Supplementary information

Active Pharmaceutical Ingredient Incorporated Supramolecular Materials as Antibacterial Agents

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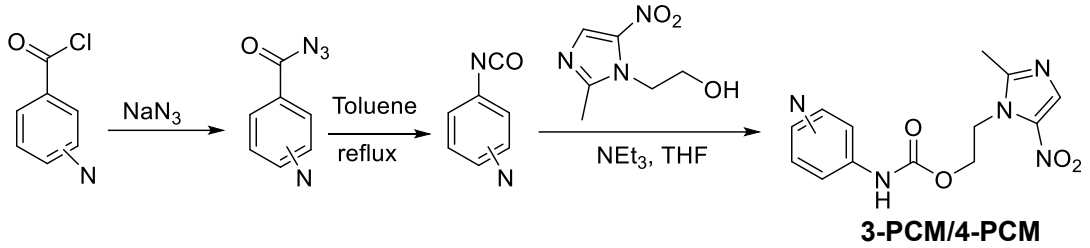
Contents

1. Materials and methods

All the starting materials, reagents, and solvents were obtained from commercial suppliers (Sigma Aldrich, Fluorochem and TCI Europe) and used without further purification. The metronidazole was purchased from Accel Pharmtech, USA. ^{1}H and ^{13}C NMR spectra (Fig. S1-S10, see Supporting Information) were recorded on a Bruker AVANCE 400 spectrometer (Rheinstetten, Germany) and SEM analysis was performed on a Leo Supra 25 microscope (Carl Zeiss, Oberkochen, Germany). The rheological experiments were performed on Anton Paar MCR 302 modular compact rheometer (Graz, Austria). Single Crystal X-ray Diffraction (SCXRD) and powder X-ray diffraction (PXRD) were carried out on Bruker D8 venture (Karlsruhe, Germany) and PANalytical instrument (Almelo, Netherlands), respectively.

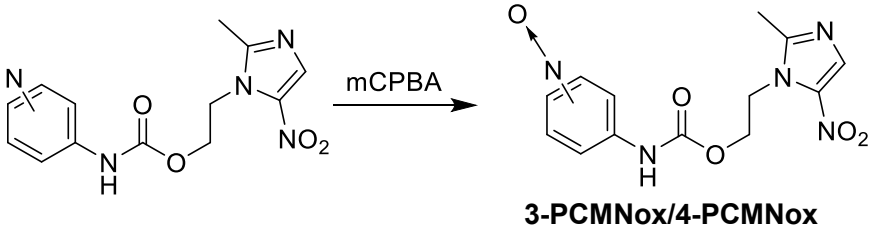
2. Synthesis



Scheme S1. Synthetic route for 3-PCM/4-PCM.

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl pyridin-3-ylcarbamate (3-PCM): A solution of nicotinoyl azide (2.96 g, 20.0 mmol) in toluene (50 mL) was refluxed for 2.0 hours under a nitrogen atmosphere and was cooled to room temperature. To this reaction mixture, a solution of metronidazole (3.42 g, 20.0 mmol) and triethylamine (3.06 mL, 21.9 mmol) in 40 mL THF was added dropwise at room temperature. The reaction mixture was refluxed overnight, after which the resulting white precipitate was collected by filtration. The solid was washed with hot water and air-dried to yield the final product as a white solid. The product was further purified by recrystallizing in ethyl acetate. Yield: 4.65 g, 80.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.56 (d, J = 2.8 Hz, 1H), 8.22 (dd, J = 4.8, 1.5 Hz, 1H), 8.05 (s, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.31 (dd, J = 8.3, 4.7 Hz, 1H), 4.61 (t, 2H), 4.48 (t, 2H), 2.49 (s, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO-d_6) δ 153.56, 152.13, 144.26, 140.83, 138.99, 135.93, 133.61, 125.88, 124.09, 63.08, 45.74, 14.43. HRMS (APCI): calcd for $C_{12}H_{13}N_5O_4Na$ $[M + Na]^+$, 314.0860; found, 314.0857.

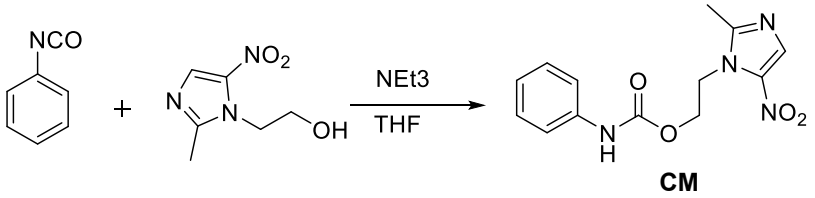
Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl pyridin-4-ylcarbamate (4-PCM): A similar procedure was followed as in the case of 3-PCM using isonicotinoyl azide (1.48 g, 10.0 mmol), metronidazole (1.71 g, 10.0 mmol) and triethylamine (1.53 mL, 10.9 mmol). The crude compound was recrystallized from ethanol/water (1:1, v/v). Yield: 2.20 g, 75.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.38 – 8.32 (m, 2H), 8.03 (s, 1H), 7.39 – 7.33 (m, 2H), 4.60 (t, 2H), 4.48 (t, 2H), 2.47 (s, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO-d_6) δ 152.58, 151.63, 150.26, 145.71, 138.51, 133.13, 112.45, 62.75, 45.17, 13.96. HRMS (APCI): calcd for $C_{12}H_{13}N_5O_4$ $[M + H]^+$, 292.1040; found, 292.1031.



Scheme S2. Synthetic route for *N*-oxides.

Synthesis of 3-(((2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)carbonyl)amino)pyridine 1-oxide (3-PCMNox): To a solution of 3-PCM (3.00 g, 10.3 mmol) in 30.0 mL DMF, 3–chloroperoxybenzic acid (3.91 g, 22.6 mmol) was added in portions, and the solution was stirred overnight at room temperature. The solution was dried to obtain the crude product, which was purified by column chromatography using 10.0-20.0% of methanol in DCM as the eluent and was recrystallized from hot water. Yield: 2.32 g, 73.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.36 (t, J = 1.9 Hz, 1H), 8.05 (s, 1H), 7.91 (dt, J = 5.8, 1.6 Hz, 1H), 7.36 – 7.28 (m, 2H), 4.61 (t, J = 5.1 Hz, 2H), 4.49 (t, J = 5.1 Hz, 2H), 2.47 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 152.71, 151.64, 138.53, 138.22, 133.25, 133.14, 129.23, 126.22, 115.05, 62.97, 45.14, 13.96. HRMS (APCI): calcd for $C_{12}H_{13}N_5O_5Na$ $[M + Na]^+$, 330.0809; found, 330.0811.

Synthesis of 4-(((2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy)carbonyl)amino)pyridine 1-oxide (4-PCMNox): A similar procedure was followed as in the case of 3-PCMNox using 4-PCM (2.00 g, 6.9 mmol) and 3–chloroperoxybenzic acid (1.71 g, 15.1 mmol). Yield: 1.55 g, 73.4 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.10 – 8.04 (m, 2H), 8.02 (s, 1H), 7.40 – 7.32 (m, 2H), 4.59 (t, J = 5.1 Hz, 2H), 4.50 – 4.43 (m, 2H), 2.45 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 152.57, 151.63, 138.91, 138.51, 136.26, 133.14, 115.07, 62.88, 45.13, 13.97. HRMS (APCI): calcd for $C_{12}H_{13}N_5O_5Na$ $[M + Na]^+$, 330.0809; found, 330.0805.



Scheme S3. Synthetic route for CM.

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl phenylcarbamate (CM): A solution of metronidazole (1.00 g, 5.8 mmol) in THF (40.0 mL) was added dropwise to a mixture of isocyanatobenzene (0.64 mL, 5.8 mmol) and triethylamine (0.89 mL, 6.4 mmol) in dry THF (50 mL) and the mixture was refluxed overnight under a nitrogen atmosphere. It was then cooled to room temperature and the white precipitate formed was filtered and washed with water and diethyl ether, and dried in air, to obtain the product as a white solid. The compound was recrystallized from ethyl acetate. Yield: 1.45 g, 85.8%.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.05 (s, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.31 – 7.22 (m, 2H), 7.00 (tt, J = 7.3, 1.2 Hz, 1H), 4.59 (t, J = 5.1 Hz, 2H), 4.46 (t, J = 5.1 Hz, 2H), 2.48 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 152.94, 151.67, 138.71, 138.49, 133.13, 128.73, 122.71, 118.52, 62.19, 45.39, 13.94. HRMS (APCI): calcd for $C_{13}H_{14}N_4O_4Na$ $[M + Na]^+$, 313.0907; found, 313.0909.

3. NMR spectra

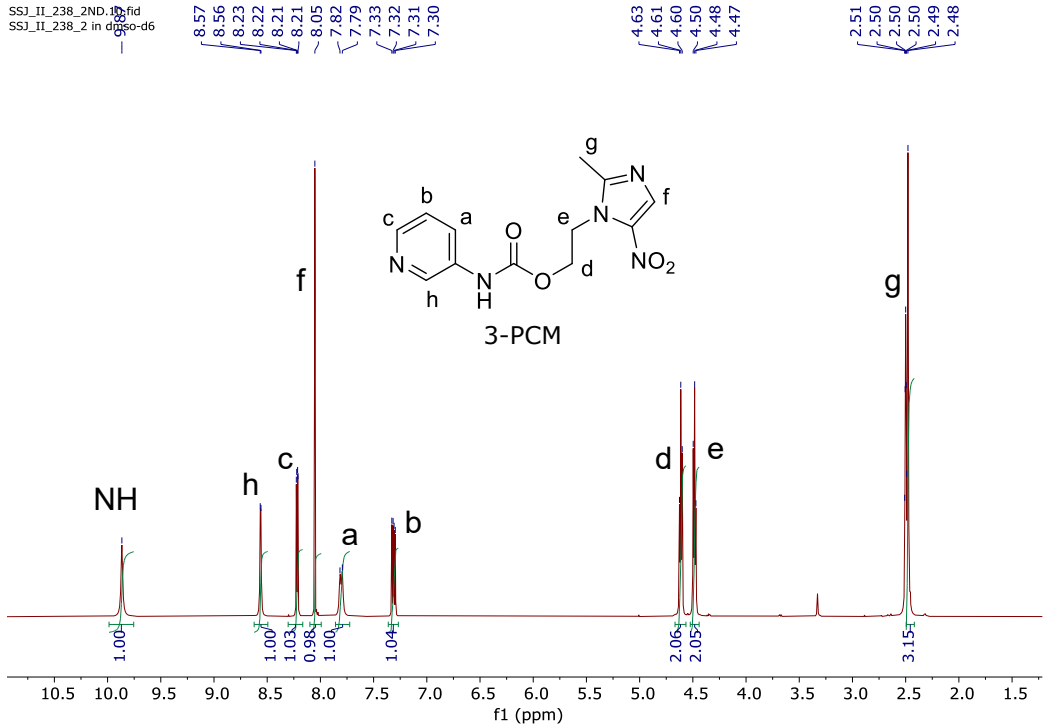


Fig. S1. ^{1}H NMR spectrum of 3-PCM.

