCMS-QP 1000 ex spectra Mass spectrometer (Tokyo, Japan) operating at 70 eV. Elemental analyses were carried out in Perkin-Elmer, Maryland, United States (model 2410 series II) C, H, N Analyzer (USA) at the Micro-Analytical center at Cairo University, Giza, Egypt.

Evaluation of the stabilizing efficiency was carried out by measuring the rate of dehydrochlorination using a continuous potentiometric determination of the evolved hydrogen chloride gas at 180°C in air. Briefly, a reference calomel electrode and silver rod as a working electrode were used in silver nitrate solution of known concentration. The evolved hydrogen chloride gas is absorbed in the silver nitrate solution. The change in potential during the assay corresponds to the change in concentration of the Ag^+ ions in the absorbent solution as a result of its reaction with the evolved gas. The amount of the hydrogen chloride absorbed is then determined by applying the Nernst equation. A detailed description of this method was given elsewhere.[44] The extent of discoloration of the degraded PVC samples was evaluated visually by subjecting the PVC in the absence and presence of various stabilizers for different degradation time intervals.

Results and Discussion

SYNTHESIS AND CHARACTERIZATION OF THE STABILIZERS

Four maleimido phenyl thiourea derivatives **1–4** (Scheme 1) were synthesized via a low-temperature (0–5°C) solution (in dry acetone); the addition reaction between the corresponding aromatic amine and the product resulted from the reaction of potassium thiocyanate with *N*-[4-(chlorocarbonyl)phenyl] maleimide in cold dry acetone. The reaction mixture was stirred for 1 h, and then poured on crushed ice. The resulting crude was filtered, dried in an oven at 80°C, and recrystallized from boiling water.

All the derivatives are novel and were obtained in a quantitative yield. The structures of the synthesized derivatives **1–4** were ascertained on the basis of their consistent elemental analyses, FTIR (Fig. 1), ^{1}H NMR (Fig. 2), and mass spectral characteristics.

ANALYTICAL DATA OF DERIVATIVE 1

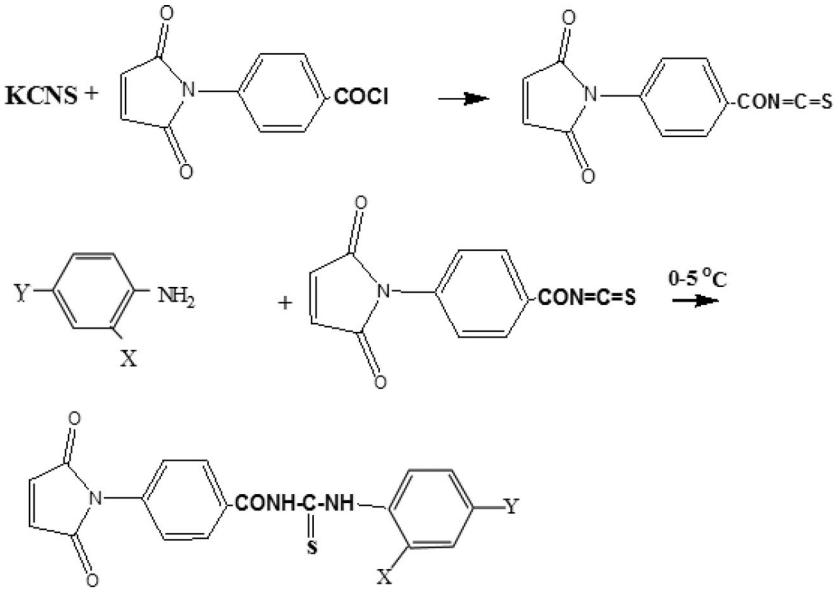
Mp (°C) 205; Yield 98%; ^{1}H NMR (270 MHz, DMSO-d_6) δ (ppm): 7.19 (s, 2H, =CH), 7.33–8.058 (m, 9H, ArH), 10.30 (s, 2H, 2NH disappearing on deuteration); FTIR (KBr pellets) ν cm^{-1}: 3463, 3318 (NH), 1649 (C=O), 1707 (C=O, imide), 1600, 1512 (Ph), 829 (maleimide moiety), 544 (N–C–S); MS m/z: 351 (M^+); Anal. calcd for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.70; N, 11.96; S, 9.11. Found: C, 61.51; H, 3.69; N, 11.95; S, 9.90.

ANALYTICAL DATA OF DERIVATIVE 2

Mp (°C) 225; Yield 96%; ^{1}H NMR (270 MHz, DMSO-d_6) δ (ppm): 7.17 (s, 2H, =CH), 7.35–8.12 (m, 8H, ArH), 11.55, 12.49 (2s, 2H, 2NH disappearing on deuteration), 2.25 (s, 3H, CH_3); FTIR (KBr pellets) ν cm^{-1}: 3456, 3365 (NH), 1673 (C=O), 1719 (C=O, imide), 1597, 1542 (Ph), 1395 (CH_3), 1075 (C=S), 541 (N–C–S), 824 (maleimide moiety); MS m/z: 381 (M^+); Anal. calcd for $C_{19}H_{16}N_3O_3S$: C, 59.83; H, 3.93; N, 11.03; S, 8.39. Found: C, 60; H, 3.93; N, 11.07; S, 8.47.

