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Supporting Information

Targeting the Antifungal Activity of Carbon Dots against *Candida albicans* Biofilm Formation by Tailoring Their Surface Functional Groups

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Supporting Information

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Carbon dots synthesis optimization

CDs-NH_2: CDs enriched in surface aminic moieties were prepared from D-glucosamine hydrochloride and 1,3-diaminobenzene as doping agent. Reaction optimization was performed keeping the same molar ratio between the two components and changing time and reaction power. Reaction trials are collected in table S1. When the reaction was carried out for 2 minutes with 600 W, incomplete carbonization of the materials was achieved. By enhancing microwave power, and increasing the time from 2 to 2.5 min no DLS signal was recorded after dialysis/filtration and freeze-drying. Therefore the reaction time was increased to 3 minutes.
Entries 1 and 2 gave a partially carbonized product with no detectable fluorescence signal. Neither change in w/w reactant ratio gave interesting results. Accordingly, the reaction voltage was enhanced to 450 W and 600 W. In this case, carbonized material was obtained but fluorescence of purified product gave ambiguous results. CDs-CO_2H were finally obtained setting the microwave power at 800 W.
A mixture of **1** and **2** in a 1:1.1 ratio was solubilized in a water/MeOH mixture (2:1) and carbonised in a microwave at 800 W for 3 min. The resulting slurry solution was diluted in MilliQ water (10 ml) and centrifuged at 4,000 rpm to remove larger particles and the supernatant was directly dialysed for five days (dialysis water changed at least 5 times per day) and then filtered through a 100 nm syringe filter. The resulting solution was freeze-dried to obtain the desired CDs.

Table S1. Reaction condition optimized for the synthesis of positive polarised CDs-CO_2H carbon dots.

Entry	Power (W)	Reaction time (min)	$GluNH_2HCl$:mDAB
1	600	3	1:1.1
2	800	2	1:1.1
3	800	2.5	1:1.1

CDs-CO_2H/NH_2: urea (**3**) and citric acid (**4**) were dissolved in water at 70 °C for 15 min, followed by 20 min sonication to ensure complete solubilization. Then, the mixture was irradiated at 800 W for 30 min. The resulting slurry solution was centrifuged at 4,000 rpm to remove larger particles and the supernatant was directly dialysed for five days and then filtered through a 100 nm syringe filter. The solution was freeze-dried to obtain the desired CDs.

CDs-CO_2H: CDs enriched in surface carboxylic acid were prepared from D-glucose and sodium polyacrylate as passivating agent. Reaction optimization was performed keeping 4 minutes as time of reaction, increasing the voltage from 300 W up to 800 W and changing the ratio between the two components. Reaction trials are collected in table S1. Entries 1 and 2 gave a partially carbonized product with no detectable fluorescence signal. Neither change in w/w reactant ratio gave interesting results. Accordingly, the reaction voltage was enhanced to 450 W and 600 W. In this case, carbonized material was obtained but fluorescence of purified product gave ambiguous results. CDs-CO_2H were finally obtained setting the microwave power at 800 W.

D-glucose (**5**) and polyacrylate sodium (MW = 5100, **6**), in a 1:2 ratio, were dissolved in water. The microwave-assisted carbonisation was performed via irradiation for 4 min at 800 W. The resulting slurry solution was centrifuged at 4,000 rpm to remove larger particles and the supernatant was directly dialysed for five days and then filtered through a 100 nm syringe filter. The solution was freeze-dried to obtain the desired CDs.

Table S2. Reaction condition optimized for the synthesis of negative polarised CDs-CO_2H carbon dots.