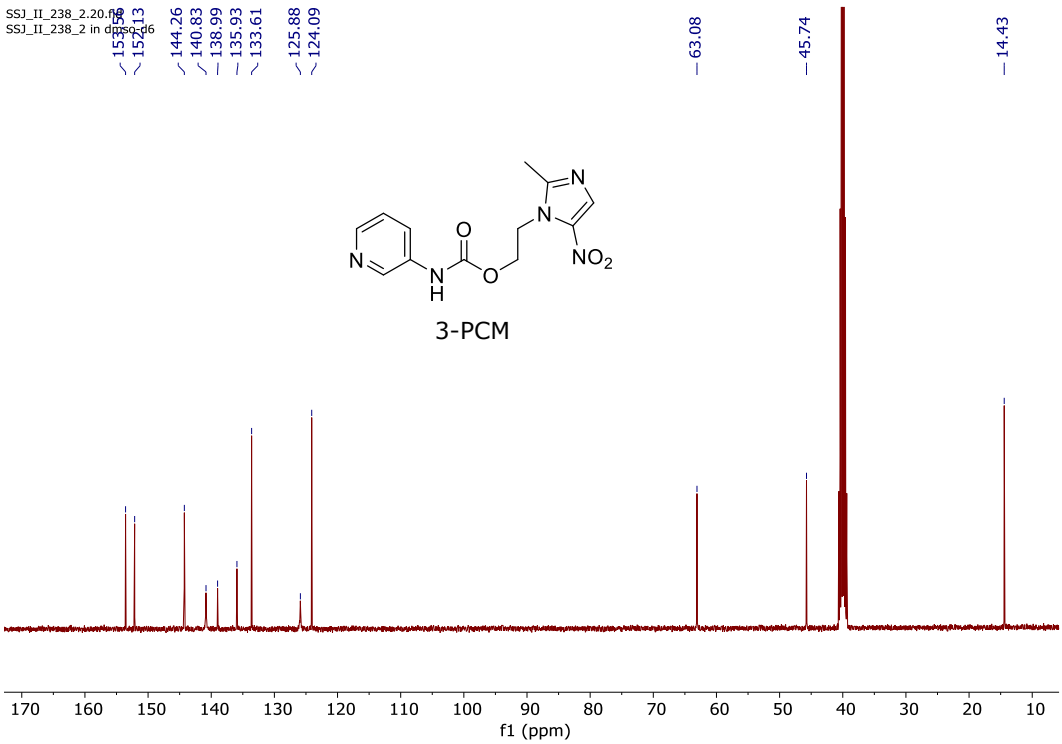


Fig. S2. ^{13}C NMR spectrum of 3-PCM.

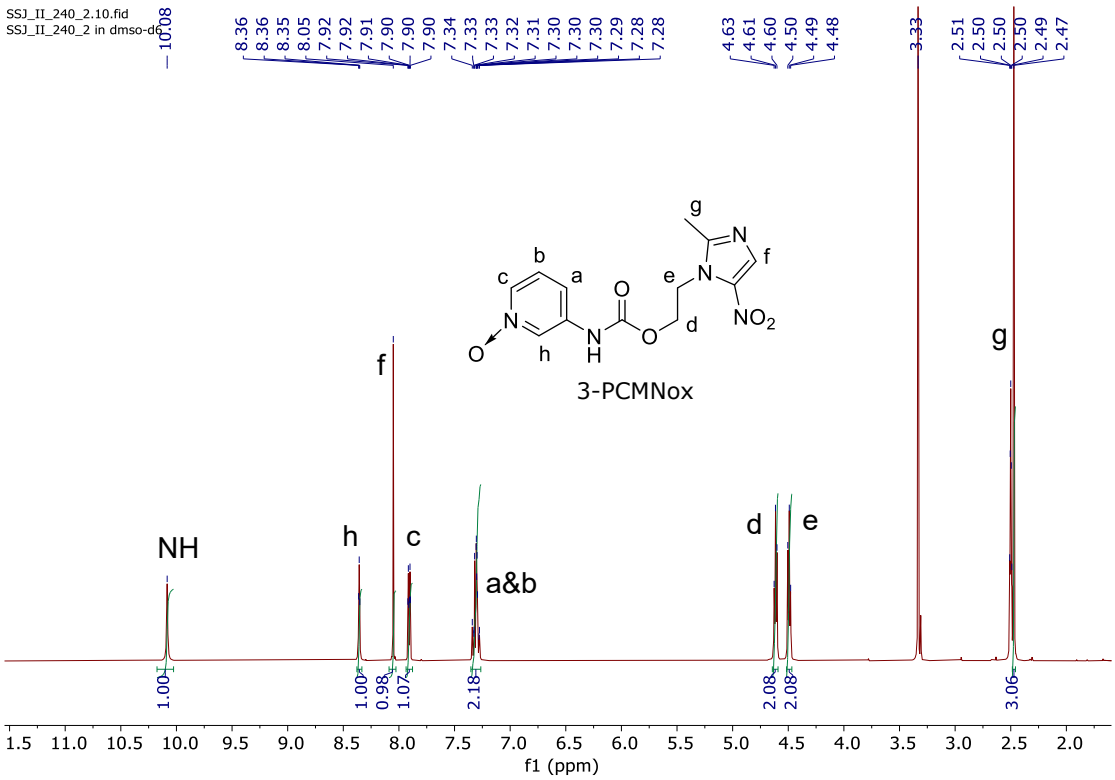


Fig. S3. ^{1}H NMR spectrum of 3-PCMNox.

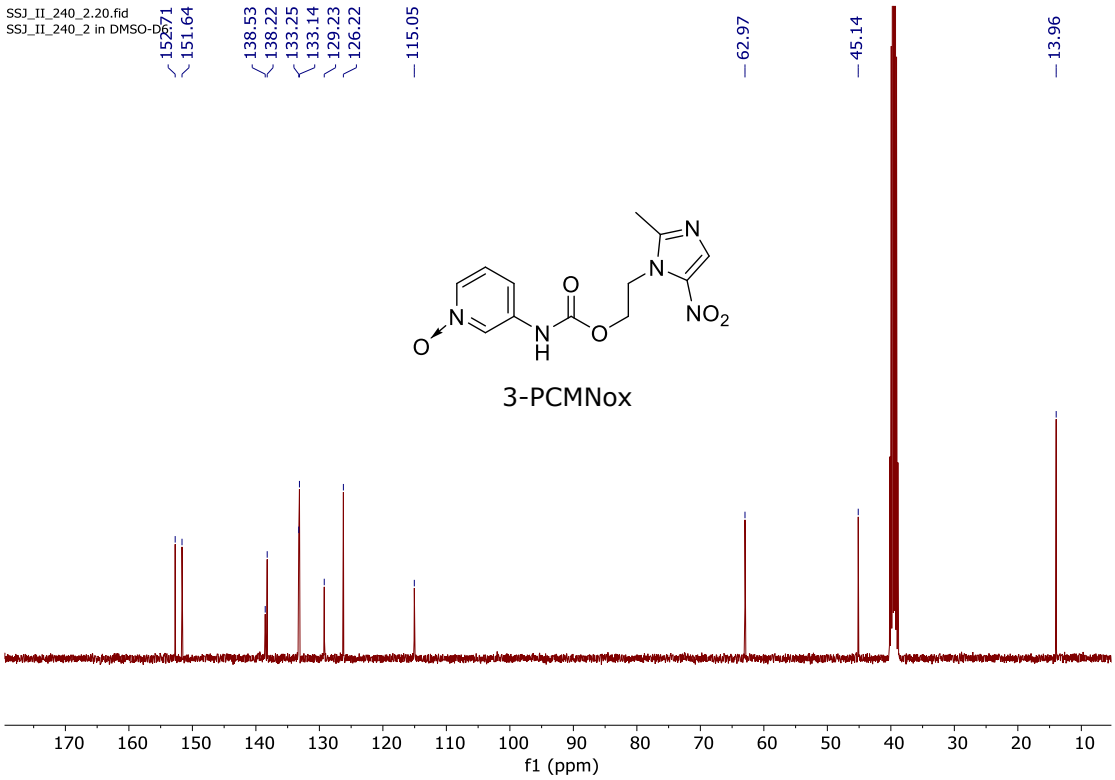


Fig. S4. ^{13}C NMR spectrum of 3-PCMNox.