ANALYTICAL DATA OF DERIVATIVE 3

Mp (°C) 156; Yield 95%; ^{1}H NMR (270 MHz, DMSO-d_6) δ (ppm): 7.19 (s, 2H, =CH), 7.33–8.19 (m, 8H, ArH), 10.14, 10.19



Derivative no.	1	2	3	4
X	H	H	Cl	H
Y	H	CH_3	H	COOH

SCHEME 1. Synthesis of maleimido phenyl thiourea derivatives.

(2s, 2H, 2NH disappearing on deuteration); FTIR (KBr pellets) ν cm^{-1}: 3456, 3275 (NH), 1655 (C=O), 1714 (C=O, imide), 1590, 1511 (Ph), 1051 (C=S), 540 (N–C–S), 830 (maleimide moiety overlapped with C–Cl); MS m/z: 385 (M^+); Anal. calcd for $C_{18}H_{12}N_3O_3S$ Cl: C, 56.01; H, 3.11; N, 10.90; S, 8.31; Cl, 9.09. Found: C, 56.05; H, 3.09; N, 10.94; S, 8.28; Cl, 9.05.

ANALYTICAL DATA OF DERIVATIVE 4

Mp (°C) >300; Yield 95%; ^{1}H NMR (270 MHz, DMSO-d_6) δ (ppm): 7.19 (s, 2H, =CH), 7.88–8.07 (m, 8H, ArH), 7.51, 7.54 (2s, H, 2NH disappearing on deuteration), 10.6 (s, 1H, 1-COOH); FTIR (KBr pellets) ν cm^{-1}: 3466, 3343 (NH, OH), 1686 (C=O), 1711 (C=O, imide and –COOH), 1599, 1509 (Ph), 1064 (C=S), 546 (N–C–S), 833 (maleimide moiety); MS m/z: 395 (M^+); Anal. calcd for $C_{19}H_{13}N_3O_5S$: C, 57.72; H, 3.29; N, 10.63; S, 8.10. Found: C, 57.88; H, 3.34; N, 10.75; S, 8.07.

ANTIMICROBIAL ACTIVITY OF MALEIMIDO PHENYL THIOUREA DERIVATIVES

Four maleimido phenyl thiourea derivatives **1–4** were screened in vitro for their antimicrobial activities against two Gram-positive bacteria *S. pneumoniae* and *B. subtilis* and Gram-negative bacteria *E. coli*, and three fungal strains *A. fumigatus, S. racemosum,* and *G. candidum* by the agar diffusion method using *ampicillin*, *gentamicin,* and *amphotericin* B as control drugs for antibacterial and antifungal activities, respectively. The results of antimicrobial evaluation are presented in Tables I and II.

THE ANTIBACTERIAL ACTIVITY OF THE STABILIZERS

The results showed that maleimido phenyl thiourea derivatives **1–4** are effective in inhibiting the growth of the bacteria as indicated from the inhibition zone diameter, which ranges from 12.3 ± 0.19 to 19.3 ± 0.44 mm against tested bacteria (Table I). Among these four derivatives compounds, **3** and **4** showed the highest antibacterial activities. This may be due to the presence of withdrawing substituent on the benzene ring (–Cl and –COOH), whereas compound **2** exhibited the lowest antibacterial activity, which may be attributed to the presence of electron-releasing group (–CH_3). These results were supported by the higher inhibition zone diameter and the lower MIC values (Table I) of compound **4** against tested bacteria, and the role of electron-withdrawing group in improving antibacterial activity is supported by the studies of Sharma et al.[45]. The electron-donating nature of the methyl group may increase the electron density on the methyl derivative **2** and consequently decrease its potency to interact with the negatively charged microbial cell membrane leading to lower antimicrobial activity compared to the chloro and carboxy derivatives of electron-withdrawing nature.

