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Supporting Information

Biocompatible non-leachable antimicrobial polymers with nonionic hyperbranched backbone and phenolic terminal units

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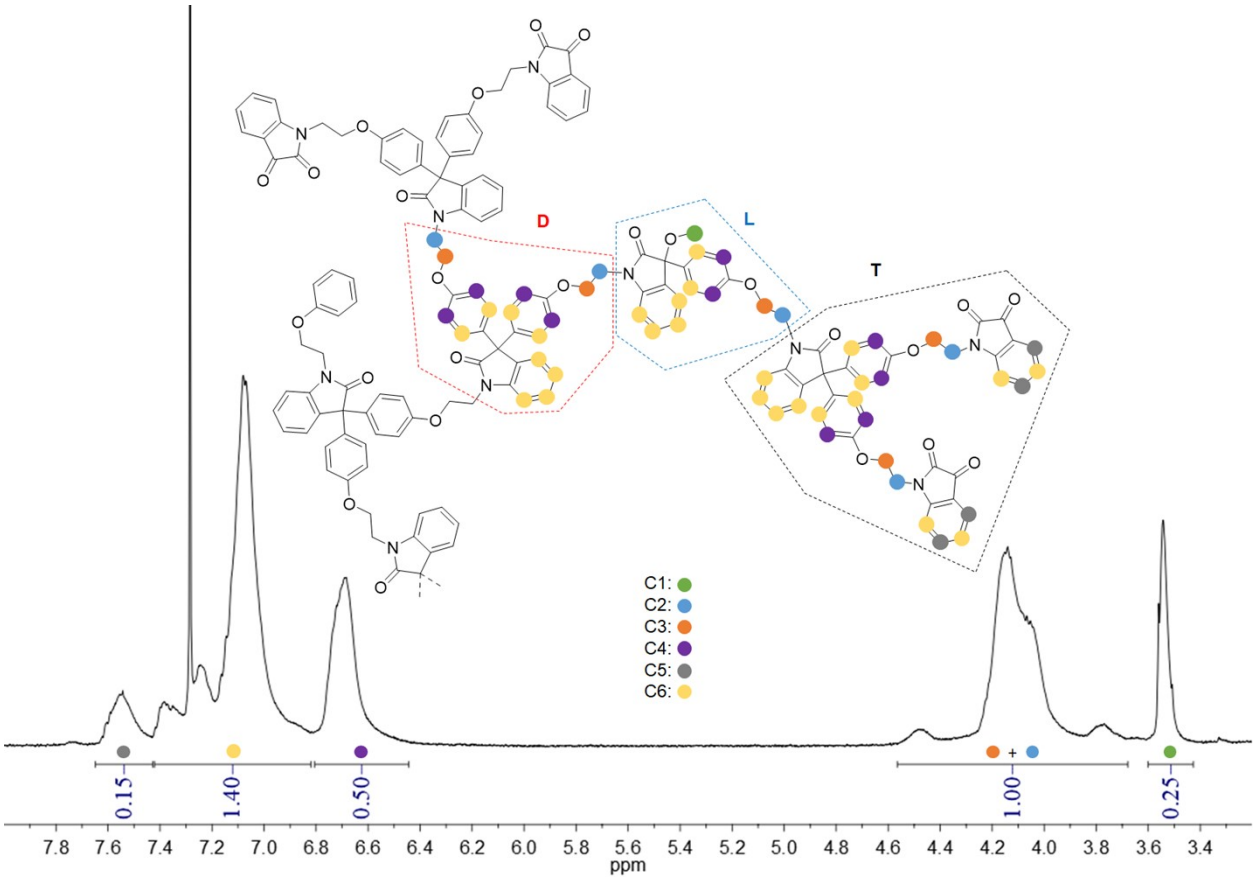

Figure S1. ^{1}H NMR spectrum of **HBP1** with the integrations of signals C1, C2, C3, C4, C5, and C6. The calculation of degree of branching based on the integrals was shown below.