Entry	Power (W)	Reaction time (min)	Glu:PAA
1	300	4	2:1

2	300	5	2:1
3	300	4	2:2
4	300	4	1:1
5	450	4	2:1
6	600	4	2:1
7	800	4	2:1

NMR

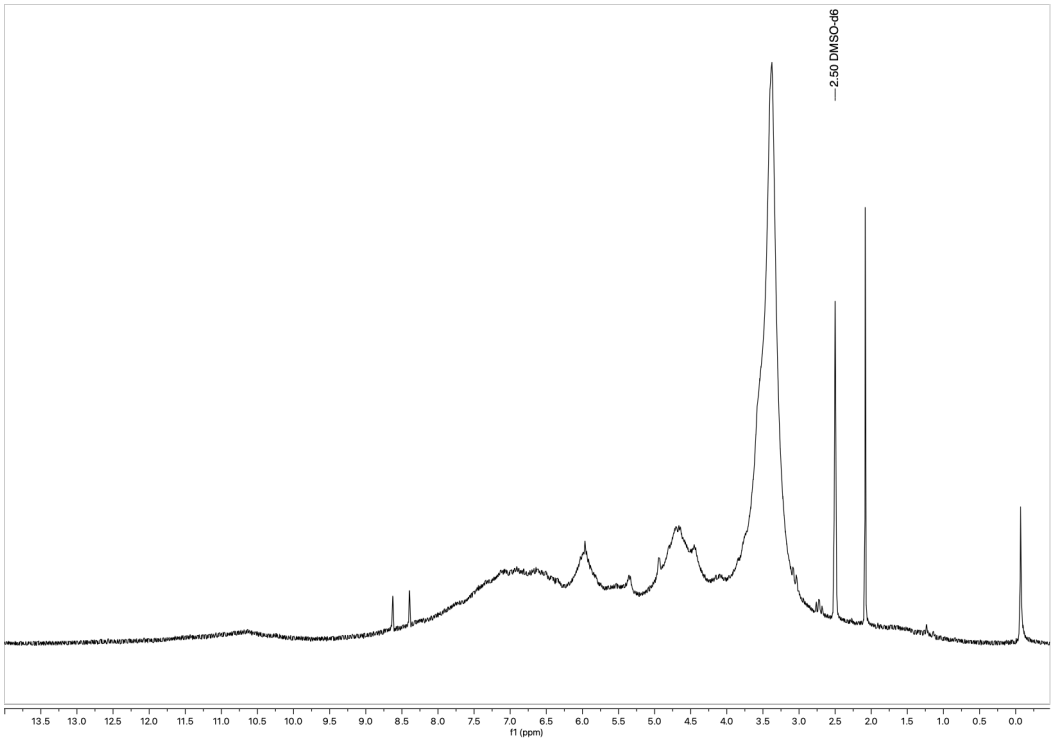

Figure S1. 1H NMR in DMSO-d_6 of **CDs-NH_2**.

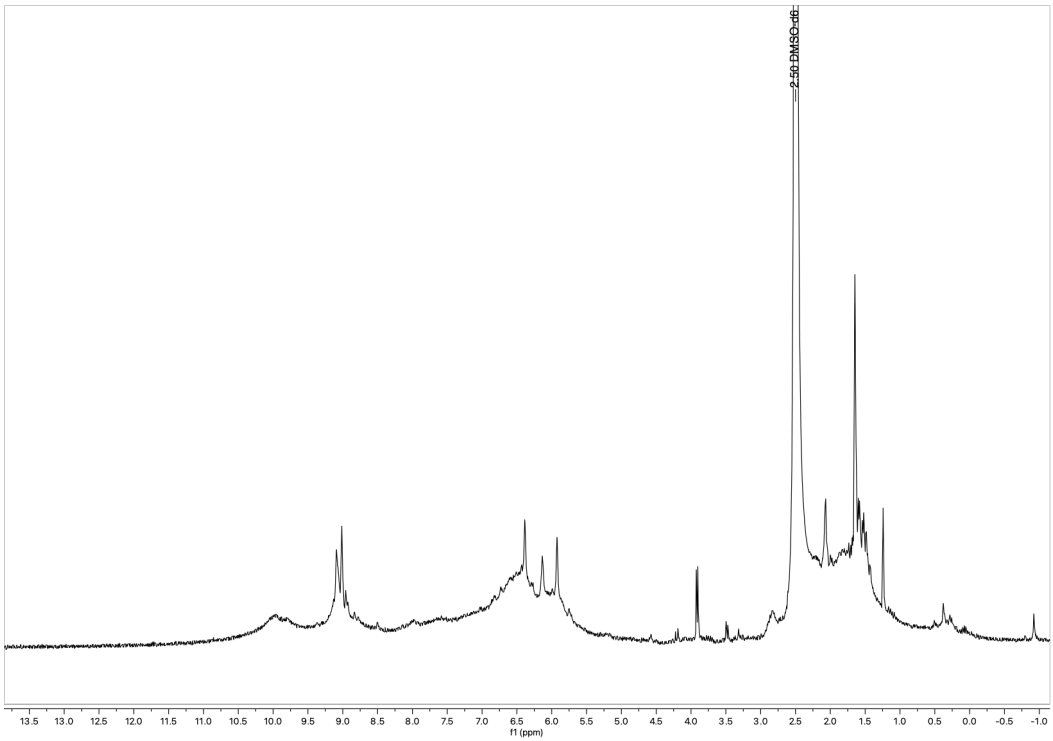

Figure S2. 1H NMR in DMSO-d_6 of **CDs-CO_2H/NH_2**.

