Electronic supplementary materials *Mendeleev Commun.*, 2020, **30**, 642–644

Novel zinc(II)/chitosan-based composite: ultrasound-assisted synthesis, catalytic and antibacterial activity

Andreii S. Kritchenkov, Alexander I. Kurachenkov, Anton R. Egorov, Niyaz Z. Yagafarov, Elena A. Fortalnova, Nikolai N. Lobanov, Artem P. Dysin, Anna S. Khomik and Victor N. Khrustalev

In this study, we used crab shell chitosan (Bioprogress, Russia) with a viscosity-average molecular weight (MW) of 3.0×10^4 and a degree of acetylation of 25% (determined from ^{1}H NMR and elemental analysis). Pyridoxal, MeI, $NaBH_4$, $ZnCl_2\times6H_2O$, $Zn(OH)_2$, ZnO were puchsed from Aldrich. Other chemicals, solvents and materials were obtained from commercial sources and were used as received.

The viscosity of the chitosan solutions in 0.3 M of NaCl/2% AcOH was measured at 20 °C in an viscometer. The intrinsic viscosity of the samples was calculated by extrapolation of the dependence $\ln(\eta_r)\times C^{-1}$ to an infinite dilution using the least squares method.

The viscosity-average molecular weight of chitosan sample was calculated using the Mark–Kuhn–Houwink–Sakurada equation: $[\eta] = K\times M_W^{\alpha}$, where $[\eta]$ is the intrinsic viscosity and K and α are empirical constants.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II spectrometer (Germany) at operating frequencies of 400 MHz and 100 MHz, respectively.

X-Ray diffraction analysis was performed by Polycrystalline X-ray diffractometer Dron-7.

Thermogravimetric analysis of the films was performed on a TA Instruments TGA Q500 using a heating rate of 10 °C/min in the temperature range from 30 °C to 600 °C.

SEM images were obtained by electron microscope JEOL JSM - 6490LV at 15kV, SEM detector, electron beam size 30, in high vacuum. The test samples were coated with 20nm (40sec at 40mA) with a platinum layer in a JEOL auto fine coater JFC - 1600.

IR spectra were measured in KBr pellets on a Shimadzu IRPrestige-21 spectrometer (Japan).

Ultrasonic treatments were carried out in an ultrasonic bath (USB300X, ITA) which could work at frequencies of 45 kHz, 80 kHz, 100 kHz and 300 kHz with a variable power output from 120 to 300 W.

Antibacterial activities were investigated as described elsewhere [S1].

Toxicity was studied as described elsewhere [S2].

Preparation of N-methyl pyridoxal iodide (**2**) was performed as described [S3].

*Preparation of **4**.* Chitosan (0.1 g) was dissolved in 0.1 M HCl (10 ml), then the aldehyde (0.7 equiv., ultrasonic conditions or 1.8 equiv., common conditions) was added, and the mixture was treated by ultrasound at 80 kHz 250 W for 10 min (ultrasonic conditions) or it was stirred at 25 °C for 3 hours (common conditions). After this, $NaBH_4$ (1.5 or 5 equiv.) was added, and the

mixture was stirred at 25 °C for 5 hours. The resulting polymer **4** was precipitated with acetone (30 ml), dissolved in distilled water, dialyzed against 3% NaCl solution and then against distilled water and, finally, freeze-dried. Degree of substitution (DS) was calculated according to the formula DS = I(H***1'***), while I(H***12***) = 0.75.

*Preparation of Zn@**1** or Zn@**4*** For the synthesis of Zn@**1**, chitosan (0.1 g) was dispersed in water (1 ml) under stirring, and then 33% HCl (1 drop) was added. pH of the resultant viscous solution was adjusted to 7, and 1% $ZnCl_2$ solution (10 ml) was added. The resulting suspension was stirred at 25 ºC for 2 h. After this, 50% NaOH (0.12 ml) was added, and the mixture was stirred at 25 ºC for 12 h. The white precipitate was centrifuged, washed with water, acetone, diethyl ether, dispersed in water and freeze-dried. Synthesis of Zn@**4** differs from the above synthesis only in the amount of the used starting compound **4** (0.19 g).

