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Maleimide-*g*-pyridine Octadecene Polymers: Synthesis, Characterization, Thermal Stability, Fluorescence and Biological Studies

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The fluorescent poly(maleic anhydride-*alt*-octadecene-arylmethoxypyridine) (PMAOA) and poly(maleic anhydride-*alt*-octadecene-arylchloropyridine) (PMAOB) polymers were synthesized and characterized by FTIR, ^{1}H & ^{13}C NMR spectroscopy. The thermal stability of polymers was measured by TGA-DTA in the range 100-700 ºC. The fluorescence properties of the synthesized PMAOA and PMAOB polymers were studied by UV-Vis spectroscopy and fluorescence spectra and showed notable report due to influence of pyridine grafted to the malelic anhydride ring derivatives. The fluorescent polymers, PMAOA and PMAOB shows high efficacy of antimicrobial activity against *Candida albicans*, *Aspergillusniger*, *Staphylococcus aureus* and *Escherichia coli* pathogens.

Keywords: Fluorescent polymer, Solvatochromism, Thermal properties, Fluorescence, Antimicrobial activity, Antifungal activity.

INTRODUCTION

Recently, much interest has been focused on the fluorescent polymeric materials are being widely used in biomedical [1-3] and biological properties such as drugs, antitumor, antimicrobial and anti-tumor activities, protein labeling, sensors, neogene-sediments diagnostics, n-type organic semiconductor and electroluminescence [4-17]. According to Yan *et al*. [18] copolymer containing maleimide in poly[(maleicanhydride)-*alt*-(vinyl pyrrolidone)] and its derivatives proved that the origin of its emission was linked with the carbonyl groups is widely used in biological studies. The oligomer containing maleimide and carbazole units were related the fluorescence with their structure [19]. Hence, it is proved that maleic anhydride polymer technique is considered to be very efficient one for grafting of more functional groups onto solid surfaces such as fluorescence, nanoparticle, drug, PLED, solar cells, thin films, *etc*. [20-32].

For the past few years, reports on the various poly(amide-imide)polymer or fluorescent polymer and their antibacterial activity [32-44] have attracted the attentions to the researchers. Recently, pyridine based polymers showed an unique fluorescence properties due to the presence of polymers containing alkyl and amide units [45,46]. In addition, some organic and maleic amide may show inhibitory action on the growth of bacterial, antitumor activity drugs [47-53]. Several biologically active organic maleic amide compounds including most of the antibiotics are enzyme inhibitors. Organic compounds that are active towards the chemical protein modification through cysteine or thiol substituted group are able to inhibit cysteine protease and other proteins containing important cysteins [54-56]. So far, the synthesis using maleimide polymer-pyridine moiety has not been reported. It motivated us to to synthesis pyridine-containing polymer directly, without any protective groups so as to reduce the number of steps.

EXPERIMENTAL

The chemicals and solvents *viz*. poly(octadecene-*alt*-maleic anhydride) (MAO), 2,4,6-trimethoxybenzaldehyde (99%), 4-chlorobenzaldehyde (98%), tetrahydrofuran, malononitrile, *N*,*N*-dimethylformamide (DMF) and ammounium chloride were purchased from Sigma-Aldrich, India. The ^{1}H NMR spectrum was recorded on a Bruker 400 NMR spectrometer, and deuterated dimethyl sulfoxide (DMSO-d_6) was used as a solvent. FT-IR spectral study was carried out in Perkin-Elmer

Spectrum FT-IR spectrometer using KBr pellet with a spectrum of wave number range of 4000-600 cm^{-1}. The UV-Vis absorption spectrum was recorded on a Perkin-Elmer LAMBDA 950 spectrophotometer and fluorescence measurements on Spectra Max Fluorolog-3 at room temperature. The TGA was carried out using Perkin-Elmer simultaneous Thermal analyzer (TG/DTA), of model NETZSCH (TG-DSC-409), the samples were heated from 40 to 500 ºC with a heating rate of 10 ºC min^{-1} and Gel Permeation Chromatography (GPC) on HIMADZU LC-20AD, at a heating rate of 10 ºC min^{-1}. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37 ºC and maintained on nutrient agar (Difco, USA) slant at for 4 ºC.