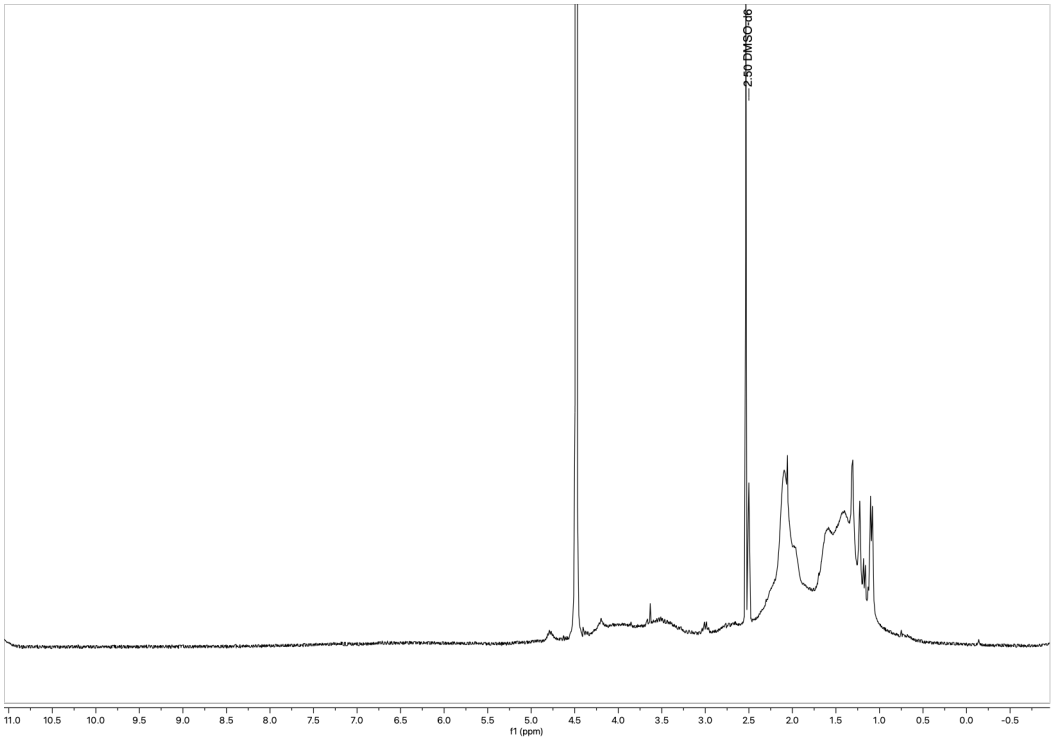

Figure S3. 1H NMR in D_2O/DMSO-d_6 1:1 of **CDs-CO_2H**.

AFM

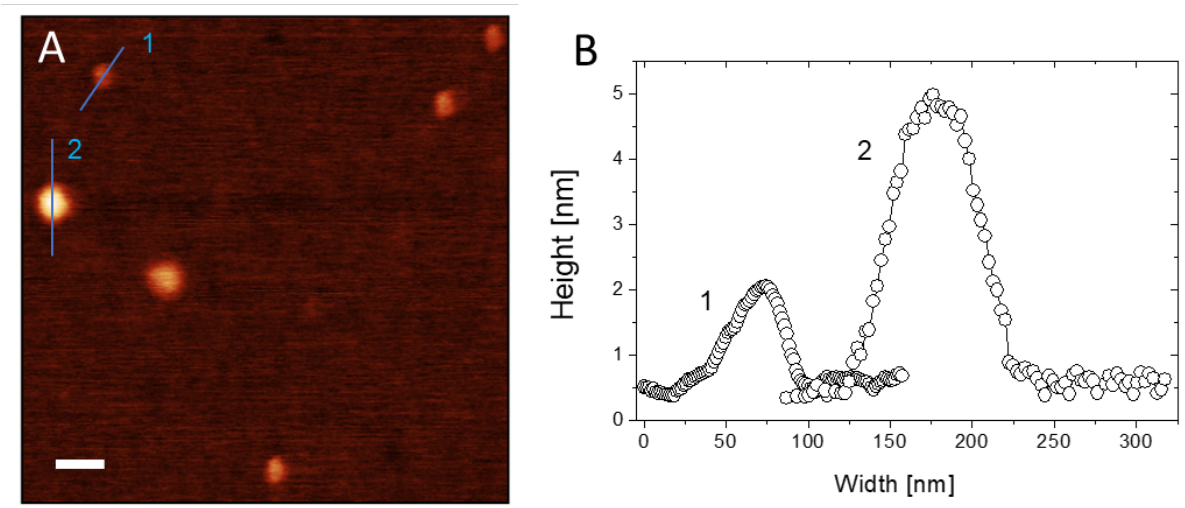

Figure S4. AFM image (height sensor) of **CDs-NH_2** in a low-density coverage condition (A, bar = 100 nm) and corresponding height profiles (B) traced on the marked sections, highlighting the maximum and minimum observed size.

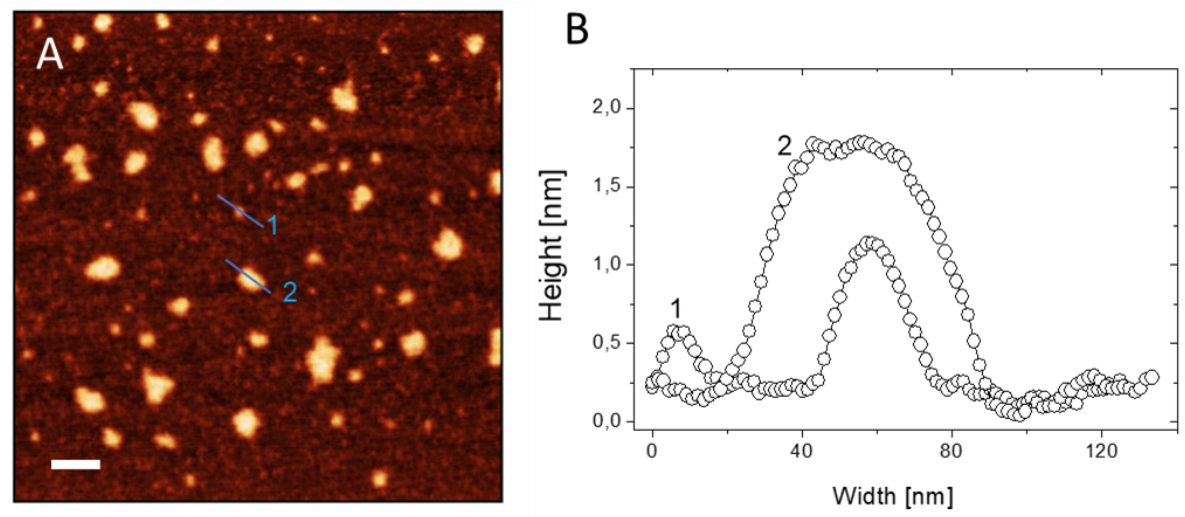

Figure S5. AFM image (height sensor) of **CDs-CO_2H/NH_2** (A, bar = 100 nm) and corresponding height profiles (B) traced on the marked sections.