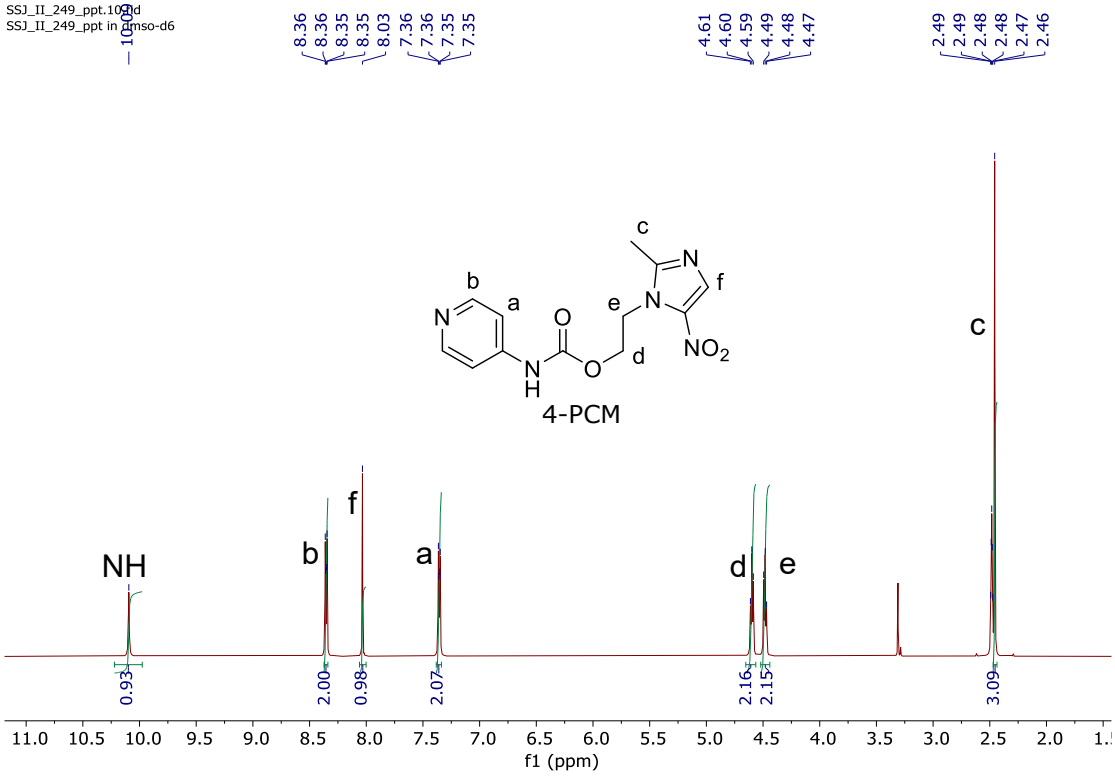


Fig. S5. ^{1}H NMR spectrum of 4-PCM.

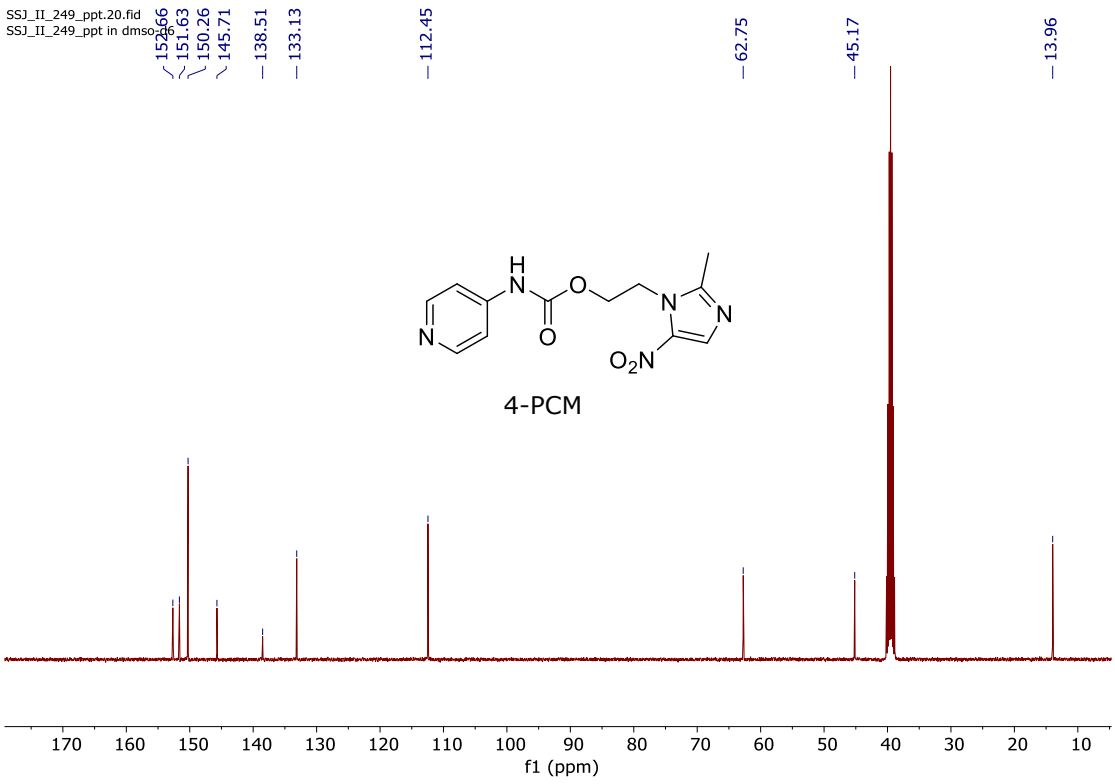


Fig. S6. ^{13}C NMR spectrum of 4-PCM.

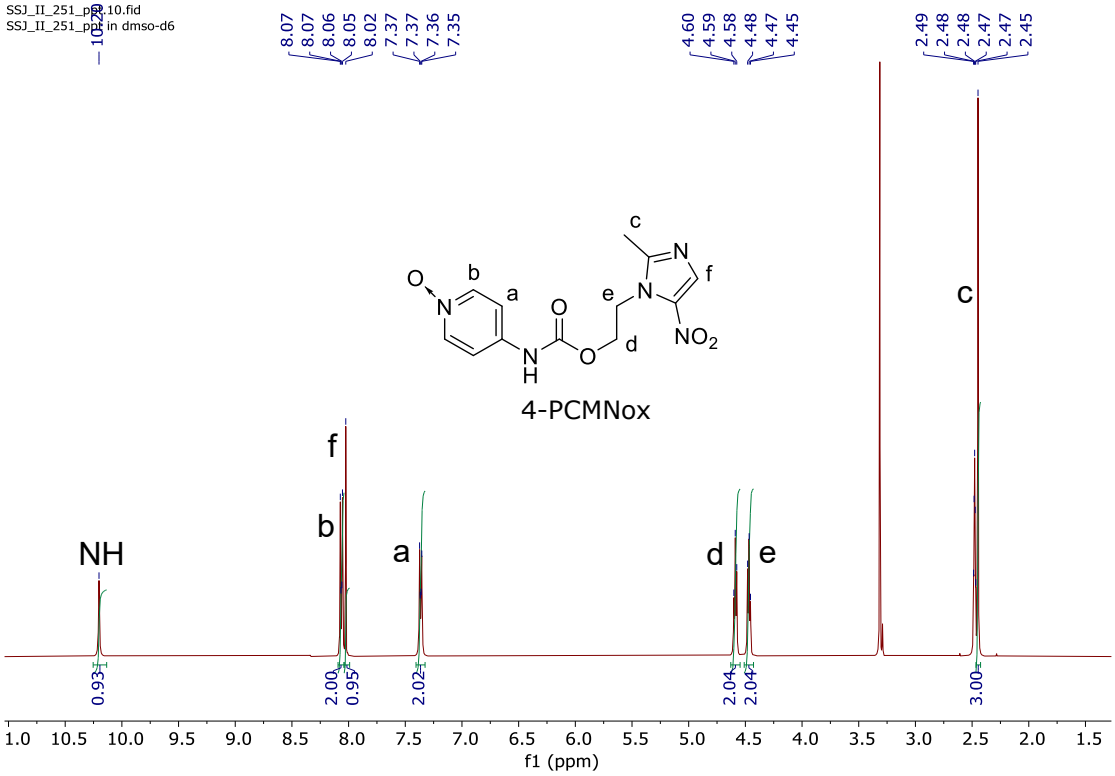


Fig. S7. ^{1}H NMR spectrum of 4-PCMNox.

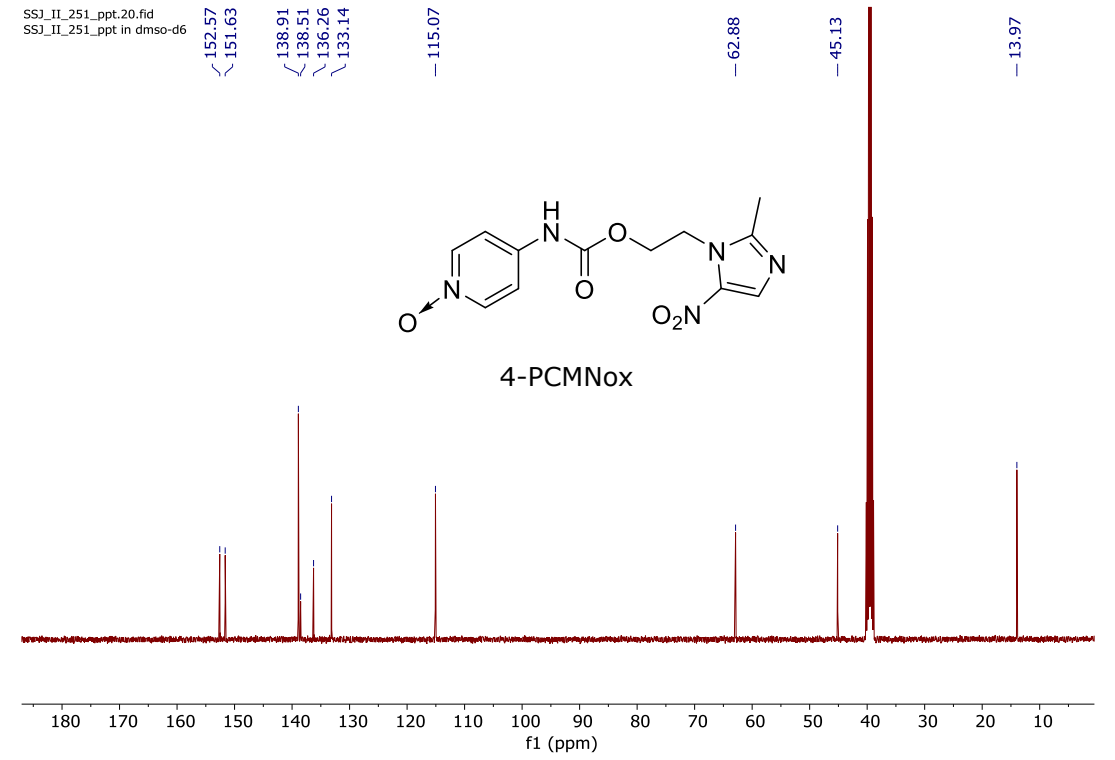


Fig. S8. ^{13}C NMR spectrum of 4-PCMNox.

