Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

A novel inclusion complex of oxybenzone with C-methylresorcin[4]arene deters skin permeation

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Number of Figures: 12

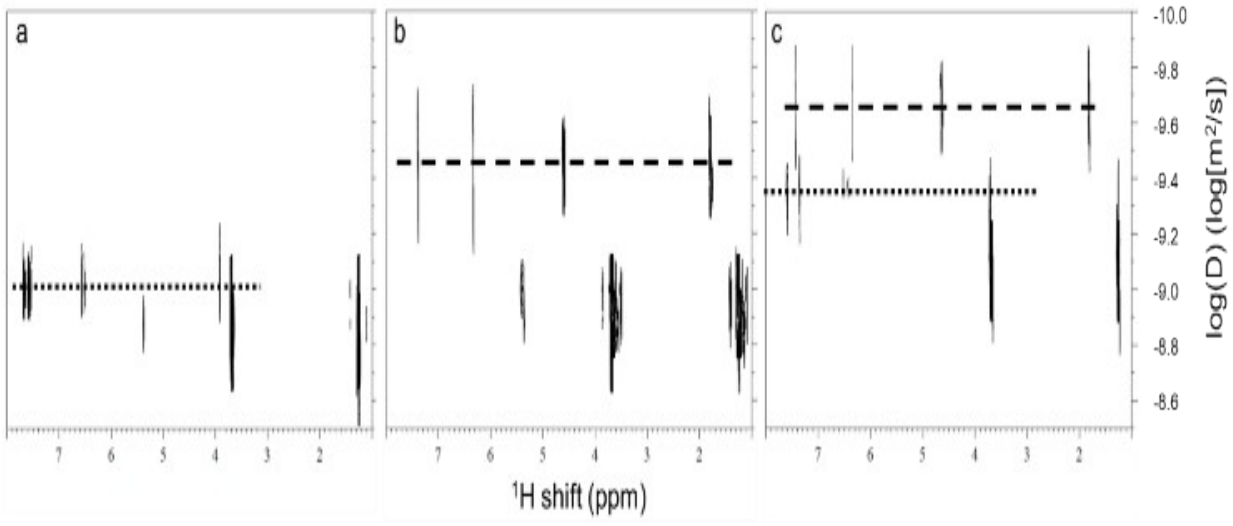

Figure S1: DOSY spectra of (a) OXB, (b) RsC1, and (c) a 1:4 mixture of OXB+RsC1 in ethanol-d_1.