Synthesis of poly(maleic acid-4-acetylphenyl-*N*-amide-*alt*-octadecene) (PMAO): 4-Aminoacetophenone (3.2 g, 1.15 mmol) in DMF (40 mL) was added to poly(maleic anhydride-*alt*-1-octadecene) (0.83 mg, 1.15 mmol based on the monomer unit weight of 347, M_n 30,000-50,000) in DMF (70 mL) and stirred at room temperature (25 ºC) for 30 min under nitrogen atmosphere. Reaction was monitored by thin-layer chromatography (TLC). After completion of reaction, water (100 mL) was poured into reaction mixture and filtered separated compound by filtration flask. The product was recrystallized from ethanol solvent to obtain pure PMAO and dried under vacuum to get 2.8 g (70%) yield. FTIR (KBr, ν_{max}, cm^{-1}): 3400 (OH *str.*), 1774 (asymm. C=O, imide), 1715 (sym. C=O *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.7 (d, 2H, Ar-H), 6.6 (d, 2H, ArH), 7.9 (s, H, NH), 2.5 (s, 3H, $-COCH_3$), 2.9-2.73 (m, 2 H, maleic anhydride proton, -CH-CH-), 2.4-1.0 (m, 2H, methyl $-CH_2-CH_2-$). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 162 (maleimide C=O), 26.0 (CH-CH).

Synthesis of poly(maleic anhydride-*alt*-octadecene-aryl-methoxy pyridine (PMAOA): PMAO (3.9 g, 0.2 mmol) in benzene (50 mL) was placed in a 250 mL round flask equipped with a magnetic stir bar, the dissolution was cooled at 20 ºC, 2,4,6-trimethoxybenzaldehyde (1.8 g, 0.2 mmol), malononitrile (0.75 g mL, 0.2 mmol), ammonium acetate (3.3 g, 8 mmol) then the mixture reflux for 8 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the crude product was filtered off and ammonium chloride was removed. The residue was recrystallized from ethanol, afterward, the polymer was purified three times by first dissolving in 5 mL of DMF followed by pouring the solution into ~200 mL of water/toluene (1:3, v/v) via a cotton filter to remove any insoluble substances. The precipitates were washed with ethyl acetate and dried in vacuum overnight at 50 ºC to a constant weight. Pale yellow powder is obtained (Yield: 2.5g (60 %)). FT-IR (KBr, ν_{max}, cm^{-1}): 3400 (OH *str.*), 3073 (-C=C), 2212 (C≡N), 1629 (symm. NH *str.*, imide). ^{1}H NMR (DMSO-d_6) δ ppm: 7.73-7.57 (d, 2H, Ar-H), 7.74-7.73 (d, Ar-H), 5.3 s (2H, NH_2), 7.2-7.4 (m, 1H, Ar-H,), 7.9 (s, NH), 1.2-0.8 (m, -CH-). ^{1}H NMR (DMSO-d_6) δ ppm: 167.4, 167.2, 129.2 (C≡N), 164.0 (maleimide C=O). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 167,163,161,158, 140, 137, 133, 131, 130 (maleimide C=O).

Synthesis of poly(maleic anhydride-*alt*-octadecene-aryl-chloro-pyridine) (PMAOB): PMAO (3.9 g, 0.2 mmol) in 50 mL of benzene was placed in a 250 mL round flask equipped with a magnetic stir bar, the dissolution was cooled at 20 ºC, 4-chlorobenzaldehyde (2.25 g, 0.2 mmol), malononitrile (0.75 g, 0.2 mmol), ammonium acetate as catalyst then the mixture reflux for 8 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered off and ammonium chloride was removed and the solvent resulted in the crude product. The crude product was recrystallized from absolute ethanol. Afterward, the polymer was purified three times by first dissolving in 5 mL of DMF followed by pouring the solution into ~200 mL of *n*-hexane/ethyl acetate mixture (1:3, v/v) *via* a cotton filter to remove any insoluble substances. The precipitates were washed with ethyl acetate and dried in vacuum overnight at 50 ºC to a constant weight. Pale yellow powder was obtained (yield: 3.5 g (65 %). FTIR (KBr, ν_{max}, cm^{-1}): 3341 (OH *str.*), 2917 (-C=C), 2207 (C≡N), 1529 (NH *str.*, amide). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.45 (s, 2H, NH_2), 6.36 (ArH), 8.0 (s, H, NH), 3.9 (s, 3H, OCH_3), 2.4-1.0 (m, 2H, methyl $-CH_2-CH_2-$). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 167, 162 (maleimide C=O), 26.0 (CH-CH).