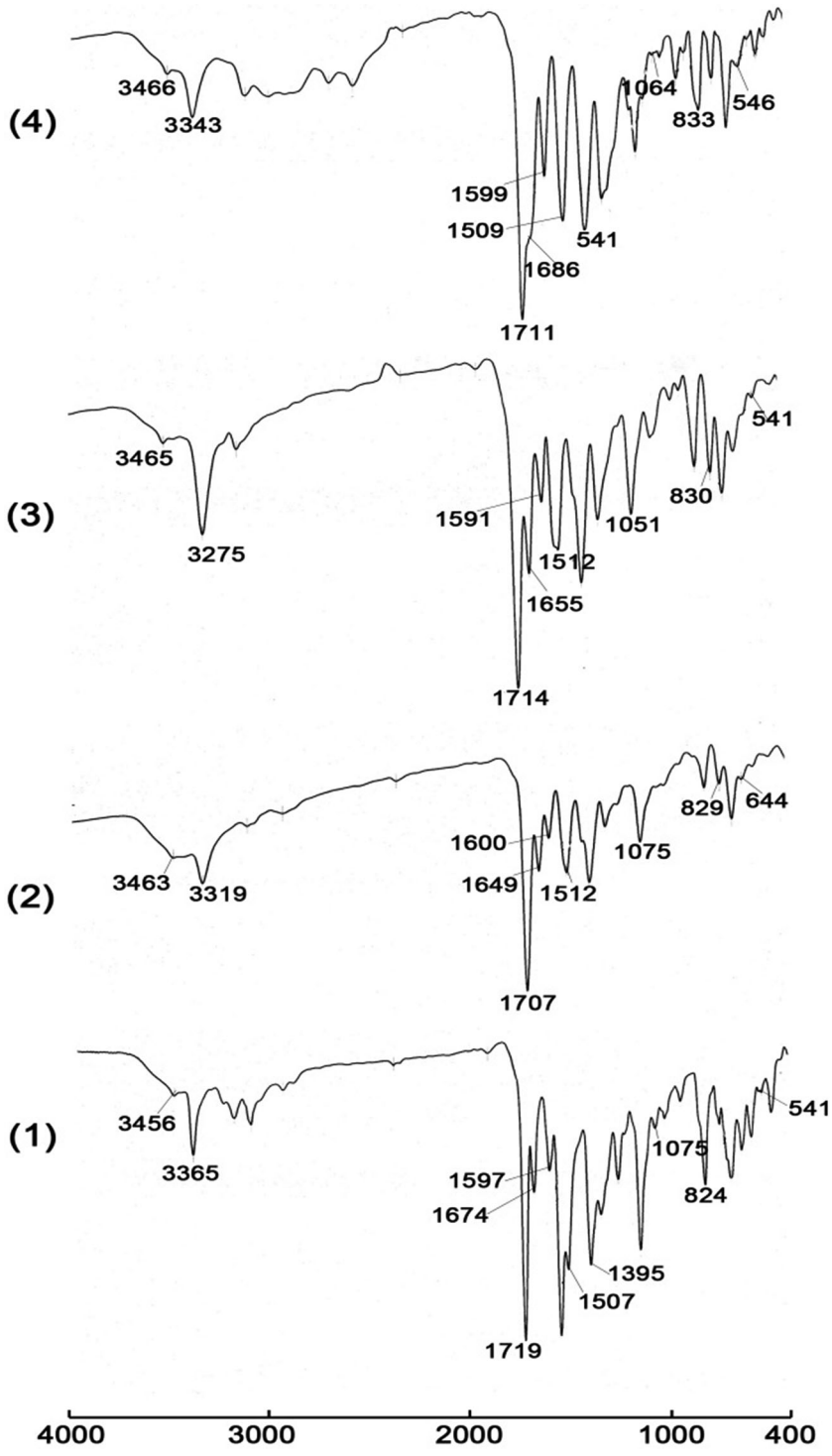


FIGURE 1. FTIR spectra of maleimido phenyl thiourea derivatives.

Moreover, it was found that the antibacterial activities of these derivatives were more effective against Gram-positive bacteria than Gram-negative bacteria (Table I). The strongest derivative **4** caused inhibition zone diameter *for B. subtilis* and *S. pneumoniae* of 19.3 ± 0.44 and 18.4 ± 0.58 mm, respectively, corresponding to 16.4 ± 0.25 mm for *E. coli*. This may be attributed to their different cell wall. The cell wall of Gram-positive bacteria is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty and allow more rapid absorption of ions into the cell. But the cell wall of the Gram-negative bacteria is made up of a thin membrane