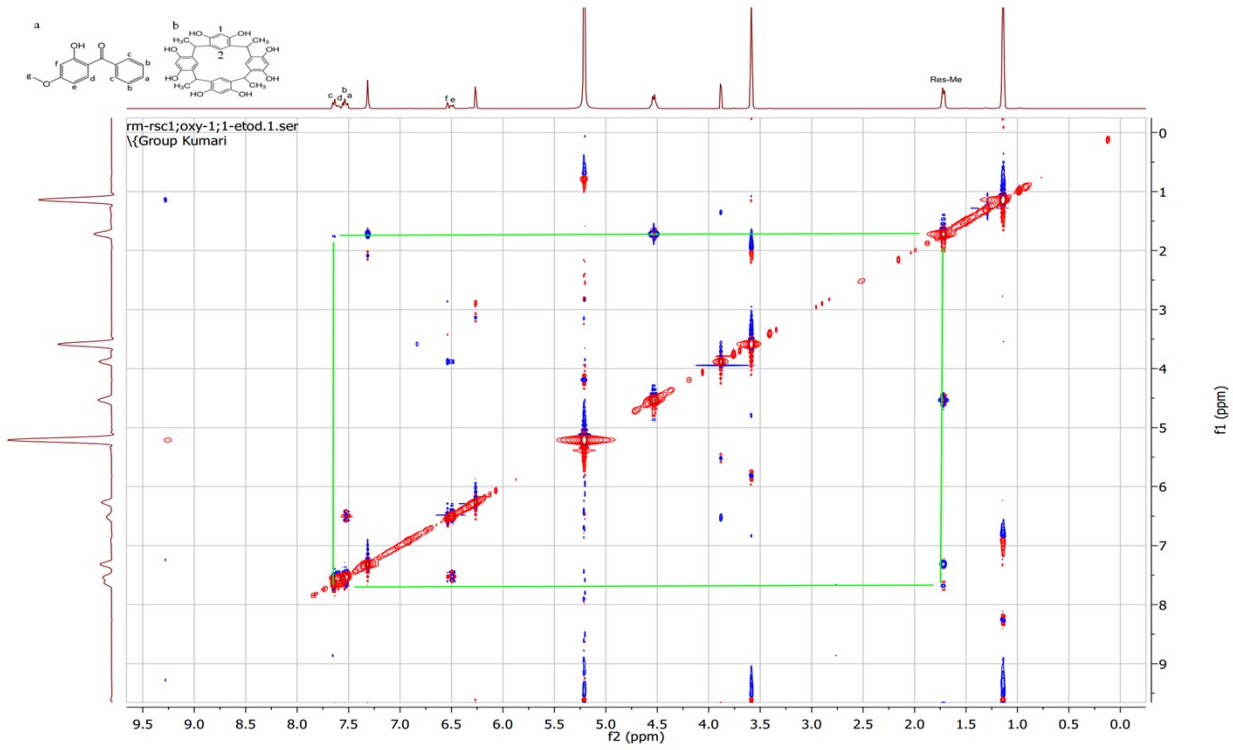

Figure S2. 2D NOESY spectrum of 1:1 RsC1-OXB complex in ethanol-d_6.

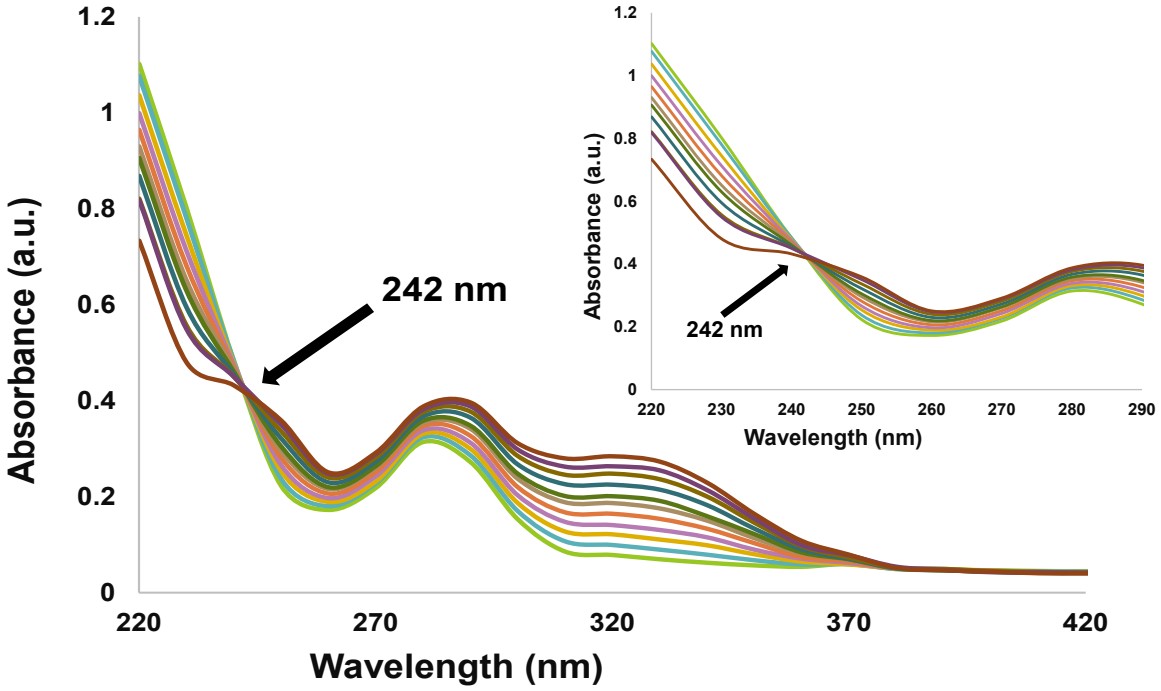

Figure S3: Absorption spectra of OXB:RsC1 (1:1) with various concentrations in EtOH at 25 °C [Inset: isosbestic point at ~242 nm for the 1:1 complex between OXB and RsC1].