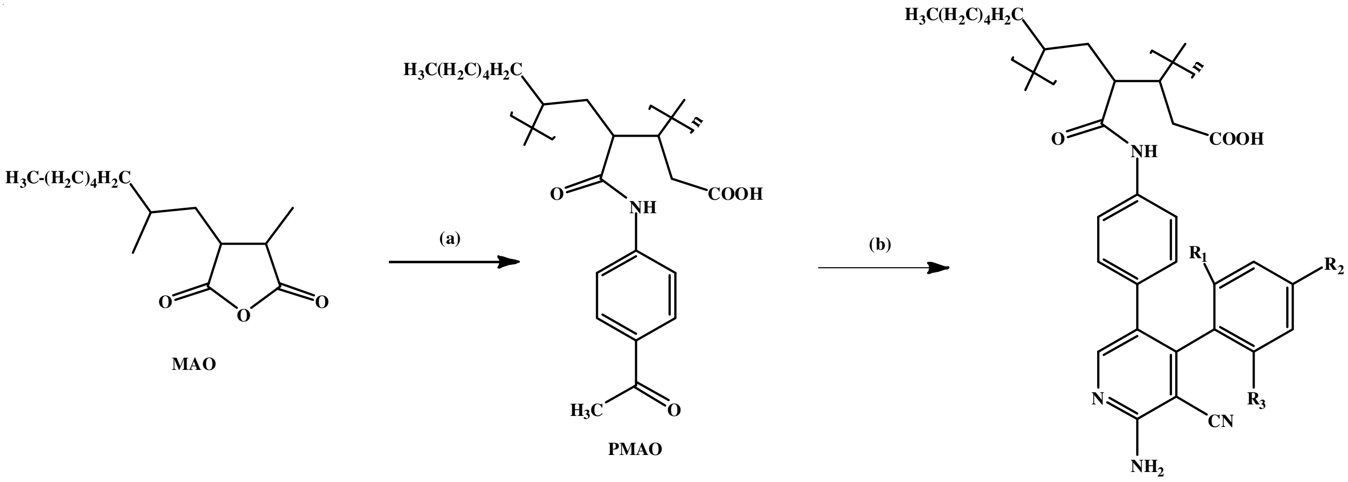
RESULTS AND DISCUSSION

Two novel fluorescent graft polymers, poly(maleic anhydride-*alt*-octadecene-arylmethoxypyridine (PMAOA) and poly(maleic anhydride-*alt*-octadecene-arylchloropyridine) (PMAOB) were synthesized *via* one spot reaction with copolymer of poly(*N*-acetylemaleic anhydride-*alt*-1-octadecene), aldehyde and malononitrile in the presence ammonium acetate as shown in **Scheme-I**. A new fluorescence emission is observed in PMAOB with maximum intensity at 510 nm in DMF solvent.

Solubility: The solubility tests were performed with 1 mg sample and 1 mL solvent at room temperature (25 ºC). The synthesized graft polymers PMAOA and PMAOB were stable at room temperature. Both compounds were soluble in highly polar solvents such as methanol, ethanol, DMSO, DMF, and solutions of NaOH and conc. H_2SO_4. Nevertheless, the PMAO was completely soluble in all solvents.

FT-IR analysis: The IR spectra of PMAO shows that the characteristic peaks at 1784 and 1668 cm^{-1} that correspond to amide (NH) and the acid groups, respectively. The peak at 1715 cm^{-1} corresponding to the keto group is disappreared in the IR spectra of PMAOA. It shows the formation of grafting at PMAOA (Fig. 1). PMAOA polymer exhibited characteristic absorption peaks centered at 2212 cm^{-1} attributed to the nitrile (C≡N) group. The pure polymer displayed broad absorption bands between 3433 and 2925 cm^{-1}, which are related to the stretching of the hydroxyl groups [35].