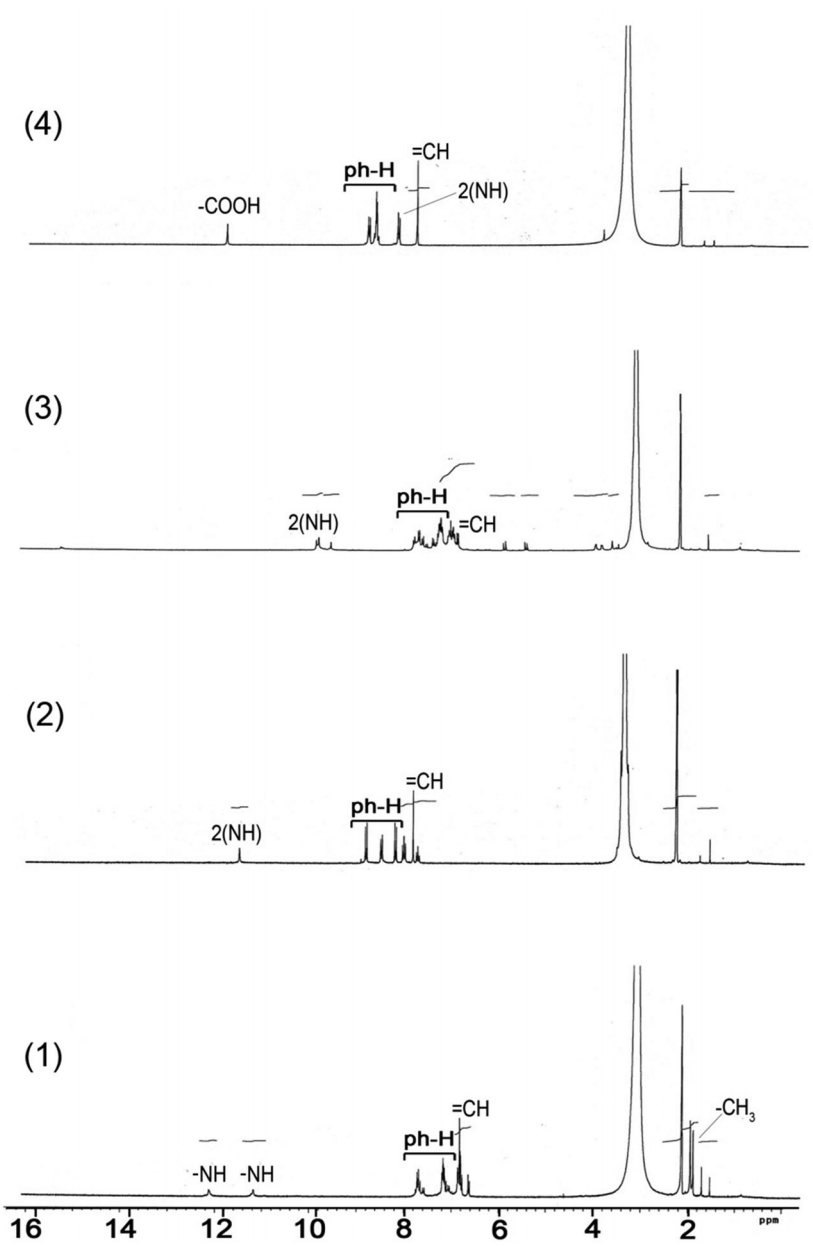


FIGURE 2. ^{1}H NMR spectra of maleimido phenyl thiourea derivatives.

of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein, and phospholipids. Because of the complicated bilayer cell structure, the outer membrane is a potential barrier against foreign molecules.[46] Therefore, these derivatives have different effects on the two kinds of bacteria. An additional evidence for the greater activity of these derivatives against Gram-positive bacteria than Gram-negative bacteria comes from their minimum inhibitory concentration, since the minimum inhibitory concentration values of the derivative **4** against *B. subtilis* and *S. pneumoniae* were 7.8 and 15.6 μg/mL, respectively, and the minimum inhibitory concentration value against *E. coli* was 62 μg/mL. Furthermore, the deduced pattern of the antibacterial activity of the synthesized compounds on the tested bacteria is in the following order: *B. subtilis* > *S. pneumoniae* > *E. coli.*

THE ANTIFUNGAL ACTIVITY OF THE STABILIZERS

The results showed that maleimido phenyl thiourea derivatives **1- -4** are effective in inhibiting the growth of the fungi as indicated from their inhibition zone diameter, which ranges from 11.4 ± 0.19 to 21.4 ± 0.44 (Table II). This may be attributed to the ability of the maleimido phenyl thiourea molecules to diffuse inside the hyphae interfering on the enzymes activity responsible for the fungus growth, which may lead to a disturbance of the

TABLE I
Inhibition Indices and MIC Values of Maleimido Phenyl Thiourea Derivatives against *B. subtilis*, *S. pneumoniae*, and *E. coli*

	B. subtilis		*S. pneumoniae*		*E. coli*	
Sample	Inhibition Zone (mm)	MIC (μg/mL)	Inhibition Zone (mm)	MIC (μg/mL)	Inhibition Zone (mm)	MIC (μg/mL)
1	16.4 ± 0.25		15.2 ± 0.25		12.3 ± 0.193	
2	14.9 ± 0.37	62.5	14.1 ± 0.25	125	12.8 ± 0.44	500
3	19.3 ± 0.44		17.2 ± 0.58		15.6 ± 0.37	
4	19.3 ± 0.44	7.8	18.4 ± 0.58	15.6	16.4 ± 0.25	62
Ampicillin	29.8 ± 0.15	0.015	21.6 ± 0.21	0.98		
Gentamicin					22.8 ± 0.22	0.98

MIC, minimum inhibitory concentration.