Calculation of degree of branching (DB) of HBP1

The degree of branching (DB) was calculated according to the definitions of Frey[1] and Fréchet [2] (equations (1)), in which D, T, and L represent the three possible structural units in HBPs: dentritic (D), terminal (T), and linear (L).

$$DB = \frac{D + T}{D + T + L} \quad (1)$$

In order to evaluate the DB of **HBP1**, the structural units D, T, and L was defined first (Fig. S1), and the relative amount of each unit was assessed based on the integrals of the corresponding signals in the ^{1}H NMR spectrum of **HBP1**. As shown in Fig. S1, proton signal C1 was due to the presence of the OCH_3 groups in the linear units. Proton signals C2 and C3 were due to the

combined ethylene bridge protons of D, T and L units. Proton signal C4 belong to the aromatic protons at the ortho position on the phenyl ether moiety of D, T and L units. Proton signals C5 were caused by the unshielded aromatic protons at the ortho and para position of the carbonyl group on the isatin moiety in the terminal units. Finally, proton signals C6 were due to the rest of aromatic protons of D, T and L units. Therefore, the integrals of signals Ci (i ＝1-6), expressed as I_{Ci} would follow the equations (2), (3), (4), (5), and (6) below:

$$I_{C1} = 3L \tag{2}$$

$$I_{C2} + I_{C3} = 8D + 8T + 4L \tag{3}$$

$$I_{C4} = 4D + 4T + 2L \tag{4}$$

$$I_{C5} = 4T \tag{5}$$

$$I_{C6} = 8D + 12T + 6L \tag{6}$$

Combining Equations (1) - (3), or (1), (2), and (4), or (1), (2), (5), and (6), three different DBs, DB_1, DB_2, and DB_3, respectively, could be calculated according to the following expressions,

$$DB_1 = \frac{3(I_{C2} + I_{C3}) - 4I_{C1}}{3(I_{C2} + I_{C3}) + 4I_{C1}} \tag{7}$$

$$DB_2 = \frac{3I_{C4} - 2I_{C1}}{3I_{C4} + 2I_{C1}} \tag{8}$$

$$DB_3 = \frac{3I_{C6} - 3I_{C5} - 6I_{C1}}{3I_{C6} - 3I_{C5} - 2I_{C1}} \tag{9}$$

By applying the I_{Ci} values (shown in Fig. S1, as I_{C1} ＝0.25, I_{C2} + I_{C3}＝1.00, I_{C4}＝ 0.50, I_{C5}＝0.15, and I_{C6}＝1.40), DB_1, DB_2, and DB_3 of **HBP1** were calculated as 0.50, 0.50, and 0.53 (according to Eq. 7, 8, and 9), respectively. These values are effectively the same, considering that DB_3 value was overestimated due to the residual solvent proton signal included in I_{C6}. We therefore used the average DB＝0.51 in the main text for our discussion.