^{1}H NMR analysis: In ^{1}H NMR spectrum, the peaks appeared at 8.0 and 1.2-0.79 ppm were assigned to the amide (NH) and octadecene ($-CH_2-$) protons. The peak at 2.3 ppm was the signal of methyl protons connecting to phenyl ring. The peaks of the appeared at 7.6-7.7, 6.6-6.5 (doublets) reveals that the presence of aryl protons. The signals between 2.73 and 2.89 ppm belonged to the maleic acid protons of PMAO. The peak at 8 ppm is due to NH proton of PMAOA in the polymer and peak at 7.8 ppm and 6.7 ppm are due to aromatic



Reagents and conditions: (a) 1-(4-Aminophenyl) ethanone, 25 °C, dry DMF, N_2 atm, 30 min
(b) Aldehyde, malononitrile, NH_4OAc, benzene, 80 °C, 4 h

R_1, R_3, R_5 = OCH_3 (PMAQA)
R_2 = Cl (PMAOB)

Scheme-I: Synthetic route of polymer Poly(PMAO), PMAOA and PMAOB

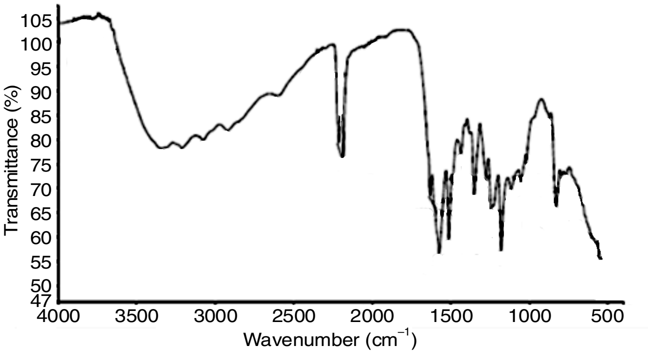


Fig. 1. FT-IR spectrum of PMAOA

protons of PMAOA units in the graft polymers. Methyl protons connecting to the maleic acid group was observed as a multiplet at 1.2 ppm. The peaks of the package of methine appeared at 0.8 to 1.2 ppm (broad, aliphatic protons) appeared in the spectra of PMAOA. The characteristic peaks of methoxy group (3.9 ppm) as shown in Fig. 2. Similar to PMAOA, polymer PMAOB showed similar observations of the proton signals and proved that both polymers are successfully synthesized by Hantzsch reaction.

^{13}C NMR analysis: The structure of PMAOA was further confirmed by ^{13}C NMR spectrum. The methyl carbons of the polymer were observed between 26 ppm. The presence of the -CONH- group of the polymer was observed at 167 ppm. The peaks between 117 and 161.3 ppm belonged to the carbons of phenyl rings of the polymer. Methyl carbon signal connected to maleicanhydride group of both polymers was observed at 26 ppm.

Fluorescence studies: The PMAOA and PMAOB (polymer exhibited a broad emission band at 500-510 nm (maximum wavelength 510 nm). Interestingly, the emission properties of poly(PMAOA) were significantly influenced by the nature of solvent used, such as DMF as shown in Fig. 3. The polymer derivative are absorption in DMF (emission 510 nm, excitation 375) and the PMAOB has fluorescence in DMF (emission 510