Catalytic experiments A reaction vial equipped with a magnetic stirring bar was charged with benzaldehyde (1.0 mmol), piperidine (1.1 mmol) and phenylacetylene (1.1 mmol), 8–10 wt% catalyst in appropriate solvent (see Table S3). The mixture was stirred at appropriate temperature (see Table S3) for 10 h (TLC control, eluent hexane/acetone, 10:1 v/v). After disappearance of benzaldehyde spot on TLC and cooling to room temperature, the mixture was centrifuged, and the supernatant was concentrated under reduced pressure to afford the crude product, which was purified by flash-chromatography on silica gel (40–60 mash) using petroleum ether/ethyl acetate (30:1, v/v) as eluent to give the pure product.

Spectroscopic data for compound **5** correspond to the previously reported [S4]. FTIR (KBr): 3051, 2935, 2749, 1594, 1487, 1451, 1312, 1159, 753, 696 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: δ, м.д. 7.63–7.61 (m, 2H), 7.54–7.49 (m, 2H), 7.37–7.26 (m, 6H), 4.81 (s, 1H), 2.55–2.53 (m, 4H), 1.63–1.57 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 138.9, 131.7, 128.2, 127.8, 127.6, 128.1, 127.5, 123.3, 87.5, 85.8, 62.3, 50.7, 26.1, 24.5. HRESI-MS, $[M + H]^+$, *m/z*: 276.1757 (calculated: 276.1752).

In the FTIR spectrum of chitosan and its derivatives, the peaks at ca. 3327 cm^{-1} are attributed to N–H and O–H stretching vibrations, at ca. 2281 cm^{-1} – to stretching CH vibrations, while the bands at 1645 and 1555 cm^{-1} are due to carbonyl stretching vibrations of $NHC(O)CH_3$ moiety, and 1372, 1415, 1200–1000 cm^{-1} correspond to stretching C–N and C–O vibrations. The spectra of **4** and Zn@**4** display also overlapped peaks at ca. 1600–1440 cm^{-1} due to stretching vibrations of aromatic ring. The spectra of Zn@**1** and Zn@**4** have strong differences from the spectra of the starting chitosan **1** and **4**, respectively, in the region of 1700–1400 cm^{-1} due to coordination of zinc(II) center to glucosamine units of chitosan polymer matrix. Figures S4–S6 demonstrate that the starting chitosan as well as **4** are characterized by predominantly amorphous structure, while diffractograms of composites Zn@**1** and Zn@**4** display more pronounced peaks due to higher degree of crystallinity of these samples. Moreover, diffractogram of Zn@**4** exhibits the highest number of peaks which indicates high enough degree of crystallinity of this composite.