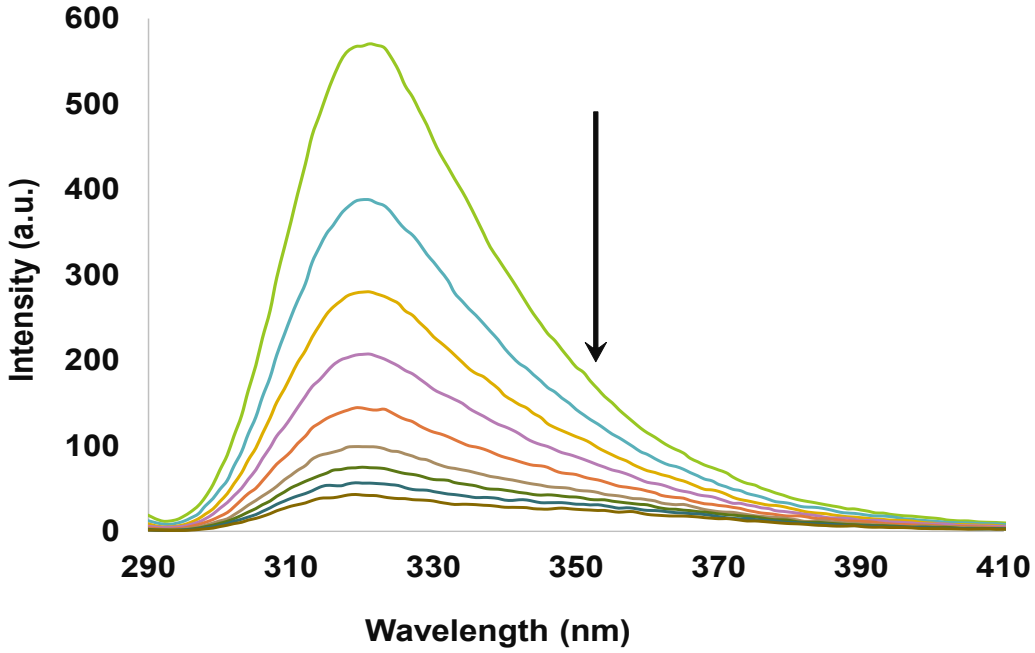

Figure S4: Fluorescence emission spectra of RsC1 (0.02 mM) in EtOH with varying amounts of added OXB, from top spectrum to bottom: 0.00, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 mM.

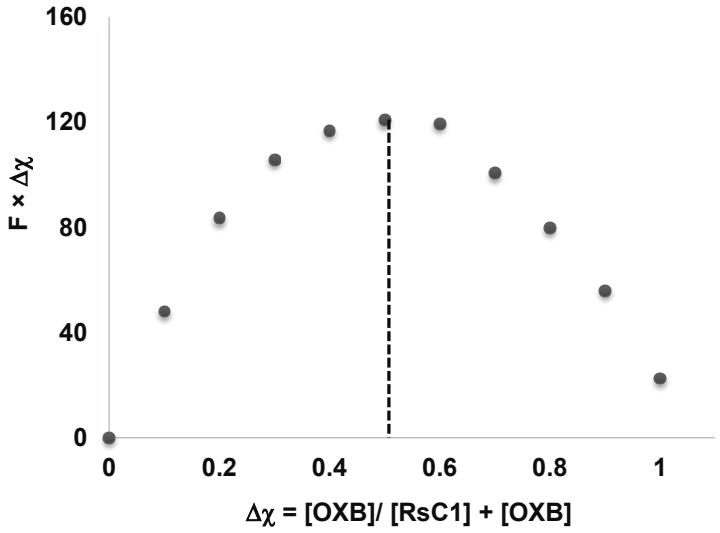

Figure S5: Job's plot from the fluorescence spectra of RsC1 and OXB with 0.02 mM each (λ_{ex} 290 nm).