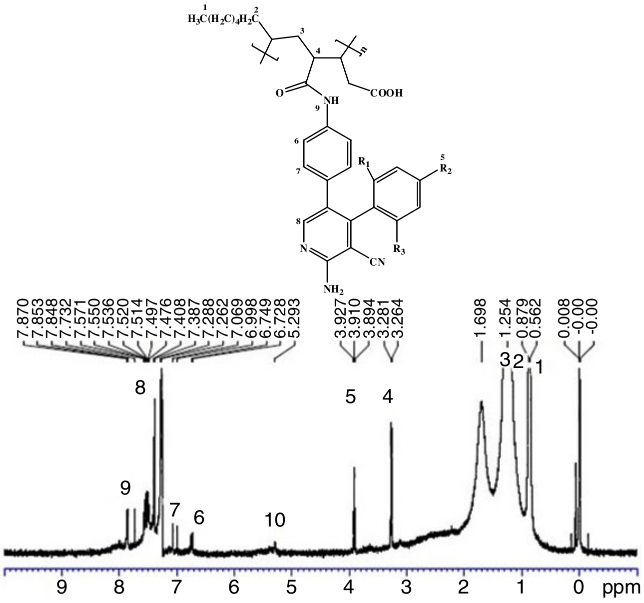


Fig. 2. ^{1}H NMR spectra of PMAOA

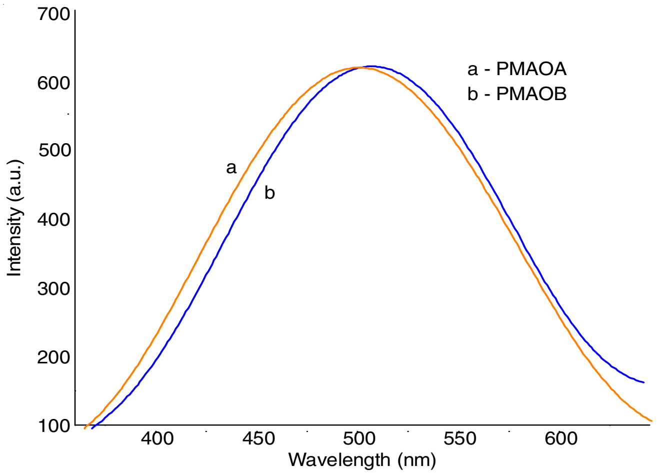


Fig. 3. Fluorescent emission (500 nm) spectra of the PMAOA, PMAOB in DMF

nm, excitation 375 nm). It is seen that both polymers exhibit similar absorption spectra in the solvent. This result indicates that the absorption spectra of these polymers are caused mainly from pyridine.

Solvatochromism has been well characterized for many pyridine dyes [38]. In these cases, solvatochromic shifts can be associated to the interaction of the ground state solvent molecules with the excited state of solute molecules. The fluorescence properties of PMAOA in DMF were investigated at room temperature. The fluorescence spectra of polymer (PMAOA) were taken for same concentration in order to determine the effect of polymer solvents on the fluorescence properties. The pyridine polymer are known to be strongly sky-blue colored and fluorescent due to the ICT, which gives rise to a large excited state dipole moment that is polarity dependent. Hence, the absorption and emission spectra of PMAOA were recorded in different solvents with varying polarity of solvent. For this purpose, the absorption spectra of the polymer are measured in seven various solvents at a concentration 10^{-6} mol.

UV-visible analysis: The UV-Vis absorption spectra of PMAOA in both protic and aprotic solvents showed a high-energy transition at 340-350 nm and a low-energy band at 485-510 nm as shown in Fig. 4. The fluorescence emission spectra of polymer (PMAOA) exhibited solvatochromism; being significant red-shift in the emission maxima upon increasing the solvent polarity (Table-1). For example, in THF exhibited sky-blue emission at 490 nm, while in DMSO a yellow-green emission at 515 nm was observed.

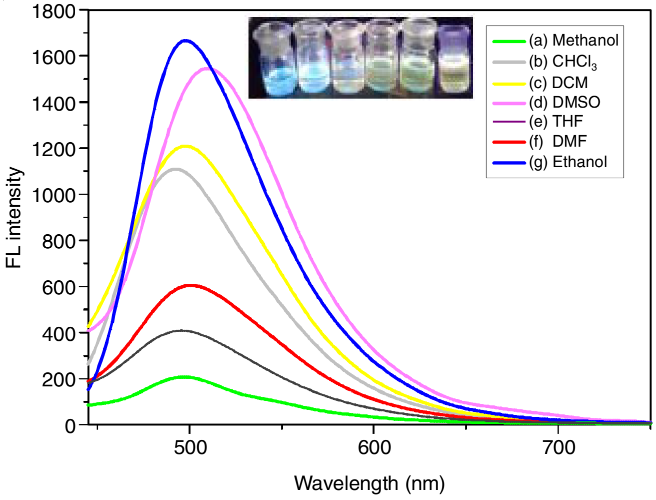


Fig. 4. Flurescence solvatochromism of PMAOA in various solvents

TABLE-1
PHOTOPHYSICAL PROPERTIES OF
PMAOA IN VARIOUS SOLVENTS

Solvents	Appearance	λ_{ab} (nm)	λ_{em} (nm)
DMSO	Colourless	445	490
DMF	Colourless	440	500
Ethanol	Blue	430	495
Methanol	Blue	430	495
$CHCl_3$	Blue	425	485
DCM	Blue	430	500
THF	Colourless	440	480