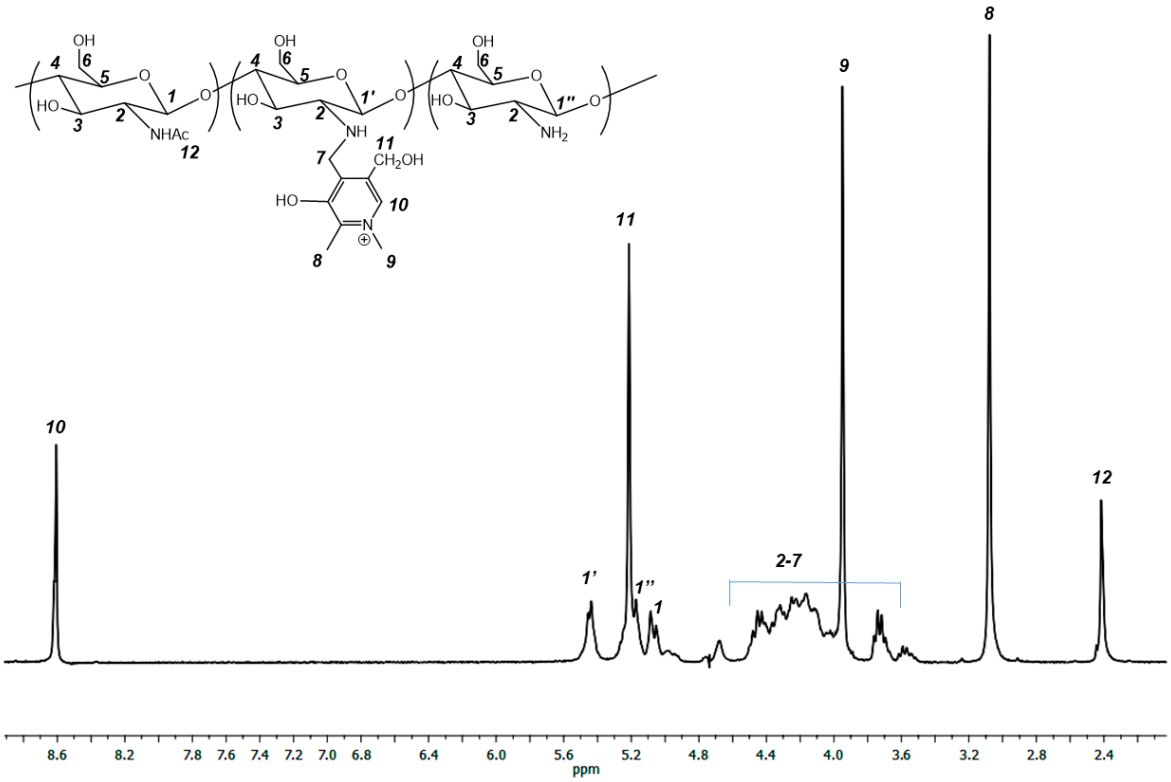

Figure S1. ^{1}H NMR spectrum of **4** (D_2O/CF_3COOH) with signal assignment.

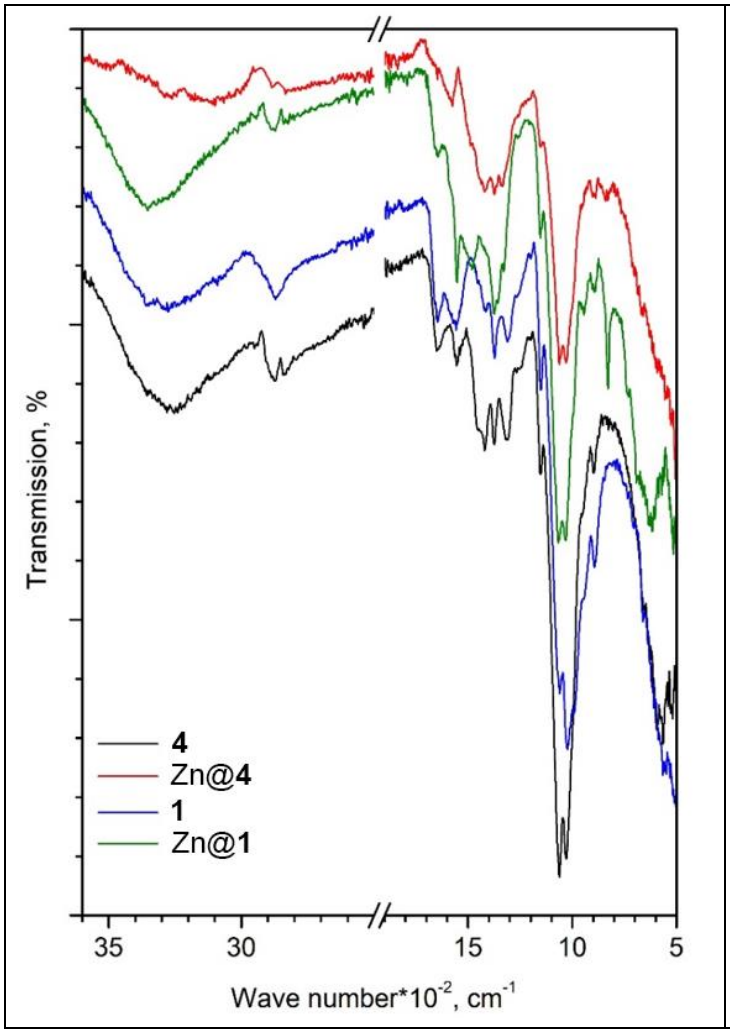

Figure S2. IR spectra of starting chitosan **1** and derivatives **4**, Zn@**1** and Zn@**4**.