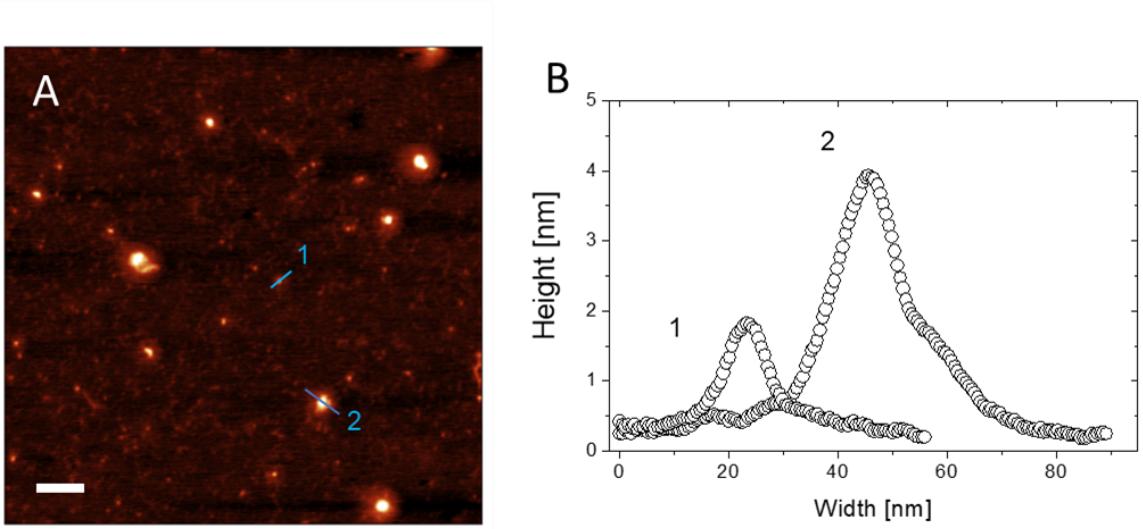

Figure S6. AFM image (height sensor) of **CDs-CO_2H** (A, bar = 100 nm) and corresponding height profiles (B)traced on the marked sections.

DLS / DELS

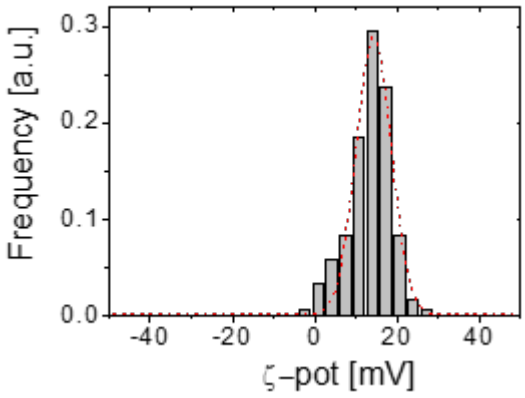

Figure S7. ζ-pot distribution (B) of **CDs-NH_2** . Dashed line shows the non-linear fit of data by a gaussian distribution, respectively, with average values of ζ-pot equal to 14.3 ± 0.21 mV

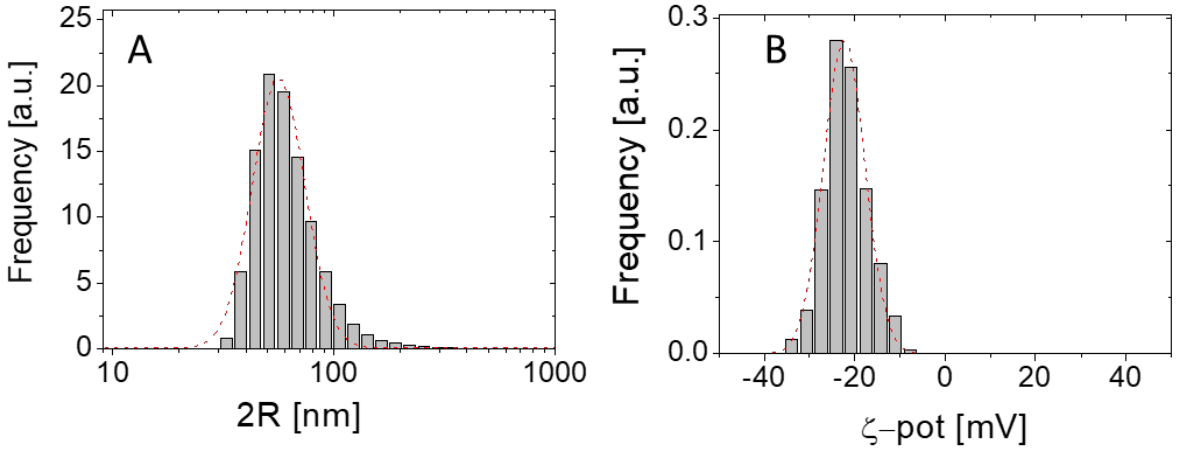

Figure S8. DLS number-weighted distribution (A) and -pot distribution (B) of **CDs-CO_2H/NH_2** . Dashed line shows the non-linear fit of data by a lognormal and a gaussian distribution, respectively, with average values of hydrodynamic diameter and ζ-pot equal to 60.44 ± 0.45 nm and -22.32 ± 0.65 mV