TABLE II
Inhibition Indices and MIC Values of Maleimido Phenyl Thiourea Derivatives against *G. candidum*, *A. fumigatus*, and *S. racemosum*

	G. candidum		*A. fumigatus*		*S. racemosum*	
Sample	Inhibition Zone (mm)	MIC (μg/mL)	Inhibition Zone (mm)	MIC (μg/mL)	Inhibition Zone (mm)	MIC (μg/mL)
1	16.2 ± 0.58		13.4 ± 0.44		13.7 ± 0.58	
2	13.2 ± 0.25	62.5	12.8 ± 0.4	125	11.4 ± 0.19	500
3	18.6 ± 0.44		16.8 ± 0.58		14.9 ± 0.25	
4	21.4 ± 0.44	1.95	19.3 ± 0.19	7.81	17.3 ± 0.25	31.25
Amphotericin B	26.7 ± 0.11	0.12	22.8 ± 0.11	0.98	20.3 ± 0.19	1.95

MIC, minimum inhibitory concentration.

enzyme activities responsible for the growth criteria, instead of the adsorption of the insoluble compounds on the fungal hyphae surface. It was found from the results that derivatives **3** and **4** are of higher antifungal activities than derivatives **1** and **2**. This may be due to its higher hydrophilicity, which allows them to introduce easily inside the hyphae of the fungi and thus enhance the diffusion of the active ingredient inside the pathogens and inhibit their growth. Moreover, as shown in Table II, derivative **4** of the highest antifungal activity exhibit highest inhibition zone diameter (21.4 ± 0.44, 19.3 ± 0.19, and 17.3 ± 0.25) and lower MIC values (1.95, 7.8, and 31.25) against tested fungi corresponding to inhibition zone diameter (26.7 ± 0.11, 22.8 ± 0.11, and 20.3 ± 0.19) and MIC values (0.12, 0.98, and 1.95) for reference standard *amphotericin* B.

STABILIZATION ACTIVITY OF THE MALEIMIDO PHENYL THIOUREA DERIVATIVES

Results of the dehydrochlorination of rigid PVC stabilized by various maleimido phenyl thiourea derivatives and thermally degraded at 180°C, in air, are shown in Fig. 3. Data of the non-stabilized blank PVC and those of the samples stabilized by

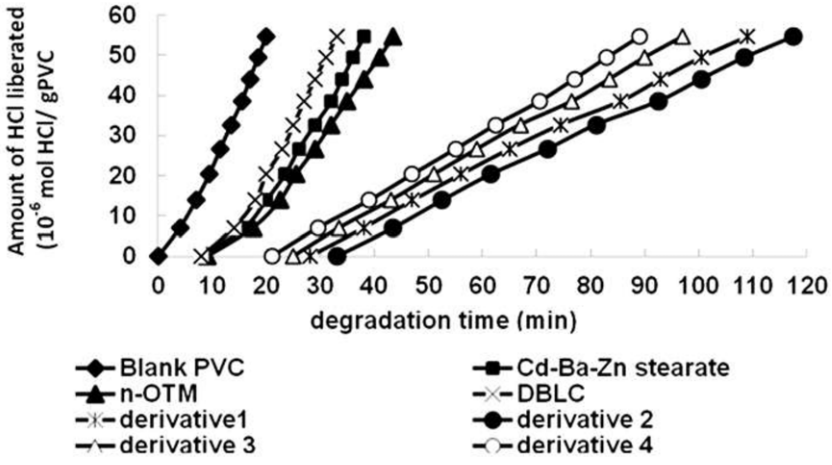


FIGURE 3. Rate of dehydrochlorination of rigid PVC at 180°C, in air, in the presence of various maleimido phenyl thiourea derivatives and reference stabilizers. All the stabilizers were used in a concentration of 2 mass% of PVC.

TABLE III
Thermal Stability (*Ts*) Period of Rigid PVC Thermally Degraded at 180°C, in Air, in the Presence of the Maleimido Phenyl Thiourea Stabilizers and Reference Stabilizers

Stabilizer Code	*Ts* (min)
Blank PVC	0
DBLC	8
Cd–Ba–Zn stearate	8
n-OTM	9
1	28
2	33
3	25
4	21

All the stabilizers were used in concentration of 2 wt%.

DBLC, *n*-OTM, and Cd–Ba–Zn stearates reference stabilizers are also given for comparison. All the stabilizers were used in a concentration of 2 mass% of PVC weight, and the results represent the average of three comparable experiments of each stabilizer. The results clearly reveal the greater stabilizing efficiency of these compounds than that of the reference stabilizers. This is indicated not only by the longer thermal stability periods (*Ts*) during which no detectable amounts of hydrogen chloride are liberated (i.e., there is no change in potential reading from the beginning of the degradation process to the end of this period) (Table III), but also by the lower rates of dehydrochlorination during the subsequent stages of degradation (Fig. 3). The thermal stability period of compound **2** is almost four times higher than the periods obtained for the reference stabilizers.