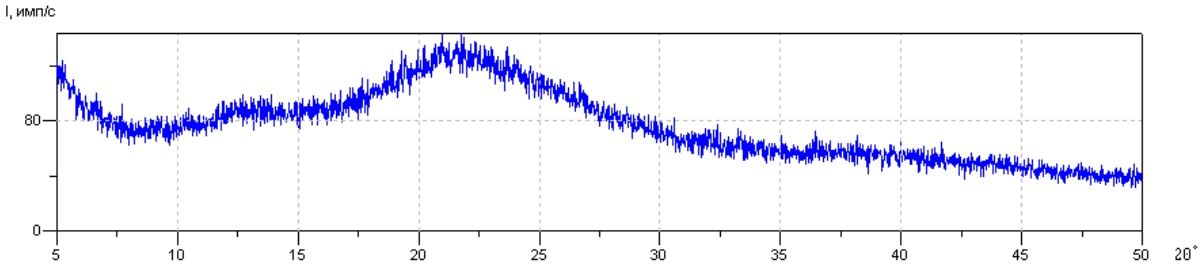

Figure S3. X-Ray diffractogram of chitosan.

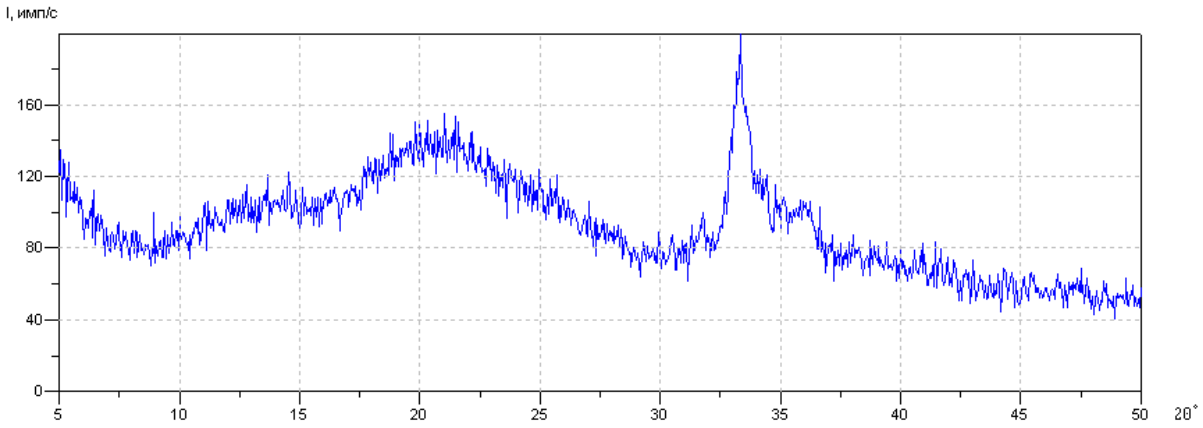

Figure S4. X-Ray diffractogram of Zn@**1**.

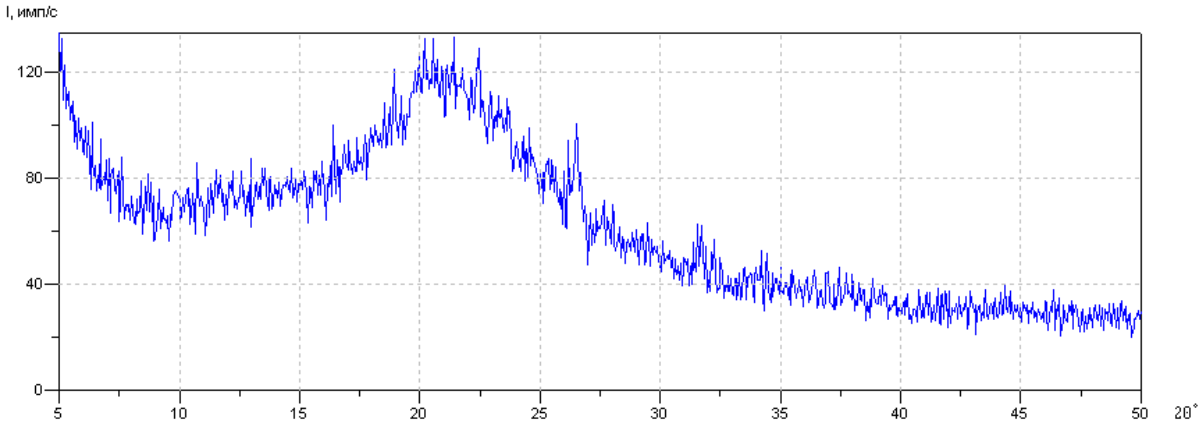

Figure S5. X-Ray diffractogram of **4**.