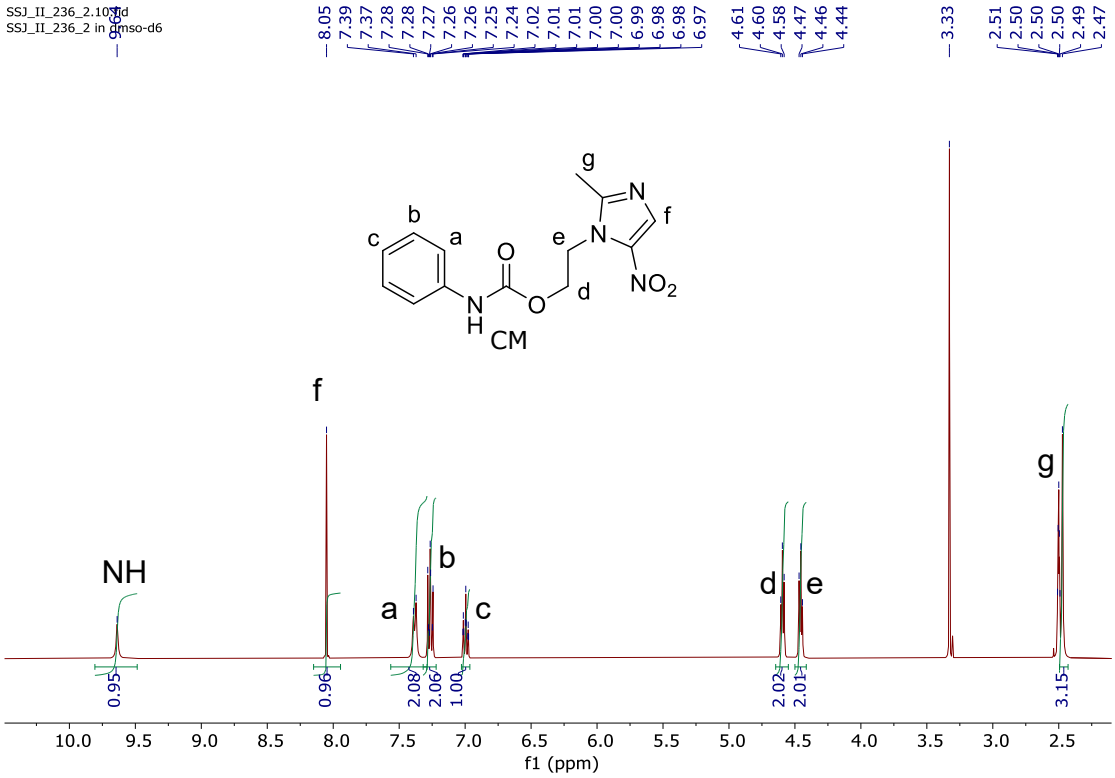


Fig. S9. ^{1}H NMR spectrum of CM.

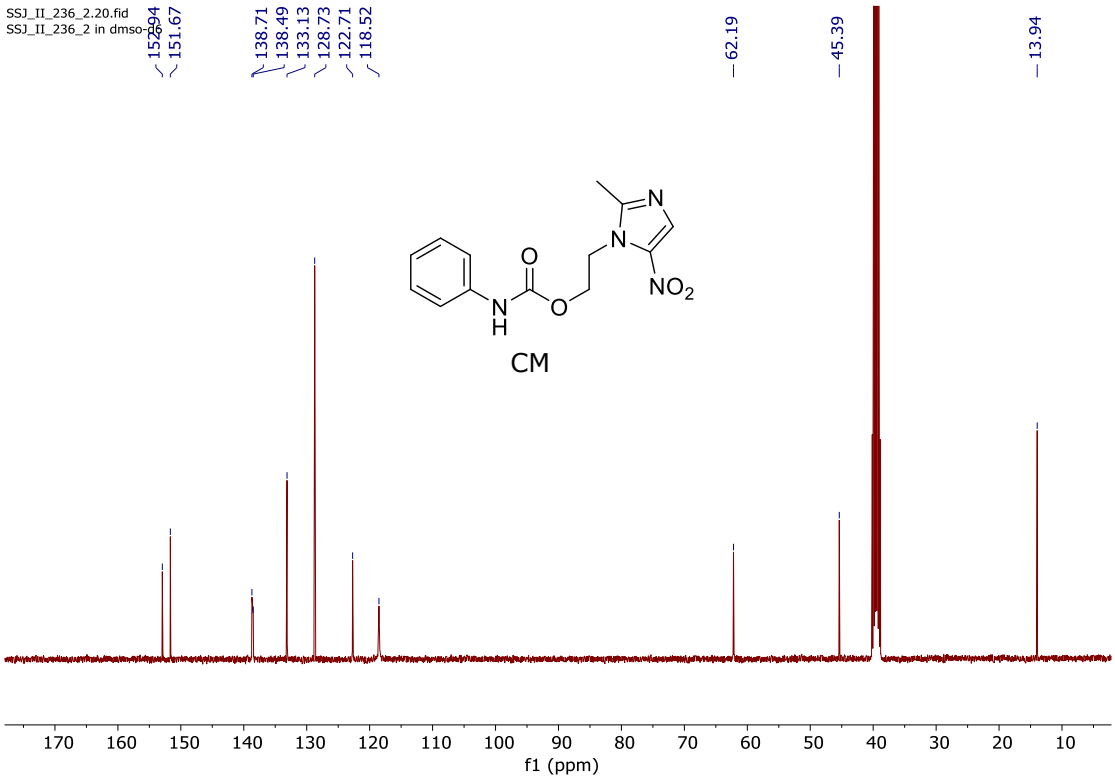


Fig. S10. ^{13}C NMR spectrum of CM.

4. Details of Gelation Experiments

We performed the gelation ability of all the compounds in various solvent/solvent mixtures (Table S1). The gelation test was carried out following a trivial method, where the gelator (10.0 mg) was dispersed in a suitable solvent (1.0 mL) in a sealed vial via sonication, and heated to give a transparent solution. The vial was left undisturbed, and the gel formation was confirmed by a vial inversion test.

Table S1. Gelation Experiments with compounds at 1.0 wt/v%

Sl No.	**Solvent**	CM	3-PCM	4-PCM	3-PCMNox	4-PCMNox
1	*o*-xylene	I	I	I	I	I
2	*m*-xylene	I	I	I	I	I
3	*p*-xylene	I	I	I	I	I
4	toluene	I	I	I	I	I
5	mesitylene	I	I	I	I	I
6	n-butanol	Cry	Cry	Ppt	Ppt	Ppt
7	EtOH	Cry	Cry	Ppt	Ppt	Ppt
8	water	I	I	I	**G**[#]	Cry
9	chlorobenzene	Cry	Ppt	I	I	I
10	Nitrobenzene	S	Cry	Ppt	Ppt	Ppt
11	EG:water	Coll*	**G***	Ppt*	S*	Coll*
12	DMF:water	Coll*	Ppt*	Cry*	Ppt*	Coll*
13	DMSO:water	Cry*	**G***	Ppt*	Ppt*	Coll*
14	EtOH/water	Ppt	Cry	Ppt	Ppt	Ppt

G= gel, [#]= 4.0 wt/v%, *= 5.0 wt/v%, Ppt= precipitate, Coll= Colloid, I= Insoluble, and aqueous mixtures were taken at 1:1, v/v.

5. Minimum gelator concentration (MGC)

The minimum amount of the gelator required to form a self-standing gel is known as the minimum gelator concentration (MGC). The gel was prepared according to the aforementioned procedure by dissolving the compounds in 1.0 mL of solvent. Incremental quantities of the solvent were added, and the gelation procedure was repeated until a minuscule amount of the solvent was detected on the surface of the gel. The concentration slightly above which a stable gel obtained is observed to be the MGC of the gelator.

Table S2. Minimum gelator concentration (MGC) studies

MGC (wt/v%)			
Sl No.	**Solvent**	3-PCM	3-PCMNox
1	water	---	3.8
2	EG:water (1:1, v/v)	3.8	---
3	DMSO:water (1:1, v/v)	4.5	---

6. Thermal stability

The gel-sol transition temperature (T_{gel}) is marked by the conversion of a gel to its solution state, which can be used to calculate the thermal stability of the gel. The required quantity of gelator was placed in a 7.0 mL standard vial, followed by the addition of 1.0 mL of solvent. The

mixture was sonicated and slowly heated until dissolution occurred and then allowed to stand undisturbed. A tiny spherical glass ball was placed delicately on top of the gel after 24.0 hours and the vial was sealed. It was then immersed carefully in an oil bath fitted with a thermosensor and magnetic stirrer. The temperature of the oil bath was increased at a rate of 10.0 °C per minute, consistently. At some point, the glass ball slowly slides down, and the temperature at which the glass ball touched the bottom of the vial was recorded as T_{gel}.

Table S3. Determination of Sol-gel Transition Temperature (T_{gel})

T_{gel} (°C)				
Sl No.	**Solvent**	Wt/v%	3-PCM	3-PCMNox
1	water	4.0	---	67.9
2	EG:water (1:1, v/v)	5.0	86.2	---
3	DMSO:water(1:1, v/v)	5.0	75.1	---

7. Mechanical stability

Understanding the physiological properties of a gel is a key factor in analysing the solid-like characteristics of the gel network.[1] Rheology is used to quantify the deformation and flow characteristics of supramolecular gels, and rheological experiments provide information about the gel structural characteristics.[2] The mechanical strength of the gel was measured using an MCR 302 Anton Paar modular compact rheometer equipped with a 2.5 cm stainless steel parallel plate geometry with a measuring gap of 1.00 mm. Rheological experiments were performed in (1:1, v/v) EG/water and DMSO/water at 5.0 wt/v% with 3-PCM and at 4.0 wt% with the aqueous gel of 3-PCMNox. The experiments were conducted carefully by placing approximately 1.0 mL of gel on the plate. To prevent solvent evaporation and ensure a consistent temperature of 20.0 °C during frequency and amplitude sweeps, a Peltier temperature control hood was employed. Initially, oscillatory rheological measurement (amplitude-sweep tests) was conducted to determine the linear viscoelastic region (LVR) at a constant frequency of 1.0 Hz. The storage modulus (G') dominates over the viscous modulus (G'') within the LVR region, and the elastic component is independent of the applied strain. The 3-PCM based gels made at 5.0 wt/v% in EG/water and DMSO/water (1:1, v/v) showed an LVR of about 0.05% (Fig. S12a), while the 3-PCMNox hydrogel at 4.0 wt% showed an LVR of 0.02% (Fig. 12b). The cross-over point at which the gel completely breaks into solution was observed to be 1.0-10.0% in the above cases. Amplitude sweeps were carried out at a constant frequency of 1.0 Hz and logarithmic ramp strain (γ) from 0.01% to 100%. Frequency sweeps were performed over the range of 0.1-10.0 Hz within the linear viscoelasticity region (at a strain of 0.02%). The results indicated that these materials can be called true gels since the elastic (G') is a magnitude higher than the viscous (G") moduli under varying frequencies.