It has been previously suggested that maleimides owe their stabilizing efficiency to the replacement of the labile chlorine atoms on PVC chains by a relatively more thermally stable stabilizer moiety.[38] The stabilizers efficiency is attributed to their radical potencies to intervene with the PVC radical degradation process. This most probably occurs not only through trapping the radical species in the degradation process, but also by blocking the radical sites created on PVC chains. The radical attack seems to occur first on the ethylenic carbon–carbon double bond, followed by cleavage of the imide linkages during the later stages of degradation. This mode of action has previously been published, together with experiments to prove it.[38] Furthermore, phenyl thiourea derivatives are efficient thermal stabilizers for rigid PVC. They exhibit their stabilizing efficiency through effective absorption of the degradation products (HCl gas) by their basic groups.[39] In view of structural similarity of the investigated stabilizers to maleimides combined with phenyl thiourea derivatives, their mechanism is outlined by assuming that their first part (maleimide) can work as radical trapper, whereas their other part (phenyl thiourea) acts as HCl absorber. The data also demonstrate how the *Ts* and the rate of dehydrochlorination are affected by the type of the substituent group

TABLE IV
Extent of Discoloration of Thermally Degraded Rigid PVC at 180°C, in Air, for Various Time Intervals in the Presence of Different Stabilizers

Stabilizer Code	Color at 0 min	Color at 20 min	Color at 40 min	Color at 60 min
Blank PVC				
DBLC				
Cd–Ba–Zn stearate				
n-OTM				
1				
2				
3				
4				

All the stabilizers were used in concentration of 2 wt%.

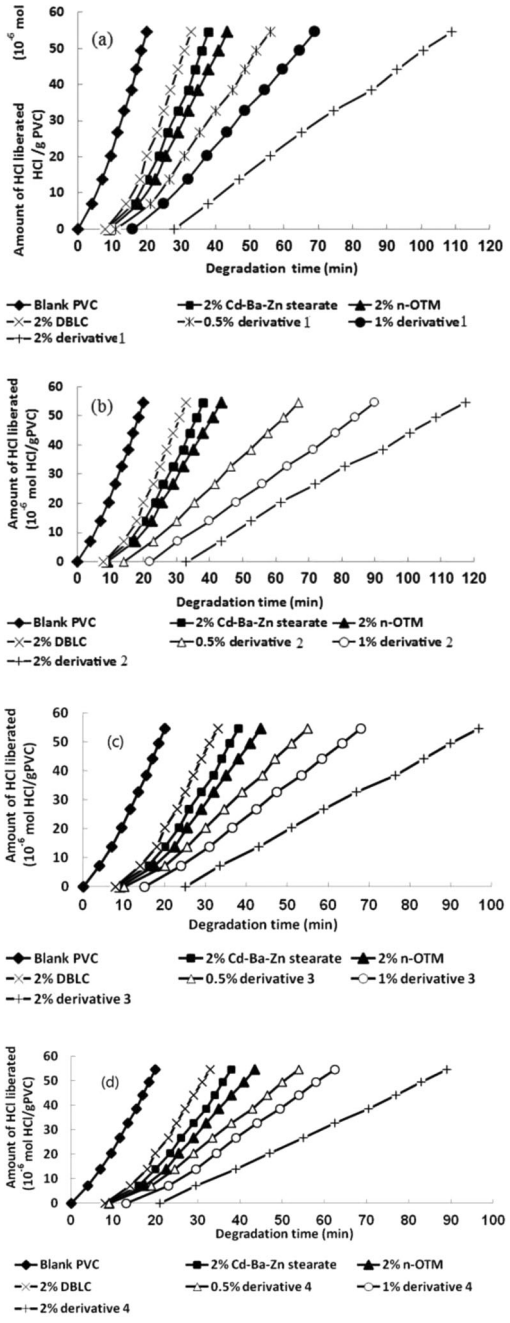


FIGURE 4. Effect of the maleimido phenyl thiourea derivatives concentration on the rate of dehydrochlorination of thermally degraded rigid PVC at 180°C in air: (a) derivative 1, (b) derivative 2, (c) derivative 3, and (d) derivative 4.

in the aromatic ring of the phenyl thiourea part (–CH_3, –Cl, and –COOH groups) of the stabilizers. This indicates the important role played by the substituent groups in the aromatic ring in the stabilization process, and the introduction of the –CH_3 group into the phenyl ring leads to an appreciable improvement in *Ts* (at early stages of degradation) and a slight lowering in the rate of dehydrochlorination (at subsequent stages of degradation). This may be attributed to the nature of this substituent (electron-releasing substituent), which can donate electrons toward both the phenyl thiourea and ethylenic carbon–carbon double bond, resulting in higher stabilizing efficiency relative to that of non-substituted derivative **1**. On the other hand, derivatives **3** and **4** exhibited lower *Ts* and higher rate of dehydrochlorination than derivative **1**. This can be explained due to the presence of electron-withdrawing groups –Cl and –COOH, which can withdraw electrons away from the thiourea group and ethylenic carbon–carbon double bond leading to a decrease in their stabilizing effect on PVC chains to some extent. Furthermore, the stabilizing effect of derivative **3** was greater than derivative **4**; this is due to the greater accepting power of the –COOH group relative to that of the –Cl group.