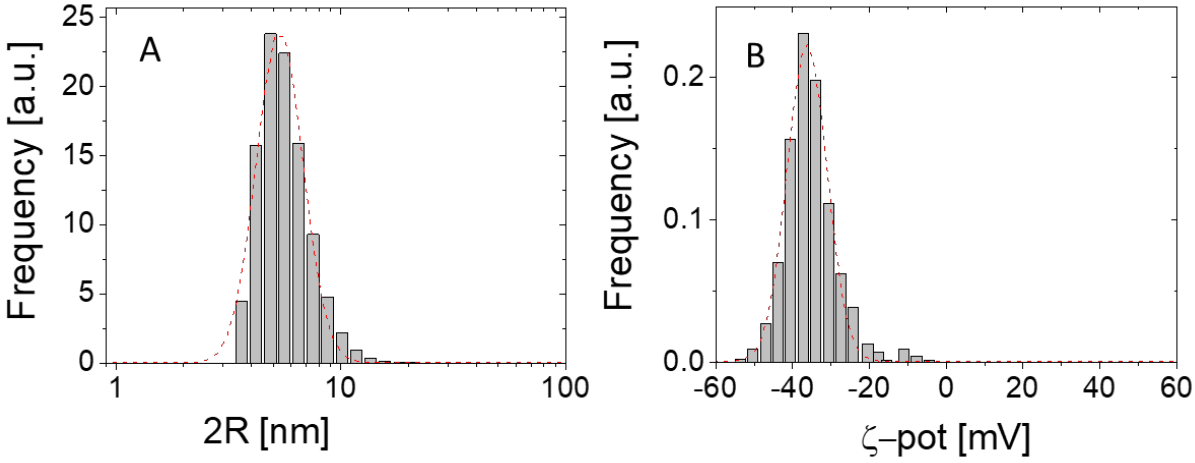

Figure S9. DLS number-weighted distribution (A) and z-pot distribution (B) of **CDs-CO_2H** . Dashed line shows the non-linear fit of data by a lognormal and a gaussian distribution, respectively, with average values of hydrodynamic diameter and ζ-pot equal to 5.65 ± 0.035 nm and -36.23 ± 0.78 mV.

XPS

XPS spectra of **CDs-NH_2**

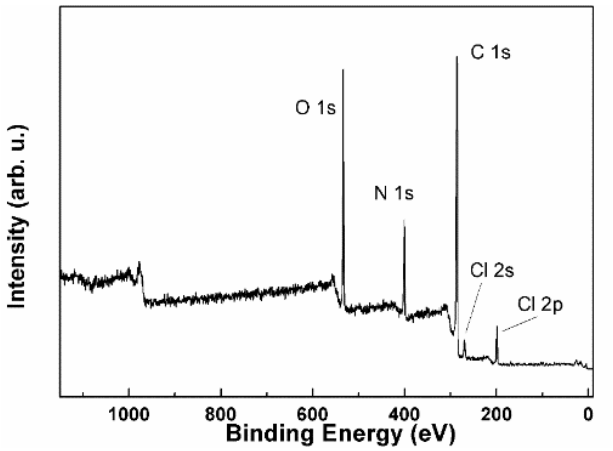

Figure S10. XPS survey spectrum of **CDs-NH$_2$.**

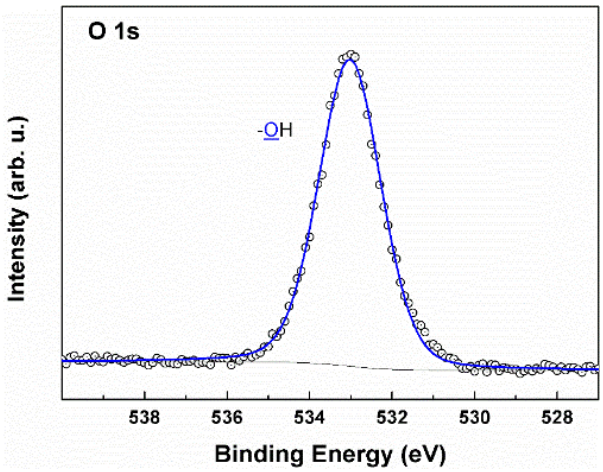

Figure S11. XPS O 1s spectrum of **CDs-NH$_2$.** Raw data are reported in dots, while curve fitting results in continuous lines. A single component assigned to –OH groups has been used.