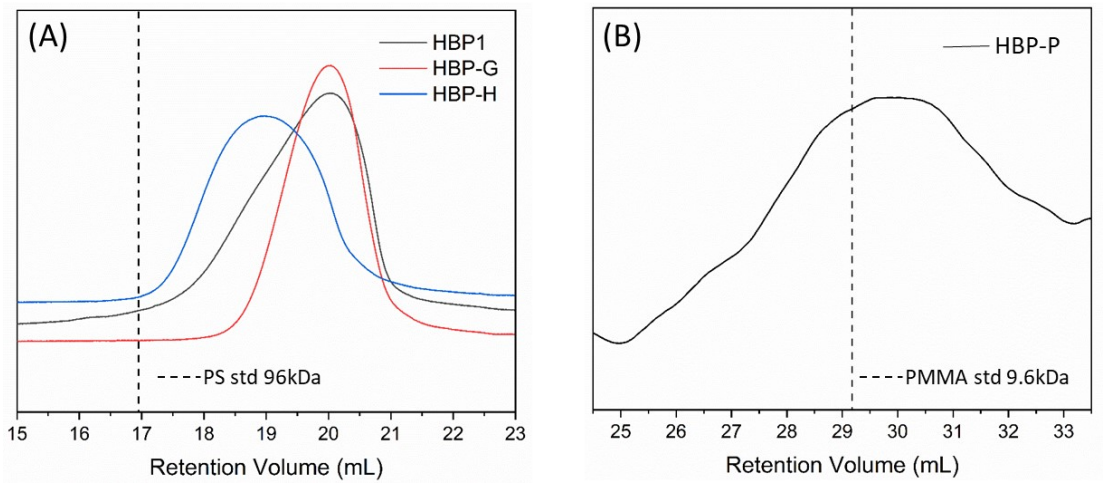

Figure S2. SEC chromatograms of the refractive index of (A) **HBP1**, **HBP-G** and **HBP-H** in chloroform; the retention volume of a polystyrene standard (PS, M_n ~96 KDa) was indicated as the dashed line and (B) **HBP-P** in DMAc; the retention volume of a poly(methyl methacrylate) standard (PMMA, M_n ~9.6 KDa) was indicated as the dashed line

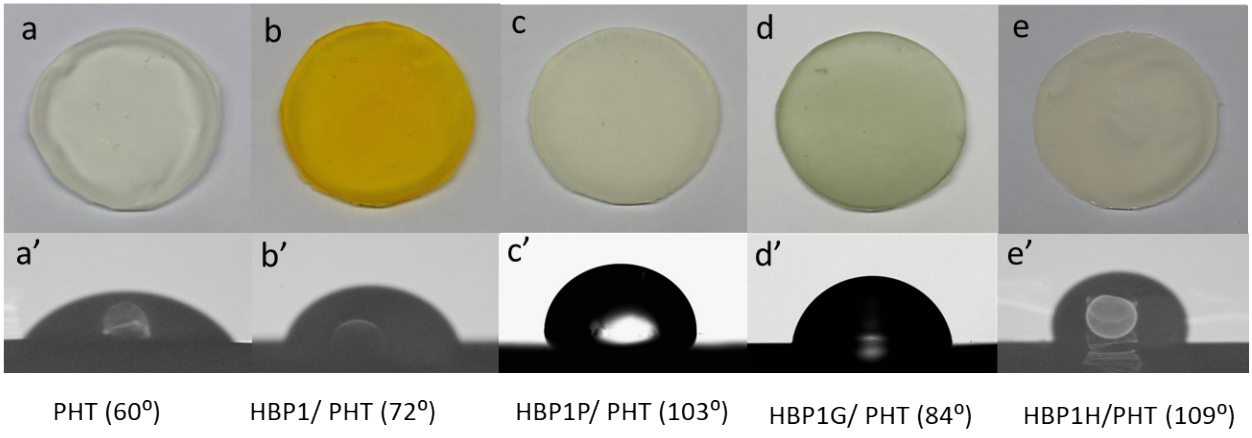

Figure S3**.** Films of pure PHT (a) and PHT with 5 wt% **HBP1** (b), **HBP-P** (c), **HBP-G** (d) and **HBP-H** (e). Water contact angle of films of pure PHT (a') and PHT with 5 wt% **HBP1** (b'), **HBP-P** (c'), **HBP-G** (d') and **HBP-H** (e').