EFFECT OF THE STABILIZERS ON THE DISCOLORATION OF THERMALLY DEGRADED RIGID PVC

It was found that maleimido phenyl thiourea stabilizers lowered the extent of discoloration of PVC samples thermally degraded at 180°C, in air, for different time intervals relative to blank PVC sample and PVC samples stabilized with any of the reference stabilizers as shown in Table IV. This reflects the greater stabilizing efficiency of the investigated stabilizers, at early stages of degradation (during the thermal stability period), through the displacement of the labile chlorine atoms by more thermally stable stabilizer moiety, which disrupts the formation of conjugated double bonds that are responsible for discoloration. While at subsequent stages of degradation, the investigated stabilizers lowered the extent of discoloration through absorption of the evolved hydrogen chloride gas, thus preventing or at least minimizing its catalytic degradation effect. The results also show that derivative **4** resulted in a higher degree of discoloration compared to the other maleimido phenyl thiourea derivatives. This may be attributed to the presence of COOH group with its electron-withdrawing power that can withdraw electrons away from both the thiourea group and the ethylenic carbon–carbon double bond leading to a decrease both in the replacement of labile chlorine at early stages of degradation and absorption of hydrogen chloride gas at subsequent stages of degradation. Moreover, the lowering of the extent of discoloration in the presence of the investigated stabilizers may be attributed to their dienophilic property, which allows them to intervene with the conjugated double bonds formed on the PVC chains at subsequent stages of degradation process by Diels–Alder type of addition. The good color of the dibutylin maleate stabilizer has been attributed to the same type of addition reaction.[47]

EFFECT OF THE STABILIZER CONCENTRATION ON THE STABILIZATION EFFICIENCY OF THE THERMALLY DEGRADED RIGID PVC

It was useful to identify the least amount of investigated stabilizers that can achieve better stabilizing efficiency compared with the reference stabilizers so the study was extended to determine the lowest amount of the investigated stabilizers, which can provide stabilizing power comparable with the reference stabilizers. Results of the *Ts* periods and the rates of

TABLE V
Effect of the Maleimido Phenyl Thiourea Stabilizers Concentration on the Thermal Stability (*Ts*) Period of Rigid PVC Thermally Degraded at 180°C in Air

	Ts (min) Stabilizer Concentration (wt%)		
Stabilizer Code	0.5	1	2
1	11	16	28
2	14	22	33
3	10	15	25
4	9	13	21
DBLC	–	–	8
Cd–Ba–Zn stearate	–	–	8
n-OTM	–	–	9

dehydrochlorination of PVC stabilized with the investigated stabilizers at different concentrations (0.5, 1, and 2 mass% of PVC) are compared with those of the three reference stabilizers taken in 2 mass% of PVC as shown in Fig. 4 and Table V.

The results show the greater efficiency of the investigated stabilizers even at lower concentration (0.5–1.0 mass% of PVC) relative to those of the reference stabilizers (2 mass% of PVC). This is illustrated not only from the higher *Ts* periods (Table V), but also from the lower rates of dehydrochlorination at subsequent stages of degradation (Fig. 4). The greater efficiency of the investigated stabilizers is most probably not only due to their possession of various centers of reactivity that can act as traps for radical species resulting during the degradation process, but also due to the ability to react with the evolved HCl gas.

Conclusions

The prepared maleimido phenyl thiourea derivatives are efficient antimicrobial agents against *B. subtilis* and *S. pneumoniae* as Gram-positive bacteria and against *E. coli* as Gram-negative bacteria, and against *A. fumigatus*, *S. racemosum*, and *G. candidum* fungi. They are also efficient stabilizers for rigid PVC against thermal degradation even if they are used in lower concentrations compared with industrial stabilizers such as DBLC, Cd–Ba–Zn stearate, and *n*-OTM. The investigated stabilizers lower the degree of discoloration of the degraded PVC samples. From the above, it is possible to recommend the use of maleimido phenyl thiourea derivatives as antimicrobial thermal stabilizers for rigid PVC to obtain thermally stable antimicrobial PVC/maleimido phenyl thiourea composites.