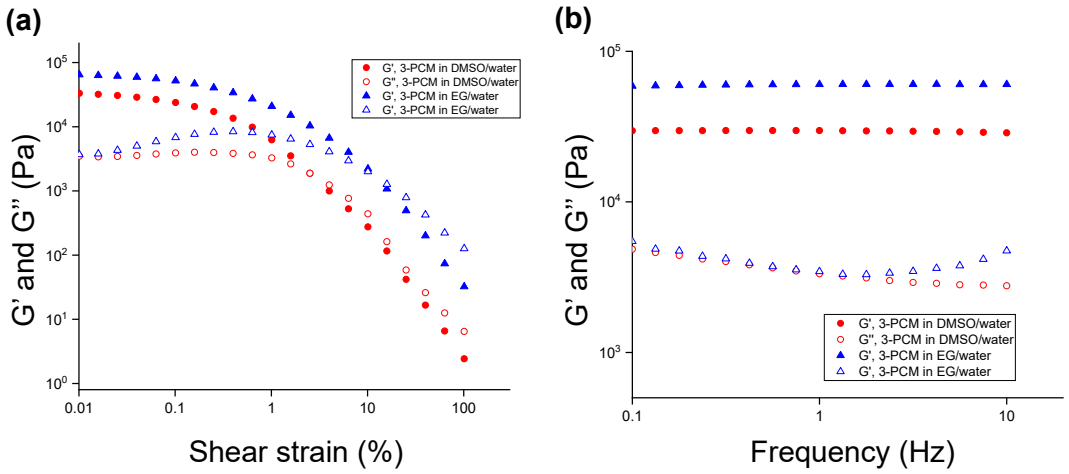


Fig. S11. Rheological experiments with 3-PCM based gels (5.0 wt/v%) in DMSO/water and EG/water 1:1, v/v, at 20.0 °C, (a) amplitude sweep experiments measured at a constant frequency of 1.0 Hz, and (b) frequency sweep experiments measured at a constant strain of 0.02%.

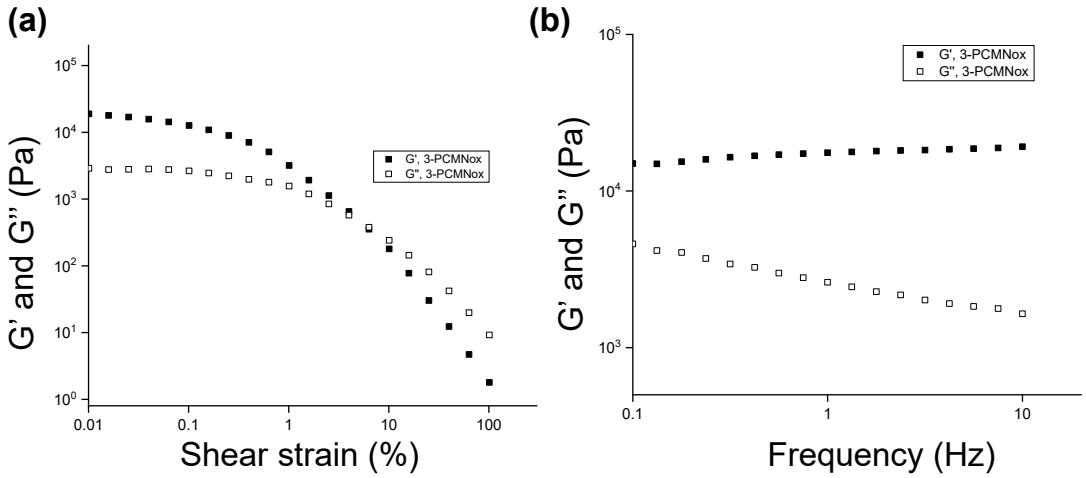


Fig. S12. Rheological experiments with 3-PCMNox based gels (4.0 wt%) in water at 20.0 °C, (a) amplitude sweep experiment measured at a constant frequency of 1.0 Hz, and (b) frequency sweep experiment measured at a constant strain of 0.02%.

8. Scanning electron microscopy (SEM)

The surface morphologies of the xerogels were examined using SEM. Gels of compounds 3-PCM in (1:1, v/v) EG/water and DMSO/water at 5.0 wt/v% and the aqueous gel of 3-PCMNox (4.0 wt%) were prepared, and the gels were filtered after 24.0 h, dried in air to obtain the xerogel. A tiny portion of the xerogel was placed on a pin mount, with the carbon tab positioned on top. The mount was then coated with a layer of gold for a duration of 5-6 minutes to obtain a thickness of gold coating of about 9.0-12.0 nm. Subsequently, the mount was loaded onto a Leo Supra 25 microscope, which was operated at a voltage of 3.0 kilovolts and a working distance of 3.0-4.0 millimeters. The SEM pictures were recorded using an in-lens detector.

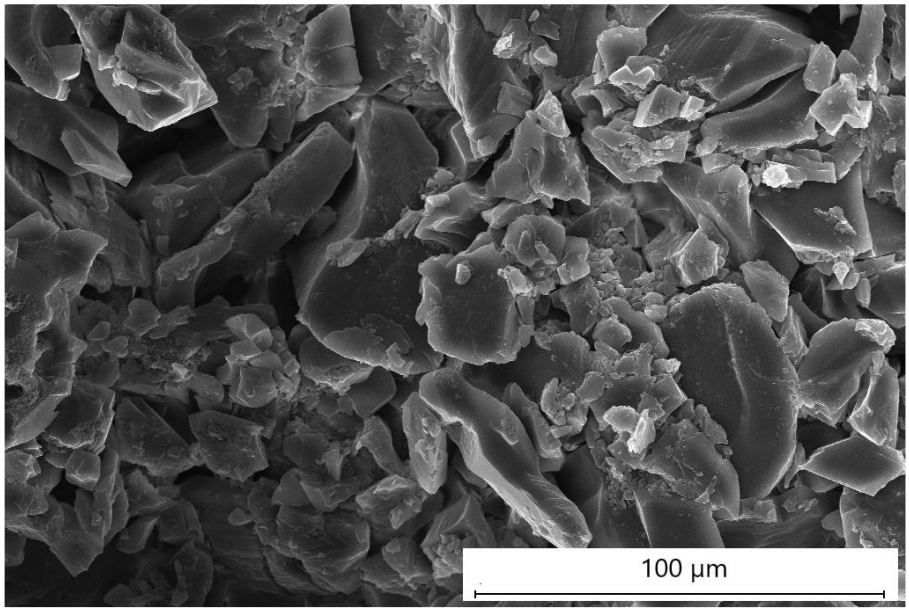


Fig. S13. SEM images of the xerogel of 3-PCM (5.0 wt/v%) from EG/water (1:1, v/v).

9. Single crystal X-ray Diffraction

Single crystals of the compound CM were obtained by the slow evaporation of 20.0 mg/mL of CM in ethyl acetate, resulting in colourless rhombus-shaped crystals. The 3-PCM crystal was obtained by the slow evaporation of 20.0 mg/mL of 3-PCM in ethanol, resulting in colourless block-shaped crystals and the crystals of 3-PCMNox obtained from water also displayed block-shaped morphology. Similarly, block-shaped X-ray quality crystals of 4-PCM were obtained by the slow evaporation of 20.0 mg of the corresponding compound in 1.0 mL DMF. X-ray analysis was conducted on a Bruker D8 Venture (Photon100 CMOS detector) diffractometer, and the crystal data were collected at room temperature using MoKα radiation (λ = 0.71073 Å). A 2015 apex-III software suite (Bruker AXS: Madison, WI) is used for unit cell determination, data collection, data reduction, structure solution/refinement, and empirical absorption correction. The crystal structures were solved using the direct method and refined by the full-matrix least-squares on F^2 for all data using SHELXTL. All non-disordered non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and refined using a riding model. The solvent molecule in 4-PCM was found to be disordered in a special position, and we were unable to assign this to a proper model. The structure was refined by excluding the electron densities from these disordered solvent molecules using PLATON/SQUEEZE,[3] that corresponds to 18 electrons per unit cell, which can be assigned to half a DMF molecule. Crystallographic data for the structures are deposited to Cambridge Crystallographic Data Centre as supplementary publication and the CCDC numbers are 2414629 (4-PCM), 2414632 (3-PCM), 2414634 (3-PCMNox), and 2414636 (CM).