$$\Delta F = F_0 + F.K\,[OXB] / 1 + K\,[OXB] \qquad (1)$$

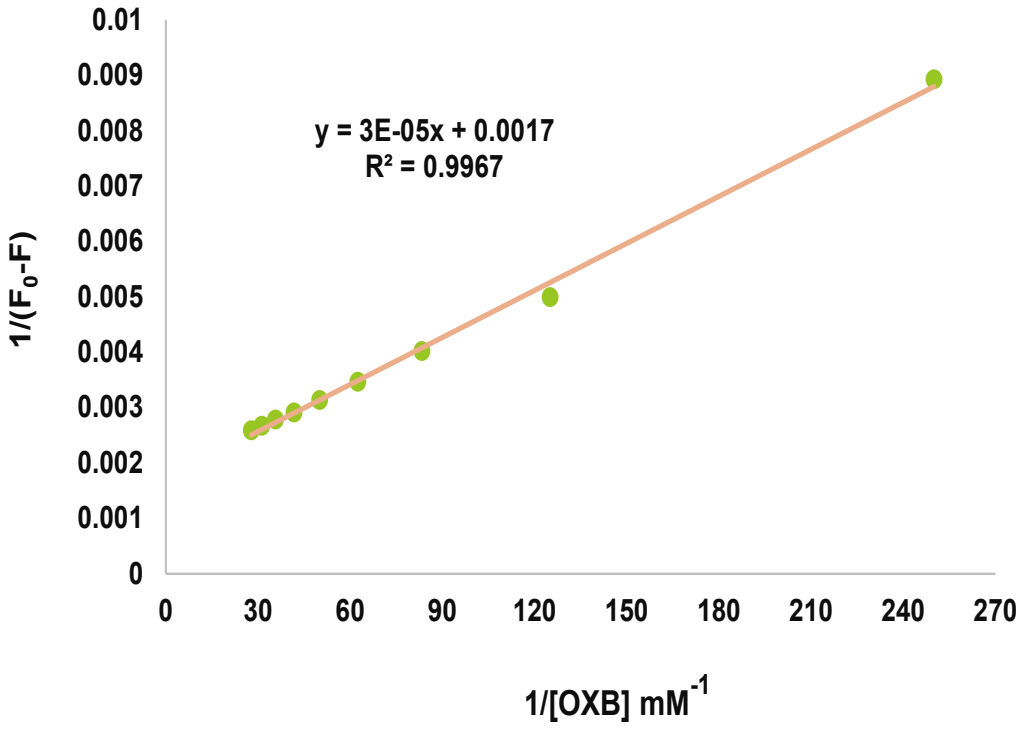

Figure S6: The binding curve for the RsC1–OXB complex. The solid lines are the fitting of the experimental data by Eq. (1).

$$F_0/F = 1 + K_{sv}.[OXB] \quad (2)$$

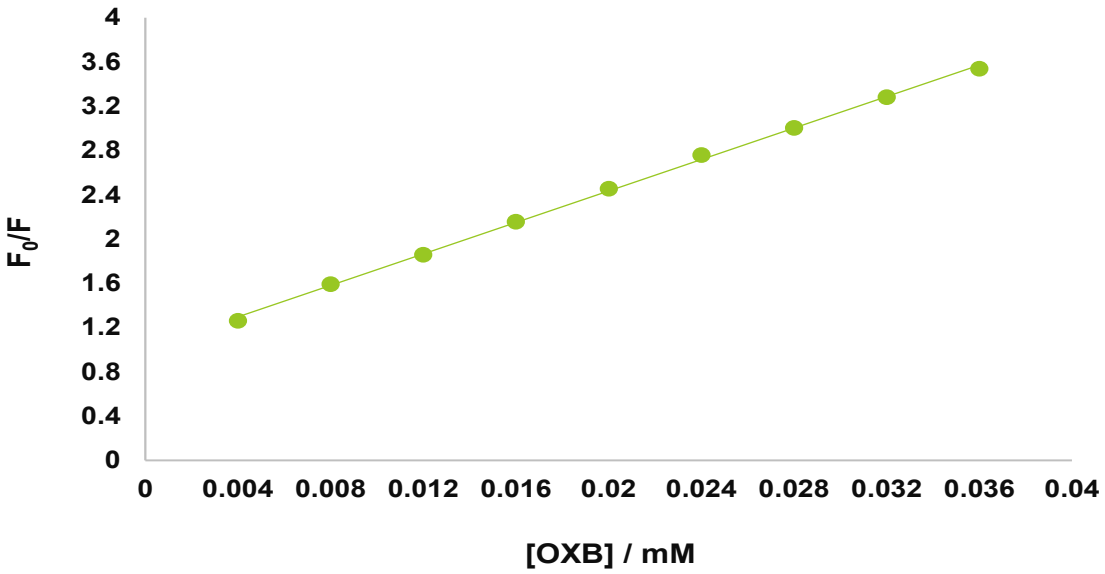

Figure S7: Stern–Volmer plot for the emission intensity (λ_{ex} 290 nm) recorded at 320 nm for RsC1 at different concentrations of OXB.

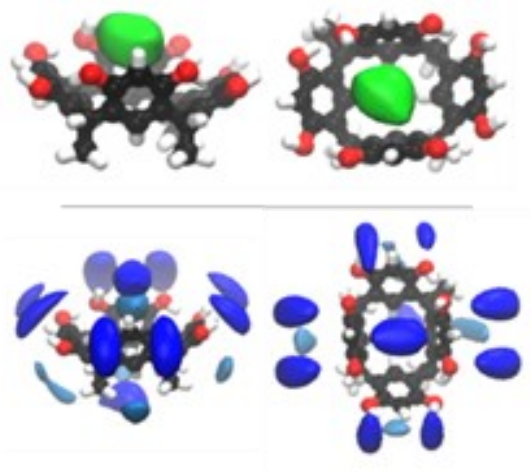

Figure S8: Spatial distribution functions of OXB (green) and solvent (blue) associated with a RsC1 molecule.