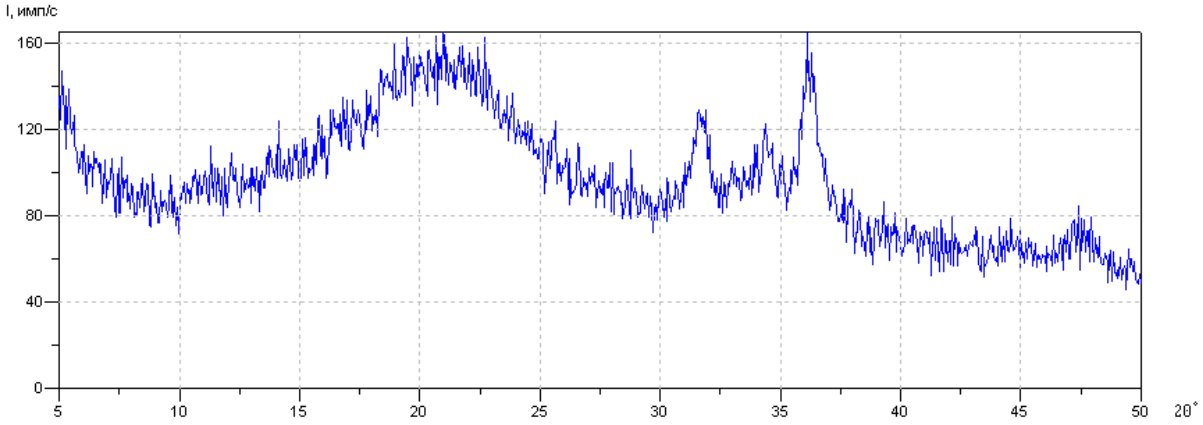

Figure S6. X-Ray diffractogram of Zn@**4**.

Table S1. Thermo-gravimetric data.

Sample	T_{max1}*	R_{max1}*	T_{on}*	T_{max2}*	T_{end}*	R_{max2}*	R_{600}*
1	72.1±0.2	78.3±0.1	257.2±0.1	304.2±0.4	377.4±0.3	41.8±0.3	15.6±0.1
Zn@**1**	99.4±0.1	79.1±0.1	271.3±0.2	319.1±0.1	407.9±0.2	45.2±0.1	16.5±0.3
4	96.5±0.2	86.9±0.3	275.1±0.1	314.8±0.3	407.7±0.3	44.2±0.1	17.8±0.3
Zn@**4**	141.3±0.2	90.3±0.2	289.4±0.1	341.2±0.3	425.2±0.1	55.1±0.1	20.4±0.2

* Mean value ± SD, n=3

T_{max1}: maximum thermal degradation temperature of water; R_{max1}: residual weight after loss of water; T_{on}: onset macromolecule degradation temperature; T_{max2}: maximum thermal macromolecule degradation temperature; T_{end}: ending macromolecule degradation temperature; R_{max2}: residual weight loss after thermal degradation of macromolecule; R_{600}: residual weight at 600 °C

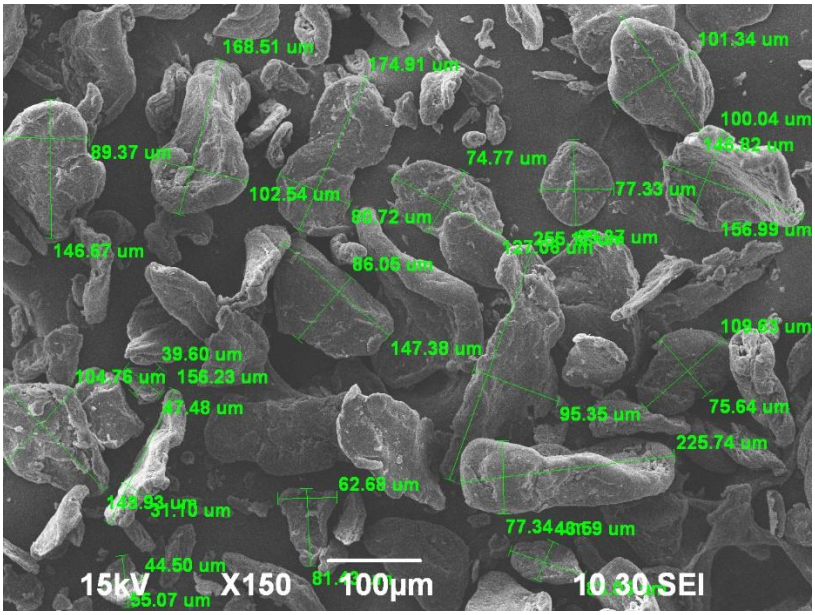

Figure S7. SEM image of **1**.