Table S4: Crystal data

Crystal data	CM	3-PCM	4-PCM	3-PCMNox
CCDC Number	2414636	2414632	2414629	2414634
Empirical formula	$C_{13}H_{14}N_4O_4$	$C_{12}H_{13}N_5O_4$	$C_{12}H_{13}N_5O_4$	$C_{12}H_{15}N_5O_6$
Color	Colorless	Colorless	Colorless	Colorless
Formula weight	290.28	291.27	291.27	325.29
Crystal size (mm)	0.250 x 0.200 x 0.080	0.190 x 0.130 x 0.070	0.230 x 0.170 x 0.040	0.190 x 0.060 x 0.040
Crystal system	Monoclinic	Triclinic	Triclinic	Triclinic
Space group	$P2_1/n$	P-1	P-1	P-1
a (Å)	10.3896(8)	7.5761(5)	9.1319(7)	6.8390(5)
b (Å)	10.9685(8)	8.6966(6)	9.4856(7)	9.0647(6)
c (Å)	11.9682(9)	10.3852(7)	9.8024(6)	12.4965(9)
α (°)	90	91.211(2)	102.462(2)	103.598(2)
β (°)	94.640(3)	103.529(2)	98.962(2)	102.087(2)
γ (°)	90	91.437(2)	106.185(2)	104.118(2)
Volume (Å^3)	1359.41(18)	664.79(8)	774.84(10)	700.39(9)
Z	4	2	2	2
$D_{calc.}$(g/cm^3)	1.418	1.455	1.248	1.542
F(000)	608	304	304	340
μ (mm^{-1}) MoK_α	0.108	0.113	0.097	0.126
Temperature (K)	305(2)	305(2)	305(2)	302(2)
Reflections collected/ unique/observed [I>2σ(I)]	49154/6634/ 4068	36735/4626/ 3367	30396/3865 / 3107	27092/3085/ 2371
Data/restraints/parameters	6634/0/195	4626/0/195	3865/0/191	3085/0/221
Goodness of fit on F^2	1.015	1.044	1.196	1.034
Final R indices [I>2σ(I)]	R_1 = 0.0555 wR_2 = 0.1475	R_1 = 0.0487 wR_2 = 0.1325	R_1 = 0.0634 wR_2 = 0.2038	R_1 = 0.0402 wR_2 = 0.0918
R indices (all data)	R_1 = 0.0960 wR_2 = 0.1721	R_1 = 0.0753 wR_2 = 0.1468	R_1 = 0.0775 wR_2 = 0.2119	R_1 = 0.0597 wR_2 = 0.1008

Table S5: Hydrogen bonding parameters

Compound CM						
No.	Donor–H···Acceptor	D–H/Å	H···A/Å	D···A/Å	∠D–H···A/°	Symmetry operation
1	N(7A)–H(7A)···N(19A)	0.918(18)	2.137(17)	3.0133(14)	161.1(15)	1-x,-y,1-z
2	C(12A)–H(12B)···O(9A)	0.97	2.50	3.1265(15)	122	1/2-x,-1/2+y,1/2-z
3	C(21A)–H(21A)···O(16A)	0.96	2.40	3.2278 (19)	144	1/2-x,-1/2+y,1/2-z
Compound 3-PCM						
No.	Donor–H···Acceptor	D–H/Å	H···A/Å	D···A/Å	∠D–H···A/°	Symmetry operation
1	N(7C)–H(2)···N(16C)	0.896(17)	2.146(17)	3.0296(15)	168.9(15)	-x,-y,1-z
Compound 3-PCMNox						
No.	Donor–H···Acceptor	D–H/Å	H···A/Å	D···A/Å	∠D–H···A/°	Symmetry operation
1	N(8C)–H(2A)···N(17C)	0.859(19)	2.184(18)	3.0287(19)	167.8(19)	x,2-y,1-z
2	O(23C)–H(1)···O(10C)	0.90(3)	2.01(3)	2.899(2)	171(2)	1-x,1-y,-z
3	O(23C)–H(2)···O(4C)	0.89(3)	1.84(3)	2.729(2)	173(3)	x,y,z
4	C(7C)–H(7C)···O(21C)	0.93	2.50	3.079(2)	120	1+x,1+y,z
5	C(12C)–H(12B)···O(4C)	0.97	2.45	3.349(2)	154	x,1-y,-z
6	C(18C)–H(18C)···O(23C)	0.93	2.35	3.221(2)	155	1+x,y,1+z
Compound 4-PCM						
1	N(7)–H(7)···N(16)	0.86	2.08	2.931(3)	171	1-x,-y,1-z
2	C(17)–H(17)···O(9)	0.93	2.53	3.385(3)	153	x,y,1+z
3	C(18)–H(18)···O(1)	0.95	2.55	3.281(3)	134	1/2-x,2-y,1/2+z
4	C(27)–H(27A)···O(11)	0.98	2.45	3.389(3)	161	1-x,-1/2+y,1/2-z

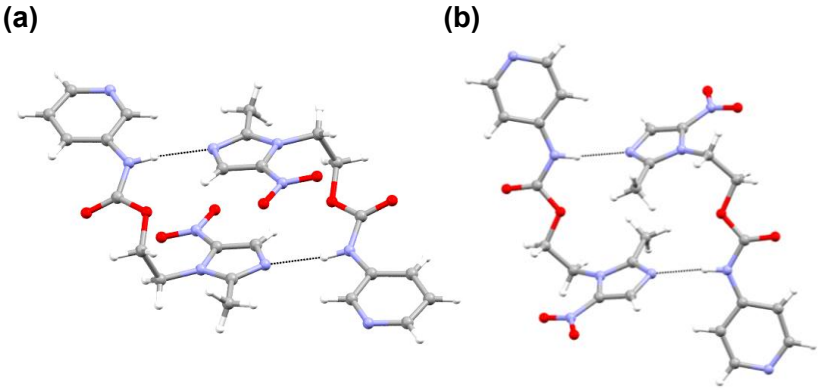


Fig. S14. Hydrogen bonded dimers of (a) 3-PCM, and (b) 4-PCM.

10. Powder X-ray Diffraction

The crystals of the compounds were ground to fine powder and about 20.0 mg of the sample was used to record PXRD. We have also recorded the PXRD of the xerogels, and the samples were prepared using SEM sample preparation protocol. The PXRD experiments were recorded on a PANalytical instrument with 2θ ranging from 4.0−60.0° with a step size of 0.026.

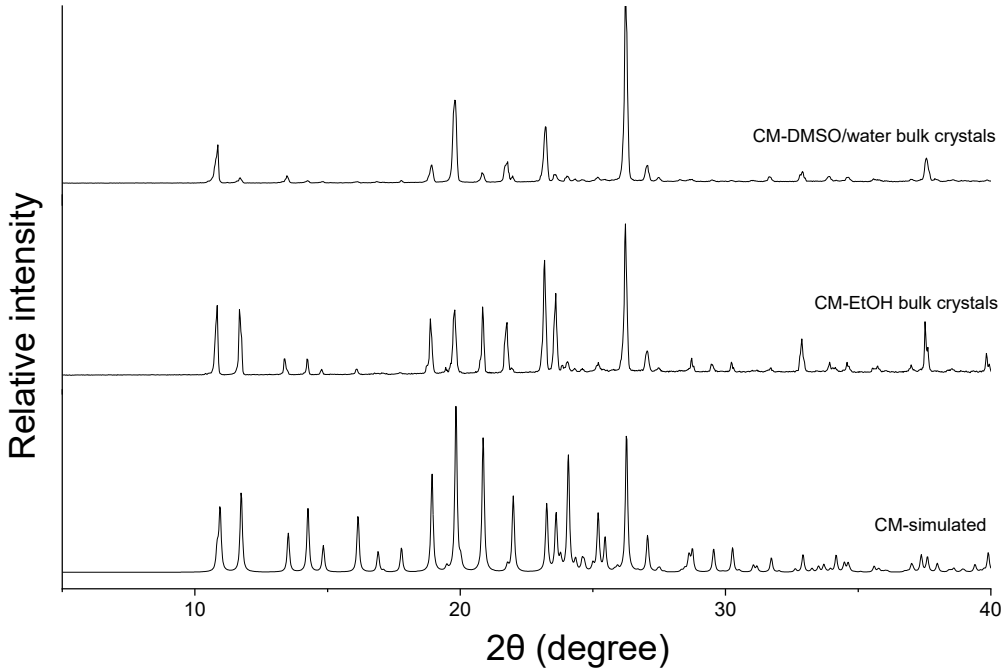


Fig. S15. Comparison of PXRD pattern of the crystals of CM obtained from ethanol and DMSO/water (1:1, v/v) at 2.0 wt/v% with that of the simulated pattern obtained from the single crystal X-ray structure of the CM.

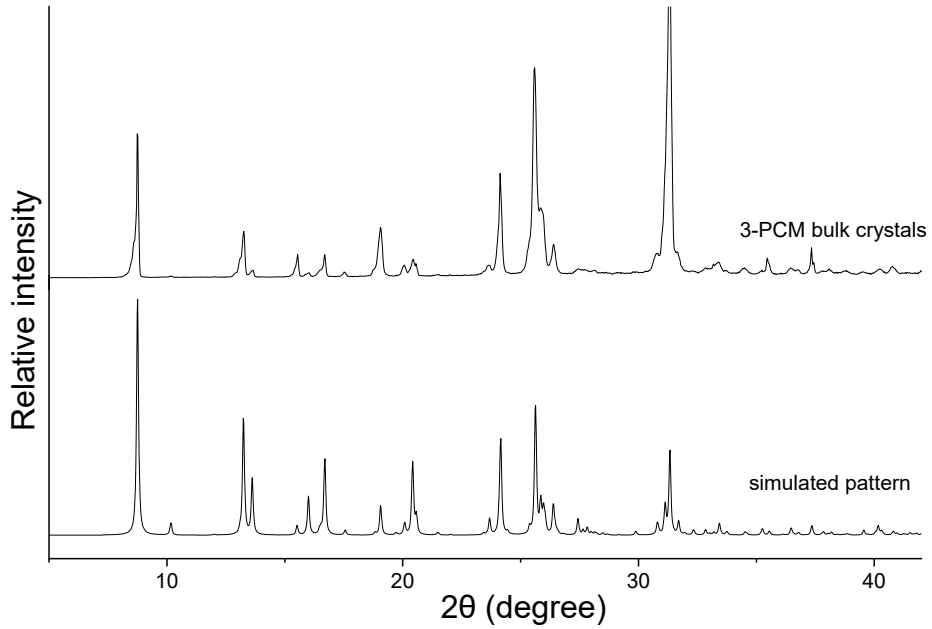


Fig. S16. Comparison of PXRD pattern; simulated pattern of 3-PCM, and the bulk crystals of 3-PCM (2.0 wt/v%) obtained from ethanol.