	Recovery		Average
OXB-1	98.2	OXB	98.3
OXB-2	98.1		
OXB-3	98.6		
OXB+RsC1-1	140.7	OXB+RsC1	135.6
OXB+RsC1-2	126.2		
OXB+RsC1-3	140.0		
RsC1-1	38.6	RsC1	37.0
RsC1-2	34.8		
RsC1-3	36.3		

Figure S9. Control recovery (%) of dosing in an empty vial. Group1: OXB; Group 2: OXB+RsC1; Group 3: RsC1. All the compounds were dissolved in ethanol, n=3. Solutions of 0.3% OXB and OXB+RsC1 in ethanol were applied in empty vials. The vials were left open for 24 h. The vials were then rinsed using 4 ml of 85:15 methanol:water solvent. The amounts of OXB were determined by HPLC at 294 nm. The average recovery from Group 1 was 98.3%. For the OXB+RsC1 complex (Group 2), it was found that the recovery was significantly higher than 100% (average of 135.6%). The reason for the higher than 100% recovery was the interference of RsC1 in the HPLC assay. RsC1 had similar HPLC retention time as OXB and UV absorption at 294 nm. The absorbance corresponding to RsC1 at 294 nm was 37%, resulting in the higher than 100% recovery (98% from OXB + 37% from RsC1 = 135% total).

Skin Name	**OXB Group**	**OXB+RsC1 group**
Skin 1	86.9	130.1
Skin 2	83.7	138.6
Skin 3	82.3	149.3
Skin 4	81.7	131.7
Average	83.6	137.4

Figure S10. Control recovery (%) of dosing on skin in a vial. Group 1: OXB; Group 2: OXB+RsC1. Split thickness skin was cut into 1.5 cm x 1.5 cm pieces and thawed in a petri dish with PBS at room temperature for 2 h. The skin was patted dry with Kimwipe and then put in a vial with the stratum corneum side facing up (n=4 for each group). Solutions of 0.3% OXB and OXB+RsC1 in ethanol were applied directly on the skin surface in the vials. The skin was left in the open vials for 24 h and then rinsed using 1 ml of 85:15 methanol:water solvent. The skin was cut into very small pieces, and then 1 ml of 85:15 methanol:water solvent was added to the vials

for extraction. The extraction was performed in the same vials to avoid mass loss. The vials were sonicated for 2 min and then centrifuged. This procedure was repeated 3 times. After the centrifugation, fresh 85:15 methanol:water solvent was added to the skin residue to repeat the extraction overnight. The amount of OXB extracted from the skin was determined by HPLC at 294 nm. This result was used to correct the skin surface rinse, skin cut-up, and donor chamber cap data in the skin permeation recovery study.

Skin name	OXB+RsC1 group at 325 nm	OXB+RsC1 group at 285 nm
Skin 1	1.63	1.66
Skin 2	0.95	0.94
Skin 3	2.25	2.26

Figure S11. OXB concentrations (μg/ml) determined at 285 and 325 nm in the HPLC assay using the OXB calibration curves at these two wavelengths, respectively, for the OXB+RsC1 group in the skin permeation study. The UV absorption peak of RsC1 was at 285 nm, and its absorbance at 325 nm was negligible compared to that of OXB (the extinction coefficient of OXB was at least 200x higher than that of RsC1 at 325 nm). HPLC assays at 285 and 325 nm were used to determine the concentration of RsC1 in the receptor chamber after 24 h in the skin permeation study. The calibration curve of OXB at 325 nm was used to calculate the concentration of OXB in the receptor chamber as the absorbance of RsC1 at this wavelength was negligible. Using the calibration curve of OXB at 285 nm, the concentration of OXB was also calculated. Student's unpaired t-test was performed, and there was no significant difference between the concentration of OXB at 325 nm (at this wavelength, the absorbance of RsC1 was negligible) and the concentration of OXB at 285 nm (at this wavelength, both OXB and RsC1 contributed to the absorbance), $p=0.22$. The lack of difference between the OXB concentrations determined at 285 and 325 nm indicates no contribution of RsC1 to the absorbance in the receptor chamber samples. The maximum concentration of RsC1 in the receptor chamber samples was also estimated by the difference between the absorbance measured at 280 nm and the absorbance at 280 nm calculated using the OXB concentration determined at 325 nm; the OXB absorbance at 280 nm was determined by OXB concentration obtained at 325 nm and the calibration curve at 280 nm. The maximum RsC1 concentration from this estimation was 0.06 μg/ml. This analysis suggests no significant permeation of RsC1 through the skin under the conditions studied.