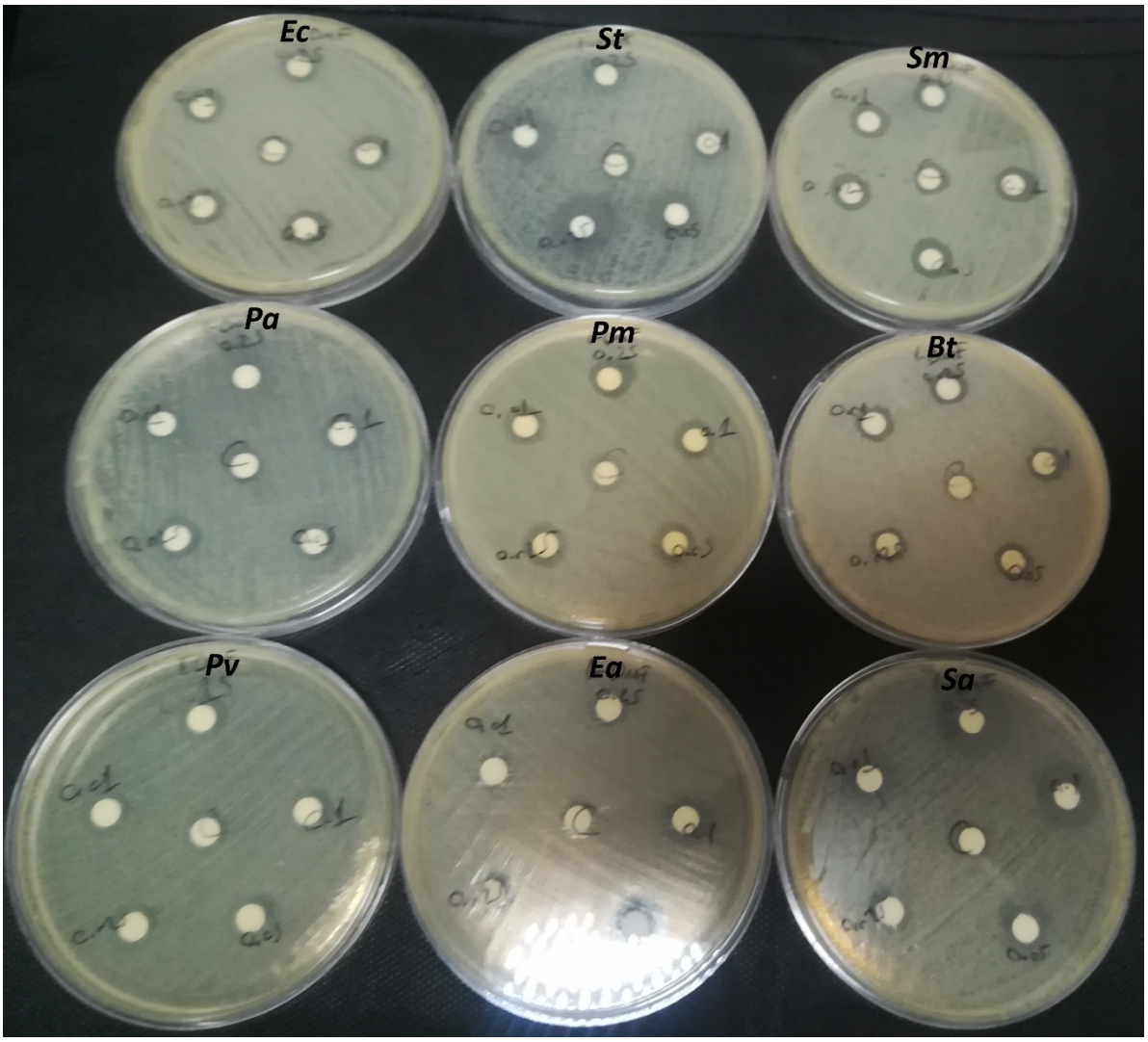

Figure S4. Example images of disk diffusion assay of **HBP1** against 9 tested microorganisms including *Escherichia coli* ATCC 25922 (*Ec*), *Salmonella typhimurium* SL 1344 (*St*), *Streptococcus mutans* ATCC 25175 (*Sm*), *Pseudomonas aeruginosa* ATCC 27853 (*Pa*), *Proteus mirabilis* ATCC 14153 (*Pm*), *Bacillus thuringiensis* (*Bt*), *Proteus vulgaris* ATCC13315 (*Pv*), *Enterobacter aerogenes* ATCC13048 (*Ea*), and *Staphylococcus aureus* ATCC 25923 (*Sa*).