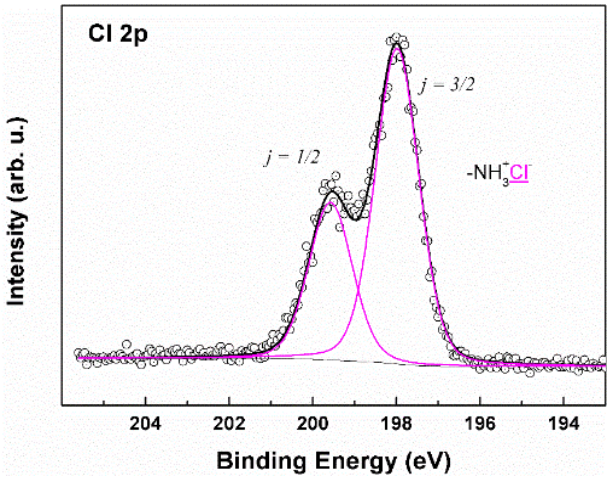

Figure S12. XPS Cl 2p spectrum of **CDs-NH$_2$.** Raw data are reported in dots, while curve fitting results in continuous lines. A single spin-orbit split component assigned to Cl^- anions has been used.

XPS spectra of **CDs-CO$_2$H/NH$_2$**

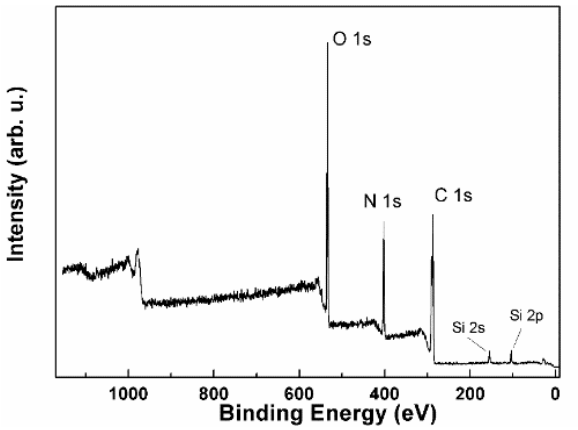

Figure S13. XPS survey spectrum of **CDs-CO_2H/NH_2**

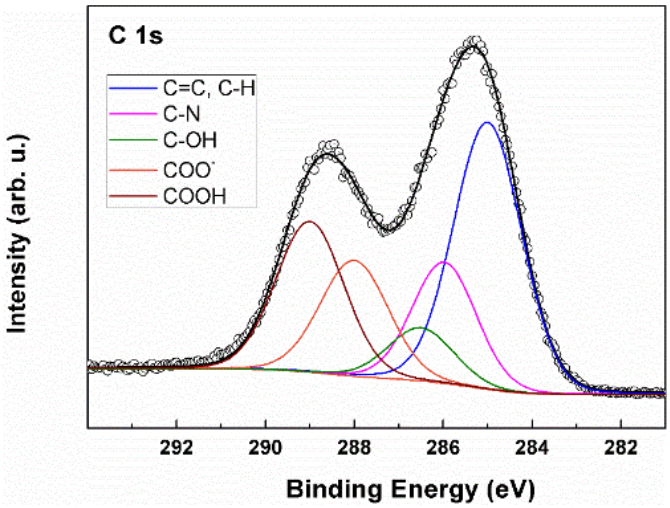

Figure S14. XPS C 1s spectrum of **CDs-CO_2H/NH_2.** Raw data are reported in dots, while curve fitting results in continuous lines.

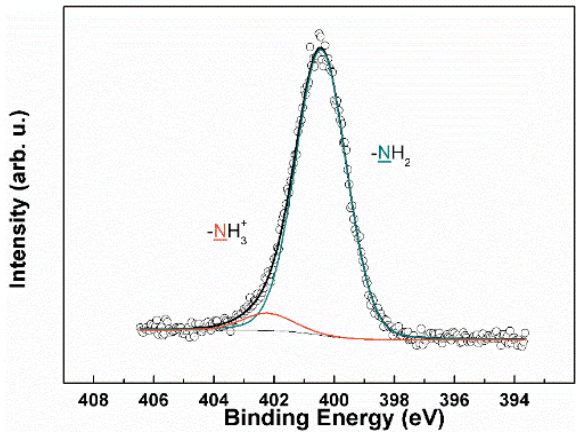

Figure S15. XPS N 1s spectrum of **CDs-CO_2H/NH_2.** Raw data are reported in dots, while curve fitting results in continuous lines.

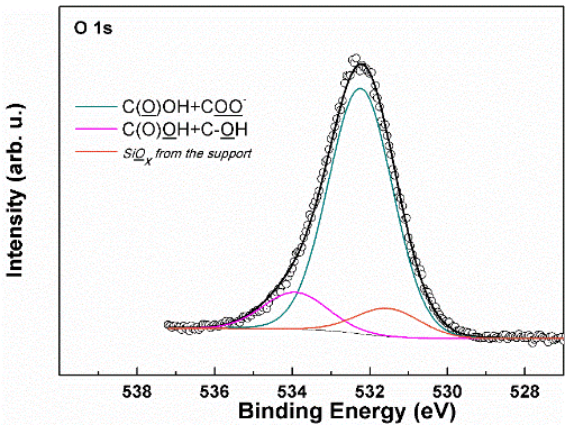

Figure S16. XPS O 1s spectrum of **CDs-CO_2H/NH_2.** Raw data are reported in dots, while curve fitting results in continuous lines.