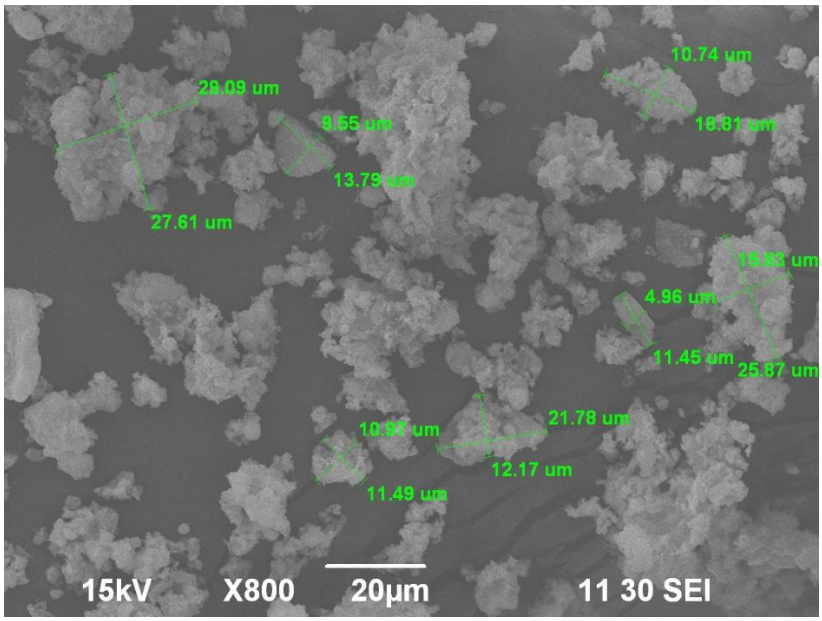

Figure S8. SEM image of Zn@**1**.

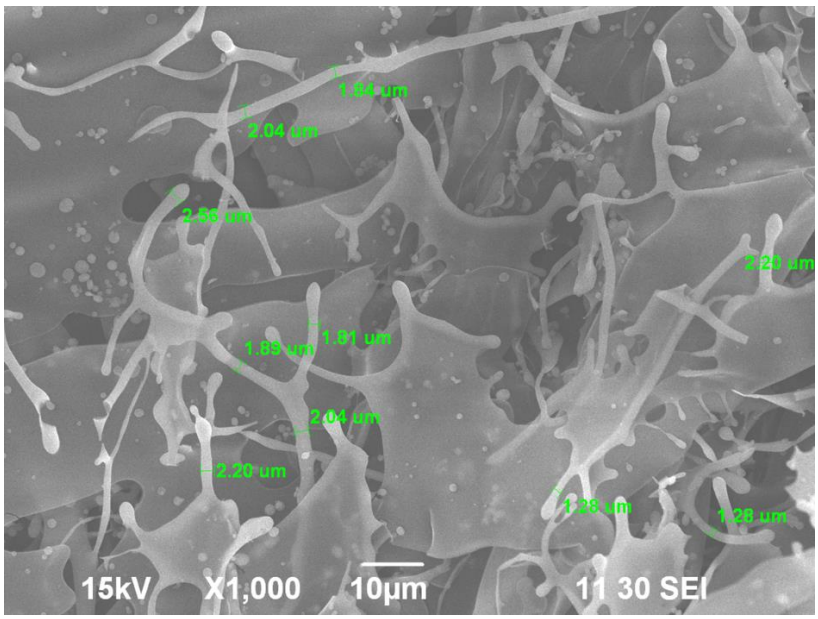

Figure S9. SEM image of **4**.