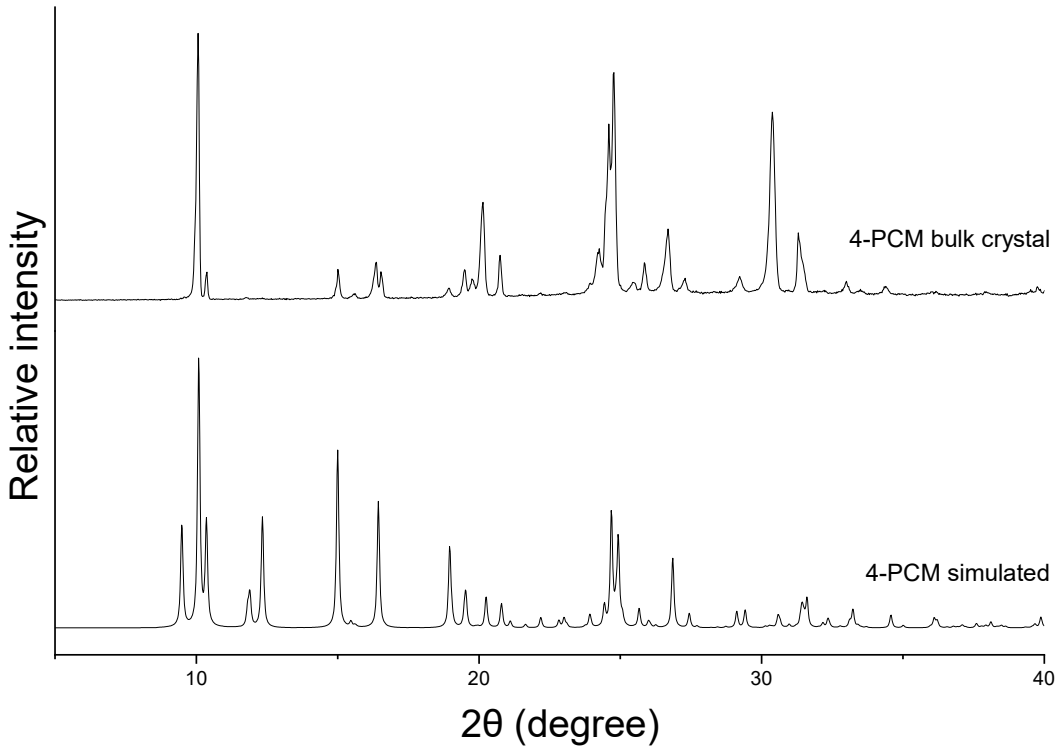


Fig. S17. Comparison of PXRD pattern; simulated pattern of 4-PCM and bulk crystals of 4-PCM (2.0 wt/v%) obtained from DMF.

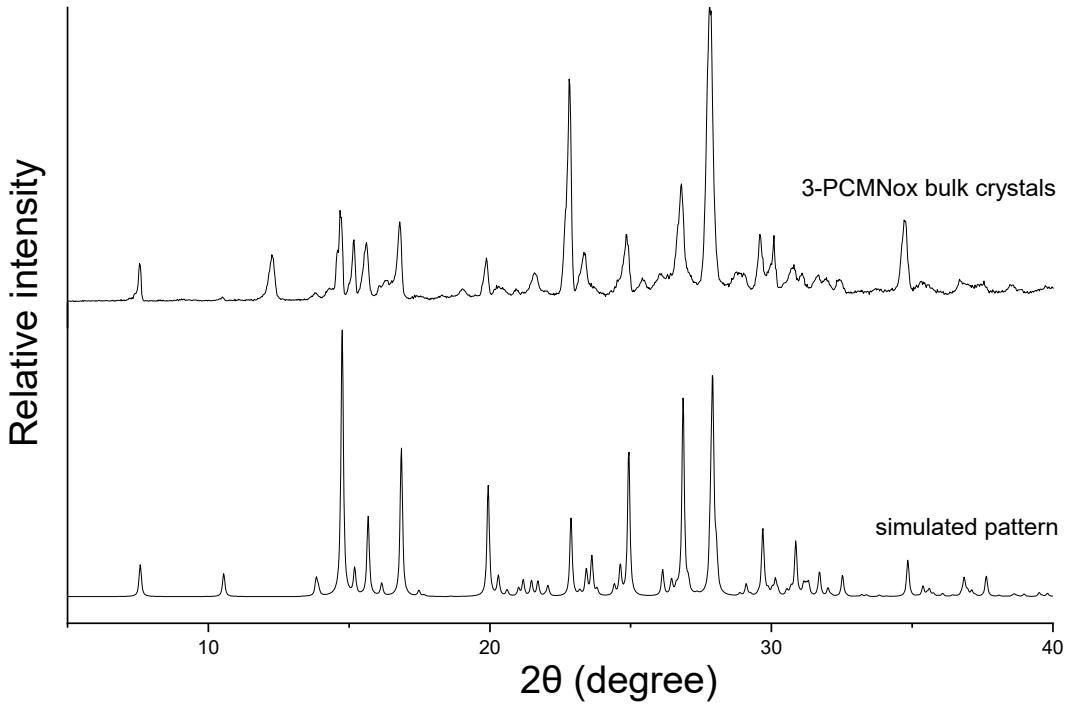


Fig. S18. Comparison of PXRD pattern: simulated pattern of 3-PCMNox and bulk crystals obtained via crystallizing in water (20.0 mg/mL).

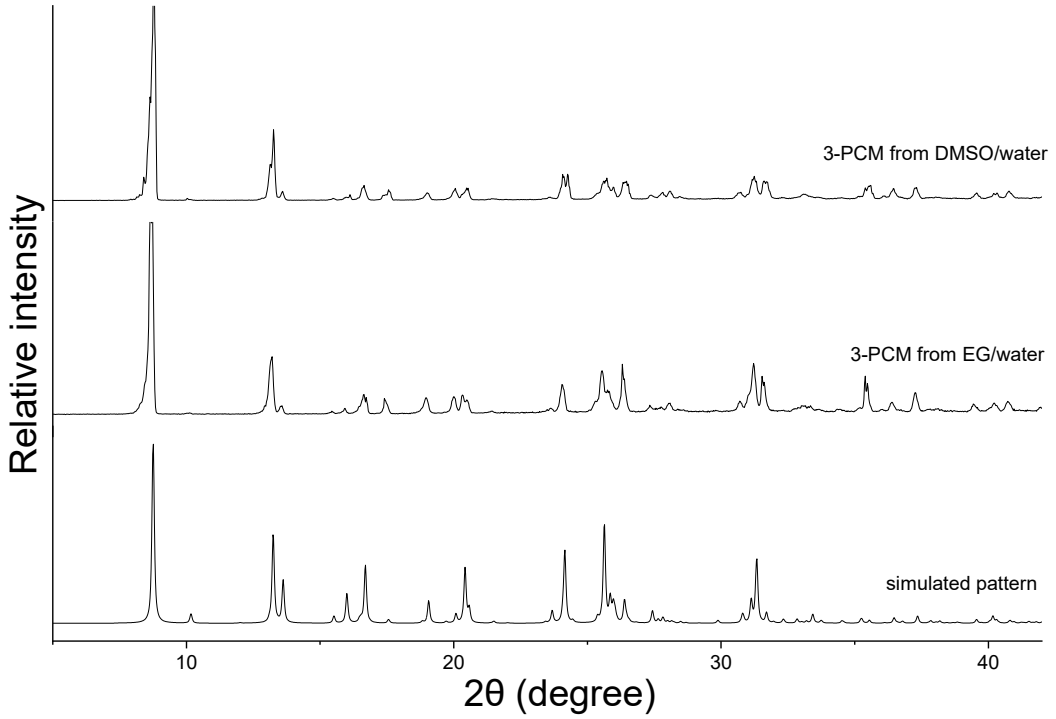


Fig. S19. Comparison of PXRD pattern; simulated pattern of 3-PCM, and the xerogel of 3-PCM obtained from EG/water and DMSO/water (1:1, v/v) at 5.0 wt/v%.

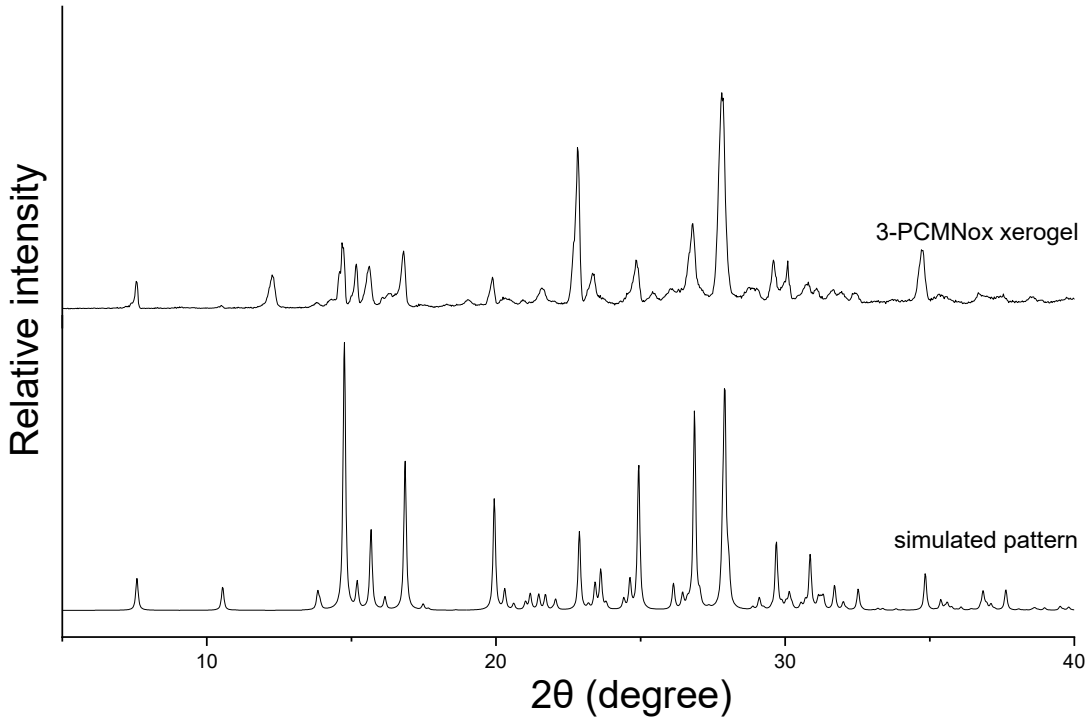


Fig. S20. Comparison of PXRD pattern: simulated pattern of 3-PCMNox and xerogels obtained from the hydrogel prepared at 4.0 wt/v%.

11. Antibacterial activity

We have examined the antibacterial efficacy of the compounds against both anaerobic and aerobic microbes.