XPS spectra of **CDs-CO_2H**

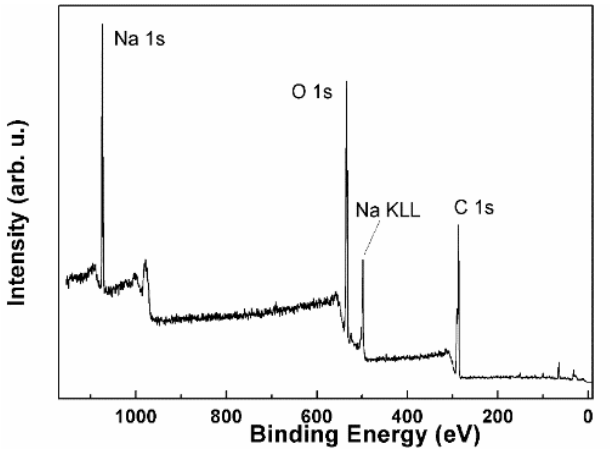

Figure S17. XPS survey spectrum of **CDs-CO_2H.**

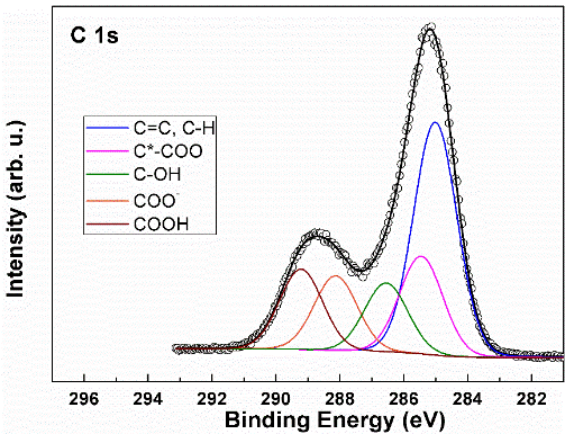

Figure S18. XPS C 1s spectrum of **CDs-CO_2H.** Raw data are reported in dots, while curve fitting results in continuous lines.

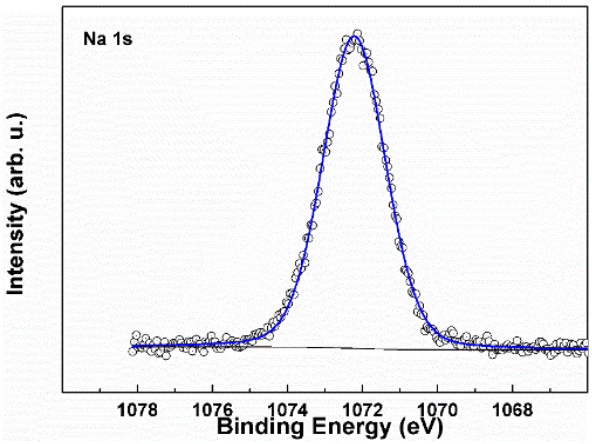

Figure S19. XPS Na 1s spectrum of **CDs-CO_2H.** Raw data are reported in dots, while curve fitting results in continuous lines. A single component assigned to Na^+ cations has been used.

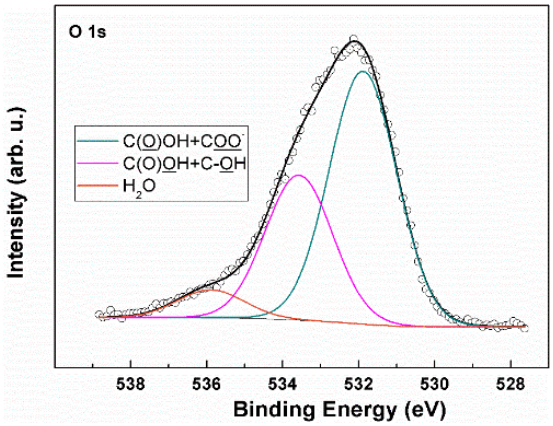

Figure S20. XPS O 1s spectrum of **CDs-CO_2H.** Raw data are reported in dots, while curve fitting results in continuous lines.

Elemental analysis

Table S3. Elemental analysis of CDs. Atoms are expressed as weight percentage.

	CDs-NH_2	**CDs-CO_2H/NH_2**	**CDs-CO_2H**
Nitrogen (%)	12.4	19.0	/
Carbon (%)	50.8	40.7	41.7
Hydrogen (%)	5.70	3.80	5.20