λ_{ab} = Absorption wavelength; λ_{em} = Emission wavelength

Thermal studies: The thermograms of PMO, PMAO, PMAOA and PMAOB are shown in Fig. 5. It can be seen that the onset decomposition temperature for the both polymers was 200 ºC, which indicated that both polymers had excellent thermal stability. In first step, between 200 and 300 ºC is observed for both polymers and decomposition takes place at comparable temperatures range of 500 to 550 ºC was due further liberation of CO_2. Subsequently, third thermal decomposition in the temperature range 550-700 ºC. On further heating, the complete decomposition of polymer occurs at 700 ºC. The presence of appreciable peak around 100 ºC in the DG analysis shows the presence of water molecules in both polymers.

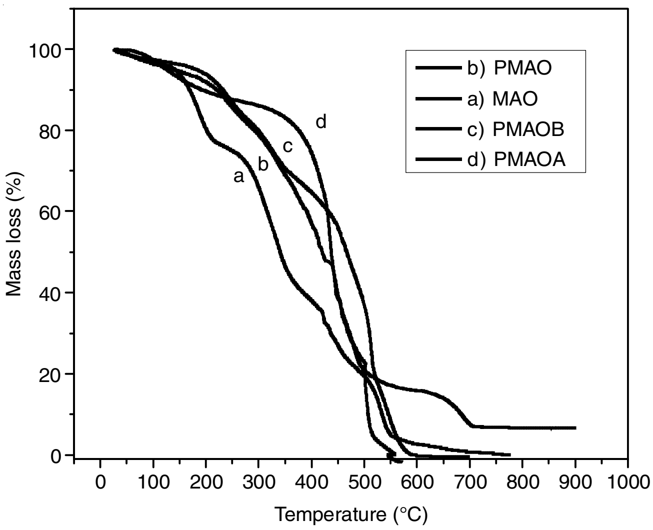


Fig. 5. Thermogravimetric analysis (TGA) profiles of (a) MAO, (b) PMAO (c), PMAOB and (d) PMAOA showing high stability polymers

Antimicrobial activity: The agar well diffusion method [56] was used to determine the antibacterial activities of the synthesized graft polymers PMAOA and PMAOB. The PMAOA showed the more activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* (Table-2).

Conclusion

In present work, two fluorescent graft polymers, PMAOA and PMAOB were synthesized *via* multi-component synthesis using Hantzsch reactions and ammonium acetate as catalysis. The structures of the newly synthesized polymers were characterized by various spectroscopic methods. Both synthesized polymers were subjected for photophysical and antimicrobial studies. The photophysical studies revealed that all maleic acid-octadecene-*g*-pyridine polymers (PMAOA and PMAOB) are fluorescent and excited by absorbing UV light around 425-430 nm while emitted fluorescence in the range of 490-500 nm wavelengths. Thermal analyses showed that both synthesized polymers are thermally stable up to 500 ºC. Also the novel grafted pyridine polymers are assessed for their *in-vitro* antibacterial activity against *E. coli* and *S. aureus*, and *in vitro* antifungal activity against *C. albicans* and *A. niger*. Conspicuously, the entire synthesized fluorescent polymers are biologically active.

TABLE-2
ZONE OF INHIBITION OF SYNTHESIZED POLYMERS AGAINST MICROBIAL PATHOGENS

Compounds and positive control	Zone of inhibition (mm) / Concentrations (μg/mL)											
	Bacterial pathogens						Fungal pathogens					
	E. coli			*S. aureus*			*C. albicans*			*A. niger*		
	20	60	100	20	60	100	20	60	100	20	60	100
PMAO	7.00	7.00	9.00	9.00	8.00	10.00	9.00	10.00	11.0	12.00	11.00	14.00
PMAOA	11.00	13.00	14.00	11.00	13.00	14.00	9.00	10.00	13.00	12.0	16.00	18.00
PMAOB	10.00	12.00	14.00	10.00	12.00	14.00	10.00	13.00	13.0	10.0	16.00	14.00

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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