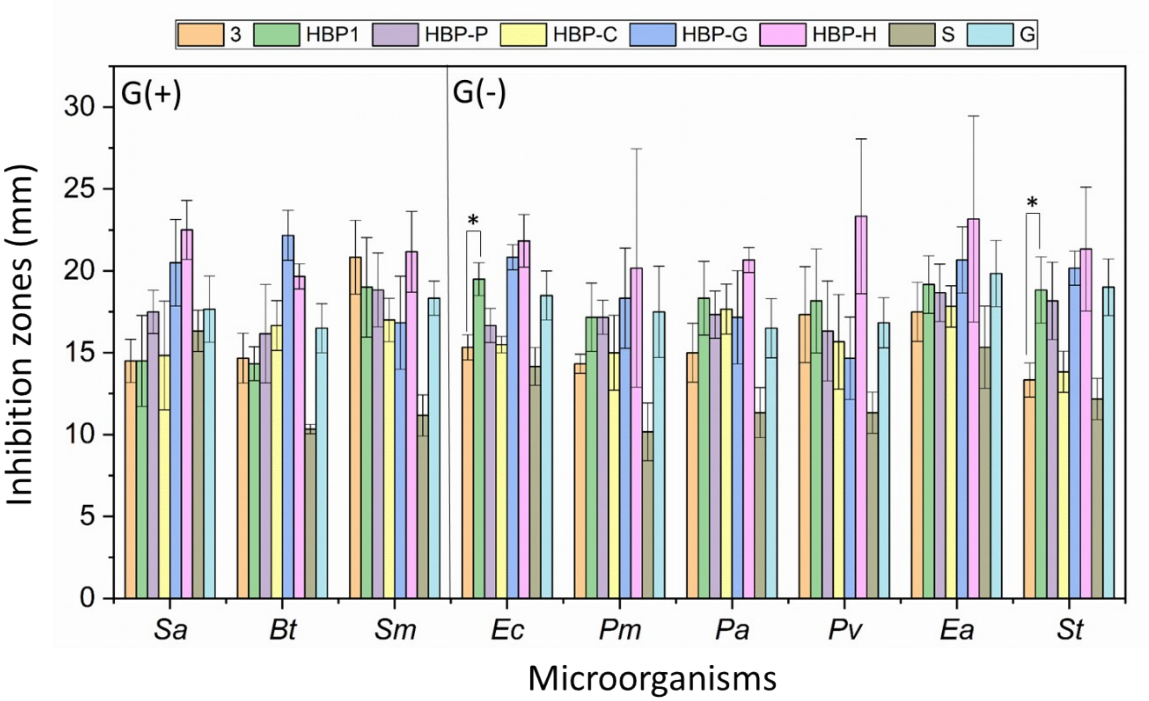

Figure S5. Inhibition zones of the obtained monomer **3** and HBPs (0.5 μg per disk), as well as commercial antibiotics streptomycin and gentamicin (marked as S and G, respectively, 25 μg per disk). The only two cases where the zones of inhibition of **HBP1** were significantly larger compared to that of monomer **3** are marked with * ($p < 0.05$).

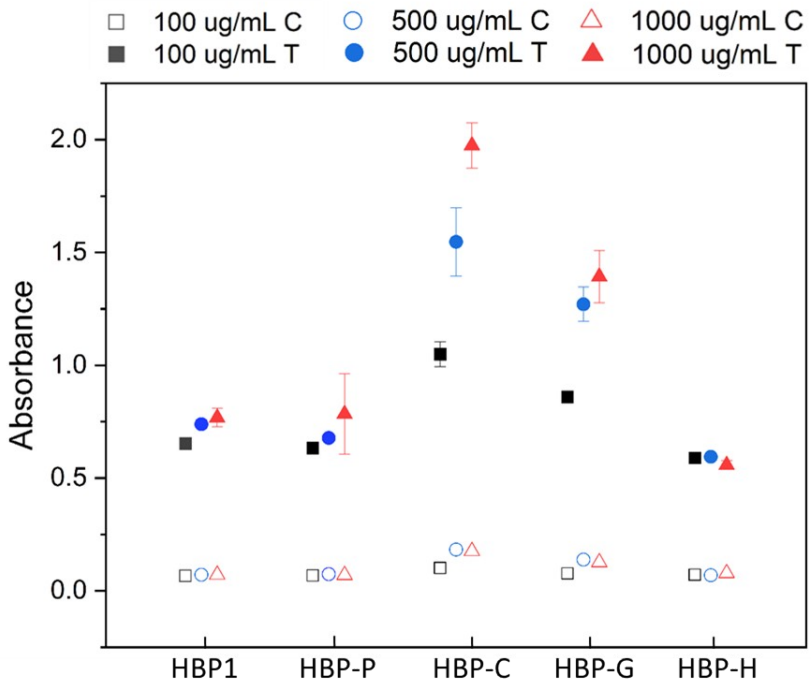

Figure S6. UV-vis absorbance at 600 nm of all HBPs with three concentrations (100, 500 and 1000 μg/mL) in MTT assay. C represents the control experiments without cells and T represents the test experiments with cells.