11.1. Anaerobic antibacterial activity

The following materials were used to perform the agar-well diffusion experiments.

a. Anaerobic Blood Agar Plate: Ready-to-use sterile anaerobic blood agar plates were used for the cultivation of anaerobic microorganisms (Himedia).

b. Fluid Thioglycollate Medium (1L): One liter of thioglycollate medium was prepared by dissolving 29.75 g of commercially available Thioglycollate (HiMedia) in 1.0 L distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15.0 lbs pressure (121.0 °C) for 15 minutes.

c. Anaerogas pack 3.5L (Himedia)

d. Penicillin (standard antibacterial agent, concentration: 10.0 mg/mL)

e. Culture of test organisms; growth of culture was adjusted according to McFarland Standard, 0.5%, *Porphyromonas gingivalis* (ATCC 33277)

Sterile anaerobic blood agar plates were seeded with a bacterial culture of *P. gingivalis* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10.0 mm were bored using a well cutter, and samples (1.0 mg dissolved in 1.0 mL of double autoclaved distilled water) at different concentrations, such as 25.0 µL, 50.0 µL and 100.0 µL, were added. We also have hydrogel of 3-PCMNox (5.0 wt%) and 3-PCM which gelled in DMSO/water (1:1, v/v) at 5.0 wt/v%. Well of approximately 10.0 mm was bored using a well cutter and a scoopful of each gel sample was carefully dispensed into the respective wells. The plates were then incubated in an anaerobic chamber with an anaerogas pack for 48.0 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.[4] Penicillin was used as a positive control.

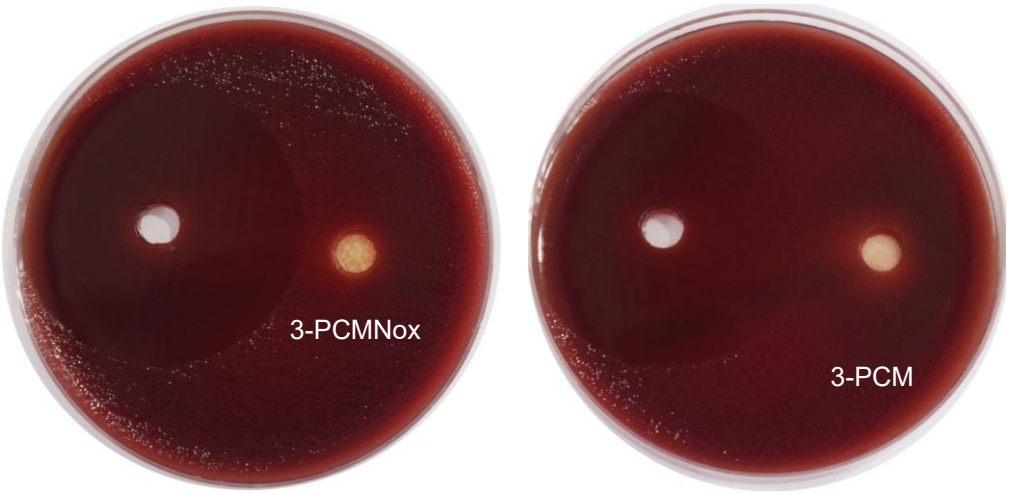


Fig. S21. Images of plates showing anaerobic antibacterial studies with gels against *P. gingivalis*.

Table S6: Activity of the Compounds against *P. gingivalis*

Sample Code	Concentration(µg)	Zone of inhibition(mm)
3-PCM	Penicillin (100 µg)	31
	25.0 µL	Nil
	50.0 µL	Nil
	100.0 µL	Nil
4-PCM	Penicillin (100 µg)	32
	25.0 µL	24
	50.0 µL	27
	100.0 µL	29
3-PCMNox	Penicillin (100 µg)	30
	25.0 µL	11
	50.0 µL	12
	100.0 µL	16
4-PCMNox	Penicillin (100 µg)	30
	25.0 µL	Nil
	50.0 µL	11
	100.0 µL	15

Table S7: Activity of 3-PCMNox hydrogel (5.0 wt%) and 3-PCM gel (DMSO/water, 1:1 v/v) against *P. gingivalis*

Sample code	Concentration	Zone of inhibition(mm)
3-PCMNox	Penicillin (100 µg)	49
	G1	Nil
3-PCM	Penicillin (100 µg)	48
	G2	41

11.2. Aerobic antibacterial activity

The Luria Bertani (LB) broth was used for inoculating the bacteria (either *S. aureus* and *E. coli*) and the inoculated cultures were incubated overnight at 37.0 °C. After the overnight incubation, the turbid grown culture was set to 0.5 (1.5 × 108 CFU/mL) as per McFarland standard. This was used for all the experiments. The aerobic antibacterial studies were assessed against one gram-positive (*S. aureus*) and one gram-negative (*E. coli*) bacterium. The LB inoculated bacterial cultures of 100 µL were spread on LB agar plates and wells of 8.0 mm diameter were punched and samples (1.0 mg dissolved in 1.0 mL of 0.1 M acetic acid) were added to the wells. The amikacin drug was kept as positive control, and 0.1 M acetic acid was kept as a negative control. The plates were then incubated at 37.0 °C in upright position for 24.0 h. After incubation, the zone of inhibition was observed.[5]

12. Biocompatible studies: *In vitro* cell viability

The *in vitro* cell viability was assessed using the Alamar blue assay with Mouse Fibroblast L929 cells. The cell viability of control cells alone (CA) and 3-PCMNox, 4-PCMNox, 3-PCM, and 4-PCM were evaluated using the Alamar blue assay, where resazurin (violet) gets converted to resorufin (pink) in the presence of live cells. The L929 cells were seeded at a density of 2.5 $x10^4$ cells/cm^2 in a 12-well plate and incubated at 37.0 °C and 5.0% CO_2 to facilitate their attachment to the well plate. After 24.0 hours, 1.0 mg/mL concentration of the samples was added to the seeded cells and incubated for 24.0 and 48.0 hours. The samples were added along with DMEM cell culture media containing 1.0% antibiotic and anti-mycotic solution and 10.0% fetal bovine serum. Cells alone without any samples were considered as a positive control. After each time interval (24.0 and 48.0 h), the cells were washed with PBS and 10.0% Alamar was added with the basal DMEM media and incubated for 4.0 h. After incubation, the samples were transferred to 96 well plate and its optical density (OD) was analyzed at 570 and 600 nm using a Biotek Synergy H1 microplate reader. The cell viability[6] was then determined using the equation:

$$\text{Cell viability (\%)} = \frac{OD_{(sample)}}{OD_{(positive\ control)}} \times 100$$

13. Stimuli-responsive properties

About 10.0 mg compounds (CM, 3-PCM, 4-PCM, 3-PCMNox and 4-PCMNox) were taken in a 7.0 mL standard vial and added 1.0 equivalent of the corresponding salts and 1.0 mL of deionized water. The mixture was sonicated and heated to dissolve and left undisturbed for 24.0 hours. The compound CM was insoluble in water at 1.0 wt%.

Table S8: Effect of salts (1.0 equivalents) on 3-PCM and 4-PCM in 1.0 mL of water

Metal salts	3/4-PCM (wt%)	Initial	Final	3/4-PCM (wt%)	Initial	Final
NaF	1.0	S	Ppt	5.0	I	Ppt
NaCl	1.0	S	Ppt	5.0	I	Ppt
NaBr	1.0	S	Ppt	5.0	I	Ppt
NaI	1.0	S	Ppt	5.0	I	Ppt
NaCN	1.0	S	Yellow solution and ppt	5.0	Partially soluble	Dark yellow solution with ppt
KF	1.0	S	Ppt	5.0	I	Ppt
KCl	1.0	S	Ppt	5.0	I	Ppt
KBr	1.0	S	Ppt	5.0	I	Ppt
KI	1.0	S	Ppt	5.0	I	Ppt
KCN	1.0	S	Yellow solution and ppt	5.0	Partially soluble	Dark yellow solution with ppt
$MgCl_2$	1.0	S	Ppt	5.0	I	Ppt
$CaCl_2$	1.0	S	Ppt	5.0	I	Ppt

I-insoluble, S-solution, Ppt-precipitate

The results indicated that the 3-PCM and 4-PCM compounds were insoluble in water at higher concentration (5.0 wt%) in the presence of salts.

Table S9: Effect of salts (1.0 equivalents) on 3-PCMNox in 1.0 mL of water

Metal salts	3-PCMNox (wt%)	Initial	Final	3-PCMNox (wt%)	Initial	Final
NaF	1.0	S	Ppt	5.0	S	Gel
NaCl	1.0	S	Ppt	5.0	S	Gel
NaBr	1.0	S	Ppt	5.0	S	Gel
NaI	1.0	S	Ppt	5.0	S	Gel
NaCN	1.0	S	Yellow solution and ppt	5.0	S	Dark orange solution
KF	1.0	S	Ppt	5.0	S	Gel
KCl	1.0	S	Ppt	5.0	S	Gel
KBr	1.0	S	Ppt	5.0	S	Gel
KI	1.0	S	Ppt	5.0	S	Gel
KCN	1.0	S	Yellow solution and ppt	5.0	S	Dark orange solution
$MgCl_2$	1.0	S	Ppt	5.0	S	Gel
$CaCl_2$	1.0	S	Ppt	5.0	S	Gel

S-solution, Ppt-precipitate

Table S10: Effect of salts (1.0 equivalents) on 4-PCMNox in 1.0 mL of water

Metal salts	3-PCMNox (wt%)	Initial	Final	3-PCMNox (wt%)	Initial	Final
NaF	1.0	S	Ppt	5.0	S	Ppt
NaCl	1.0	S	Ppt	5.0	S	Ppt
NaBr	1.0	S	Ppt	5.0	S	Ppt
NaI	1.0	S	Ppt	5.0	S	Ppt
NaCN	1.0	S	Yellow solution and ppt	5.0	S	Dark orange solution
KF	1.0	S	Ppt	5.0	S	Ppt
KCl	1.0	S	Ppt	5.0	S	Ppt
KBr	1.0	S	Ppt	5.0	S	Ppt
KI	1.0	S	Ppt	5.0	S	Ppt
KCN	1.0	S	Yellow solution and ppt	5.0	S	Dark orange solution
$MgCl_2$	1.0	S	Ppt	5.0	S	Ppt
$CaCl_2$	1.0	S	Ppt	5.0	S	Ppt

S-solution, Ppt-precipitate

14. References

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