IR

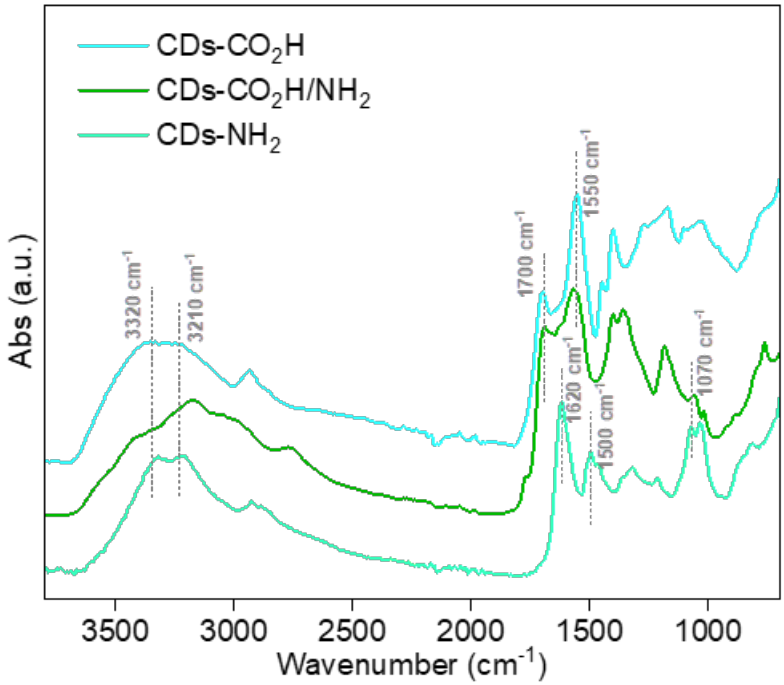

Figure S21. ATR-FTIR spectra of synthesized carbon nanodots. In the three spectra, absorption bands in the region between 3550 and 3000 cm^{-1} are related to the stretching of -NH_2 and -OH surface groups. For CDs-NH_2, the bands centered at 1620 and 1500 cm^{-1} highlighted the presence of amidic moieties, while for CDs-CO_2H/NH_2 and CDs-CO_2H the carboxylic/carboxylate groups resonated at 1700 and 1550 cm^{-1}, respectively. Lastly, the band at 1070 cm^{-1} in CDs-NH_2 and CDs-CO_2H/NH_2 is associated with the C-N stretching.

UV-Vis spectra

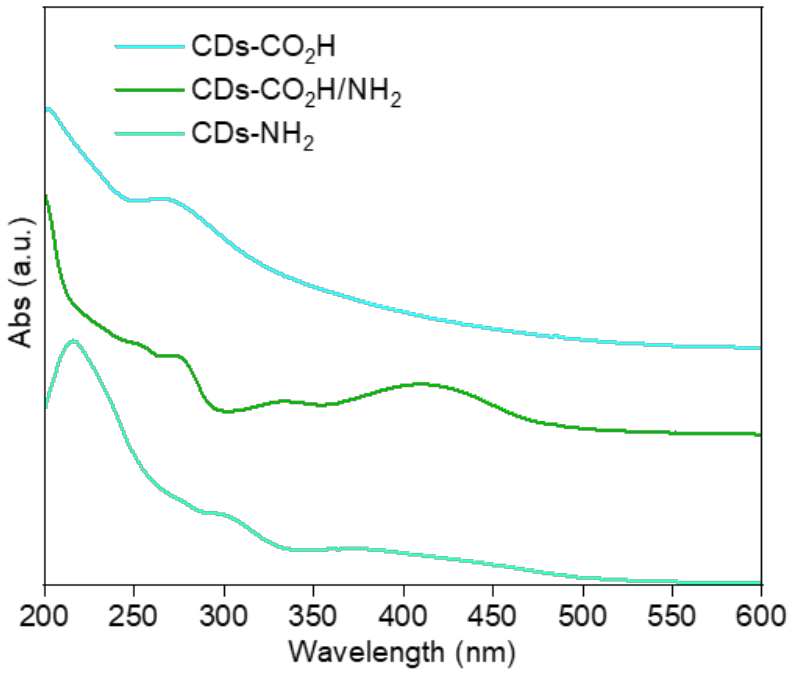

Figure S22. UV-vis spectra of synthesized carbon nanodots.

Cell cultures and cellular viability MTT assay

Table S4. Concentrations (C) of the carbon dots (CDs-NH_2, CDs-CO_2H/NH_2 or CDs-CO_2H) used in the MTT assay experiments.

	C1 (µg/mL)	C2 (µg/mL)	C3 (µg/mL)	C4 (µg/mL)	C5 (µg/mL)	C6 (µg/mL)
Carbon dots	4000	2000	1000	500	250	125

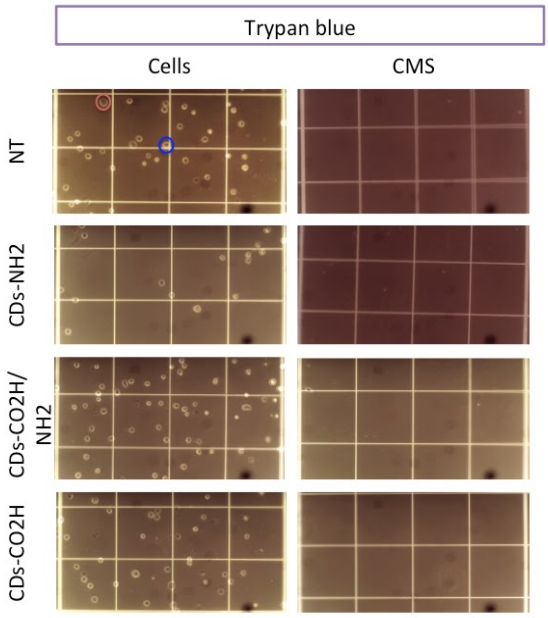

Figure S23. Trypan blue assay for live/dead cell staining of both the cells and the culture medium supernatant (CMS). Red circle dead cell, blue circle live cell.

Antifungal Susceptibility Testing

Table S5. MIC50 values of synthesized carbon nanodots

	GM MIC50 (µg/mL)	GM MIC90 (µg/mL)	GM MIC100 (µg/mL)
CDs-NH_2	397	>500	>500
CDs-CO_2H	>500	>500	>500
CDs-CO_2H/NH_2	>500	>500	>500