References

1. Chen, X.; Li, C.; Zhang, L.; Xu, S.; Zhou, Q.; Zhu, Y.; Qu, X. China Particuol 2004, 2, 226.
2. Cheng, Q., Li, C., Pavlinek, V., Saha, P., Wang, H. Appl Surf Sci 2006, 252, 4154.
3. Kameda, T., Ono, M., Grause, G., Mizoguchi, T.; Yoshioka, T. J Polym Res 2011, 18, 945.
4. McNeill, I. C., Memetea, L., Cole, W. J Polym Degrad Stab 1995, 49, 181.
5. Patel, K., Velazquez, A., Calderon, H. S., Brown, G. R. J Appl Polym Sci 1992, 46, 179.
6. Hillemans, J. P. H. M., Colemonts, C. M. C. J., Meier, R. J., Kip, B. J Polym Degrad Stab 1993, 42, 323.
7. Meier, R. J., Kip, B. J Polym Degrad Stab 1992, 38, 69.
8. Bensemra, N., Hoang, T. V., Guyot, A. Polym Degrad Stab 1990, 28, 173.
9. Dean, L., Dafeiz, Z., Deren, Z. Polym Degrad Stab 1988, 22, 31.
10. Garton, A., George, M. H. J Polym Sci, Polym Chem Ed 1974, 12, 2779.
11. Panek, M. G., Villacorta, G. M., Starnes, W. H., Jr. Macromolecules 1985, 18, 1040.
12. Crawley, S., McNeill, I. C. J Polym Sci, Polym Chem Ed 1978, 16, 2593.
13. Martinez, G., Gomez-Elvira, J. M., Millan, J. Polym Degrad Stab 1993, 40, 1.
14. Dworkin, R. D. J. Vinyl Technol. 1989, 11, 15.
15. Levai, G. Y., Ocskay, G. Y., Nyitrai, Z. S. Polym Degrad Stab 1994, 43, 159.
16. Tran, V. H., Neuyen, T. P., Molinie, P. Polym Degrad Stab 1996, 53, 279.
17. Rabek, J. F., Lucki, J., Kereszti, H., Hjertberg, T., Jun, Q. B. J Appl Polym Sci 1990, 39, 1569.
18. Cheng, W.-H., Liang, Y. C. J Appl Polym Sci 2000, 77, 2464.
19. Hoang, T. V., Micheal, A., Guyot, A. Eur Polym J 1984, 20, 7.
20. Iida, T., Kawato, J., Maruyama, K., Goto, K. J Appl Polym Sci 1987, 34, 2355.
21. Fahmy, M. M. J Appl Polym Sci 2010, 115, 2013.
22. Sabaa, M. W., Rabie, S. T., Mohamed, R. R. J Therm Anal Calorim 2012, 109, 1503.
23. Fahmy, M. M., Mohamed, R. R., Mohamed, N. A. Molecules 2012, 17, 7927.
24. Umadevi, P., Deepti, K., Srinath, I., Vijayalakshmi, G., Garakaramji, M. Int J Pharm Pharm Sci 2012, 4(Suppl 5), 285–287.
25. Madhavaa, G., Venkata, S. K., Sreenivasulu, R., Naga R. C. Der Pharmacia Lett 2012, 4(4), 1194.
26. Shweta, V., Riaz, H., Neha, K. Indo Global J Pharm Sci 2013, 3(1), 33.
27. Serkan, Y., Hilal, Y. J Chem 2013, 2013, 149693.
28. Joshua, R. B., Elton, J. N., Julian, G. H., Christophe, M., Jerrod, S. S.; Dianqing, S., Jana Kordul, k., Michael, S. S., Victoria, J., Anna, G., Rebecca, M. C., Mary, J., Michael, R. M., Richard, E. L. Med Chem 2011, 19(18), 5585.
29. Ashish, P. K., Girish, D. H., Rajesh, H. T., Atish, H. R., Satish, S. B., Vandana, M. K. Bioorg Med Chem Lett 2012, 22(10), 3445.
30. Bedia, K. K., Ahmet, O. C., Nurhayat, T., Abbas, A., Shabana, I. K., Ikhlas, A. K., David, E. W. Molecules 2013, 18, 3562.
31. Filho, V. C., Pinheiro, T., Nunes, R. J. Farmaco 1994, 49, 675.
32. Iragashi, Y., Yagami, K., Imai, R. J. Ind Microbiol 1990, 6, 223.
33. Pereiva, E. R., Fabre, S., Sancelme, M. J Antibiot 1995, 48, 863.
34. Takatori, K., Hasegawa, T., Nakano, S. Microbiol Immunol 1985, 29, 1237.
35. Watanabe, S., Igarashi, Y., Yagami, K. Plastic Sci 1992, 34, 99.
36. Watanabe, S., Igarashi, Y., Yagami, K. Int J Mater Prod Technol 1990, 5, 387.
37. Zentz, F., Valla, A.; Guillou, R. L., Labia, R., Mathot, A-G, Sirot, D. IL Farmaco 2002, 57, 421.
38. Sabaa, M. W., Mikhael, M. G., Mohamed, N. A., Yassin, A. A. Die Angew Makromol Chem 1989, 168, 23.
39. Magdy, W. S., Riham, R. M., Ahmady, A. Y. Polym Degrad Stab 2003, 81, 37.
40. Oishi, T., Fujimoto, M. J Polym Sci Polym Chem 1992, 30, 1821.
41. Furniss, B. S., Hannaford, A. J., Smith, P. W. G., Tatchell, A. R. Vogel's Text-Book of Practical Organic Chemistry, 3rd ed.; Longmans Wiley, New York, 1966.
42. Rahman, A., Choudhary, M. I., Thompson, W. J. Bioassay Techniques for Drug Development, vol. 16, Harwood Academic Publishers: The Netherlands 2001, 2024.
43. Rathore, H. S., Mittal, S., Kumar, S. Pestic Res J 2000, 12, 103.
44. Vymazal, Z., Czako, E., Meissner, B., Stepek, J. J Appl Polym Sci 1974, 18, 2861.
45. Sharma, P., Rana, N.; Gunam, V. K. Bioorg Med Chem Lett 2004, 14, 4185.
46. Zhong, Z., Xing, R., Liu, S., Wang, L., Cai, S., Li, P. Carbohydr Res 2008, 343, 566.
47. Scott, G., Tahan, M., Vyvoda, J. Eur Polym J 1979, 15, 51.