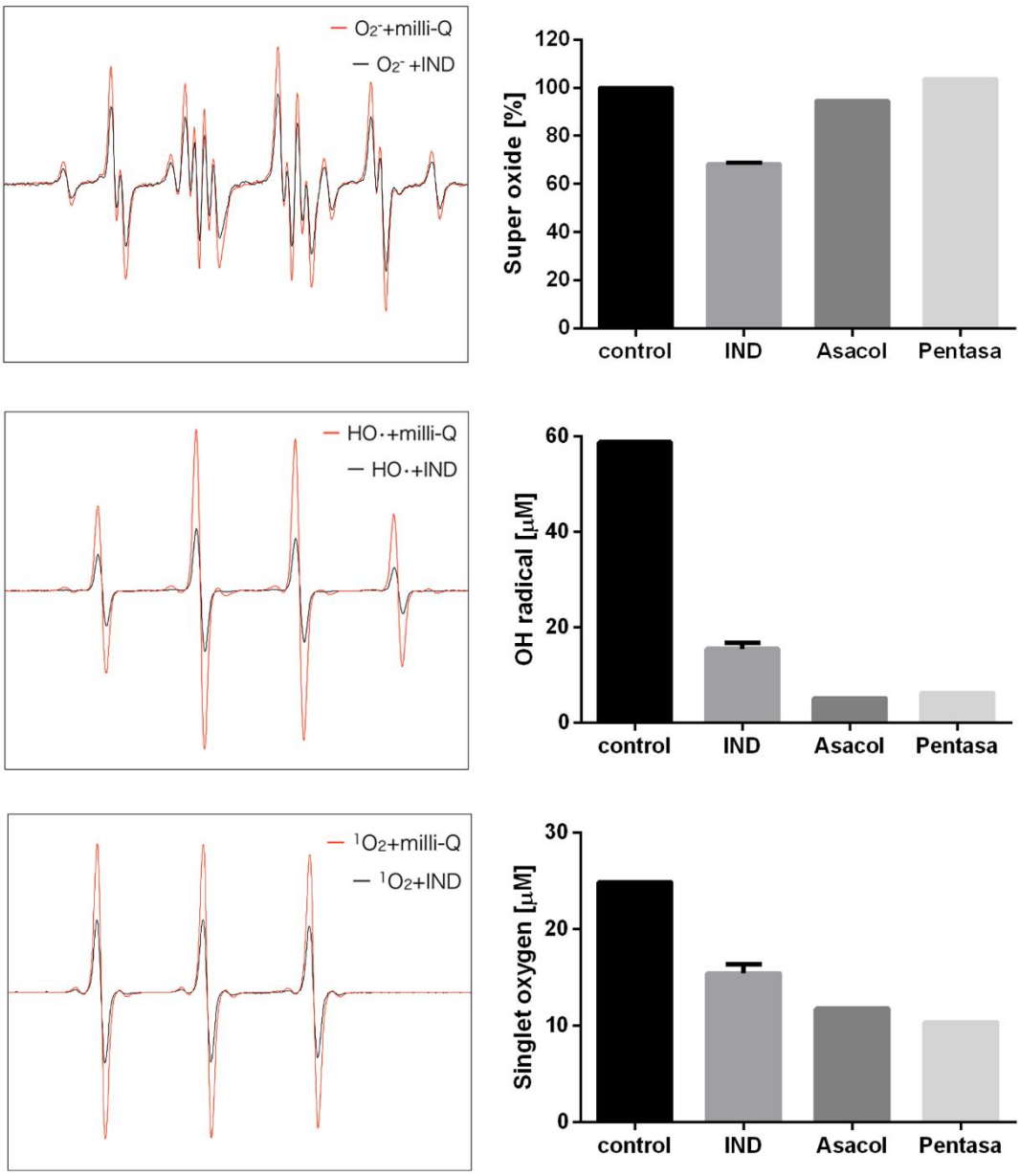

Fig S2. IND has the ability to scavenge ROS

The ability to scavenge ROS was measured using an electron spin resonance (ESR) spectrometer. The figures on the left represent the ESR spectrum from which the ROS level was calculated, which is represented by the figures on the right. The spin-trapping agents 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide (TPC) [Sigma Aldrich] as $^1O_2$ detector, and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) [LaboTec Co., Ltd.; Tokyo, Japan] as OH radical and O_2^- detector, were used. They were dissolved in phosphate buffered saline, and the concentration of TPC was fixed at 100 mM for OH radicals, and DMPO was fixed at 5 mM and 445 mM for O_2. As for ESR setup, 9.425 GHz microwave frequency, 2 min sweep time, 100 kHz modulation frequency, 335.5 ± 5 mT magnetic field,

and 0.1 s time constant were used. For OH radical and $^1O_2$ measurements, 0.1 mT modulation width was set. O_2^- measurement is fixed at 0.07 mT modulation width.

Indigo naturalis (IND) was dissolved to 2500 μg/mL in ultra-pure water. For OH radical and $^1O_2$ scavenging ability measurement, 100 μL IND solution and 100 μL TPC and DMPO solution were mixed. For O_2^- scavenging ability, 50 μL IND solution and 150 μL DMPO solution were mixed. As a positive control, 2500 μg/mL Asacol or Pentasa was mixed with DMPO as for OH radical and O_2^- scavenging measurement, and 125 μg/mL Asacol or Pentasa was mixed with TPC as for $^1O_2$ scavenging ability measurement. As a negative control, only DMPO or TPC was used.

To measure $^1O_2$ scavenging ability, 100 μM methylene blue was prepared in the IND with TPC solution, and a light source [Pencure 2000, J. MORITA MFG. CORP.; Kyoto, Japan] was used for 20 s. To measure OH radical scavenging ability, 10 mM hydrogen peroxide was prepared in the IND with DMPO solution, and UV light [Compact UV lamp UVGL-25, Funakoshi Co., Ltd.; Tokyo, Japan] was applied for 1 min. To measure O_2^- scavenging ability, the concentration of HPX and XOD was fixed at 0.5 mM and 0.1 U/mL, respectively, in the IND with DMPO solutions with 25 % added DMSO.

200 μL of solution was examined by X-band ESR spectrometer [JES-FA-100, JEOL; Tokyo, Japan], and OH radical and $^1O_2$ were calibrated with spin adducts of 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) [Tokyo Chemical Industry Co., Ltd.; Tokyo, Japan] with known amounts of radical. In the case of O_2^- spin adducts, the scavenging rate was compared.