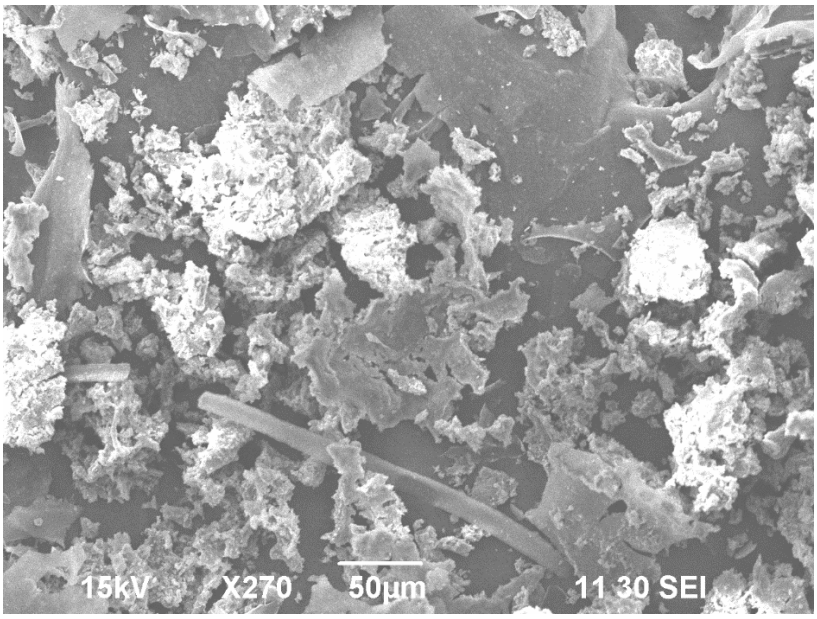

Figure S10. SEM image of Zn@**4**.

Table S2. Optimization of conditions of Zn(II)-catalyzed reaction of benzaldehyde, phenylacetylene, and piperidine (reaction time 8 h).

Entry	Catalyst (wt%)	Solvent (temperature, ºC)	Isolated yield, %
1	$ZnCl_2 \times 6H_2O$ (10)	Toluene (110)	61
2	$ZnCl_2$ (10)	Toluene (110)	60
3	$Zn(OTf)_2$ (10)	Toluene (110)	63
4	$Zn(OH)_2$ (10)	Toluene (110)	60
5	ZnO (10)	Toluene (110)	61
6	Zn@**1** (10)	Toluene (110)	77
7	Zn@**4** (10)	Toluene (110)	94
8	Zn@**4** (8)	Toluene (110)	95
9	Zn@**4** (5)	Toluene (110)	79
10	Zn@**4** (8)	Acetonitrile (82)	30
11	Zn@**4** (8)	Ethanol (78)	23
12	Zn@**4** (8)	H_2O (100)	trace
13	Zn@**4** (8)	DMF (110)	36
14	Zn@**4** (8)	None (110)	61
15	None	Toluene (110)	18

Table S3. Antibacterial activity.

Sample	Tested bacteria	
	S. aureus	*E. coli*
	Inhibition zone, mm*	
1	13.5±0.3	10.4±0.2
4	18.1±0.2	14.2±0.1
Zn@**1**	21.3±0.3	16.7±0.1
Zn@**4**	26.6±0.1	20.4±0.1
$ZnCl_2 \times 6H_2O$	26.6±0.2	20.6±0.4
$Zn(OH)_2$	25.9±0.3	19.1±0.2
ZnO	25.7±0.2	19.0±0.1
Ampicillin	30.2±0.19	–
Gentamicin	–	22.1±0.24

* Mean value ± SD, n=3

References

[S1] A.-u. Rahman, M. I. Choudhary and W. J Thomson, *Bioassay Techniques for Drug Development*, Harwood Academic Publishers, Amsterdam, 2005.

[S2] A. S. Kritchenkov, A. R. Egorov, M. N. Kurasova, O. V. Volkova, T. V. Meledina, N. A. Lipkan, A. G. Tskhovrebov, A. V. Kurliuk, T. V. Shakola, A. P. Dysin, M. Y. Egorov, E. A. Savicheva and W. M. dos Santos, *Food Chem.*, 2019, **301**, 125247.

[S3] D. Heyl, E. Luz, S. A. Harris and K. Folkers, *J. Am. Chem. Soc.*, 1951, **73**, 3430.

[S4] J. Iwanejko, E. Wojaczyńska and T. K. Olszewski, *Curr. Opin. Green Sustain. Chem.*, 2018, **10**, 27.