Table S1. *p* values to identify the "significant difference" between the inhibition zones of monomer **3**, HBPs and streptomycin. Numbers in black color indicate no significant difference ($p \geq 0.05$), and numbers in red color indicate significant difference ($p < 0.05$).

	p value					
	3	**HBP1**	**HBP-P**	**HBP-C**	**HBP-G**	**HBP-H**
Sa	0.1570	0.3573	0.3305	0.5058	0.0694	0.0083
Bt	0.0085	0.0030	0.0289	0.0021	0.0002	0.0000
Sm	0.0029	0.0146	0.0068	0.0052	0.0343	0.0033
Ec	0.2181	0.0038	0.0495	0.1404	0.0011	0.0026
Pm	0.0174	0.0112	0.0040	0.0441	0.0159	0.0819
Pa	0.0548	0.0112	0.0078	0.0071	0.0352	0.0007
Pv	0.0311	0.0257	0.0587	0.0757	0.1094	0.0131
Ea	0.2921	0.0965	0.1331	0.1986	0.0458	0.1158
St	0.2836	0.0083	0.0178	0.1801	0.0011	0.0164

Table S2. *p* values to identify the "significant difference" between the inhibition zones of monomer **3**, HBPs and gentamicin. Numbers in black color indicate no significant difference ($p \geq 0.05$), and numbers in blue color indicate significant difference ($p < 0.05$).

	p value					
	3	**HBP1**	**HBP-P**	**HBP-C**	**HBP-G**	**HBP-H**
Sa	0.0856	0.18606	0.9106	0.2761	0.2145	0.0365
Bt	0.2122	0.1090	0.8722	0.8993	0.0101	0.0311
Sm	0.1562	0.7376	0.7449	0.2420	0.4392	0.1407
Ec	0.0311	0.3911	0.1570	0.0303	0.0743	0.0584
Pm	0.1259	0.8761	0.8554	0.2960	0.7445	0.5856
Pa	0.3658	0.3331	0.5659	0.4406	0.7489	0.0211
Pv	0.8062	0.548	0.8123	0.5696	0.2714	0.0860
Ea	0.2100	0.6885	0.4924	0.2193	0.6401	0.4316
St	0.0083	0.9188	0.6481	0.0139	0.3739	0.3867

Table S3. *p* values to identify the "significant difference" between the inhibition zones of HBPs (**HBP1**, **HBP-C**, **HBP-G**, **HBP-H**) and **HBP-P**. Numbers in black color indicate no significant difference ($p \geq 0.05$), and numbers in purple color indicate significant difference ($p < 0.05$).

	p value			
	HBP-P	**HBP-C**	**HBP-G**	**HBP-H**
Sa	0.1671	0.9006	0.0538	0.0139
Bt	0.3757	0.0941	0.0018	0.0020
Sm	0.9429	0.3552	0.4183	0.3921
Ec	0.0273	0.0034	0.1404	0.0996
Pm	1.0000	0.2921	0.6137	0.5306
Pa	0.5529	0.6934	0.6073	0.1648
Pv	0.5110	0.3701	0.2089	0.1911
Ea	0.7449	0.3453	0.3868	0.3486
St	0.7292	0.0220	0.3671	0.3701

References

1 D. Hölter, A. Burgath and H. Frey, *Acta Polym.*, 1997, **48**, 30–35.

2 C. J. Hawker, R. Lee and J. M. J. Frechet, *J. Am. Chem. Soc.*, 1991, **113**, 4583–4588.