Facile synthesis of highly biocompatible folic acid-functionalized SiO_2 encapsulated rare-earth metal complexes nanoparticles and its application on targeted metal-based complex delivery

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Fluorescence microscopy images

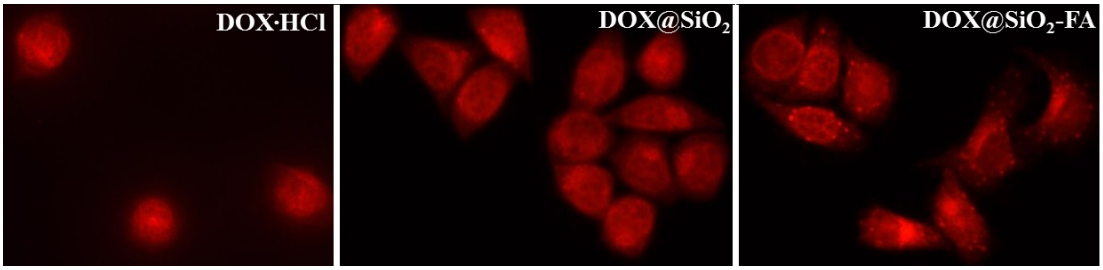

Fig. S1 Fluorescence microscopy images of HepG2 cells incubated with (a) free DOX·HCl, (b) DOX@SiO_2, (c) DOX@SiO_2–FA at 37 °C for 4 h (DOX concentration 10ugmL^{-1}).

It was difficult to imaging due to the weak fluorescent of the RC_2. To simulate this procedure of nanoparticles distribution, we choose DOX·HCl with strong fluorescent to replace the complexes (RC_2). The synthesis of DOX@SiO_2-FA was as same as RC_2@SiO_2-FA. The results show that free DOX·HCl is transported into cells via diffusion and primarily accumulated in the nuclei of the HepG2 cells where it can lead to cell death by interrupting DNA replication. DOX·HCl delivered via the DOX@SiO_2 was localized in both cytosol and nuclei of the HepG2 cells because it was taken up by the cells through a non-specific endocytosis mechanism and the DOX·HCl released from DOX@SiO_2 could be transported into cells via diffusion and accumulated in the nuclei. In contrast, DOX·HCl delivered via the DOX@SiO_2–FA was mainly localized in cytosol and dot-shaped fluorescence was observed within cytoplasm, and was considered to be SiO_2 spheres trapped in the endocytic vesicles. Thus, by analyzing the above results, we can prove that the Si-nanoparticles containing rare-earth metals are really delivered into the cells.

The stability test for nanoparticles

In order to test the stability of nanoparticles, the same concentration of RC_2@SiO_2 and RC_2@SiO_2–FA in the PBS (pH=7.4) for six days, from the picture, we can see in the first five days, RC_2@SiO_2 and RC_2@SiO_2–FA were stable enough without precipitation. But from the sixth day, the RC_2@SiO_2 began to precipitate, while the RC_2@SiO_2–FA is still stable, so that the RC_2@SiO_2–FA is sufficiently stable.

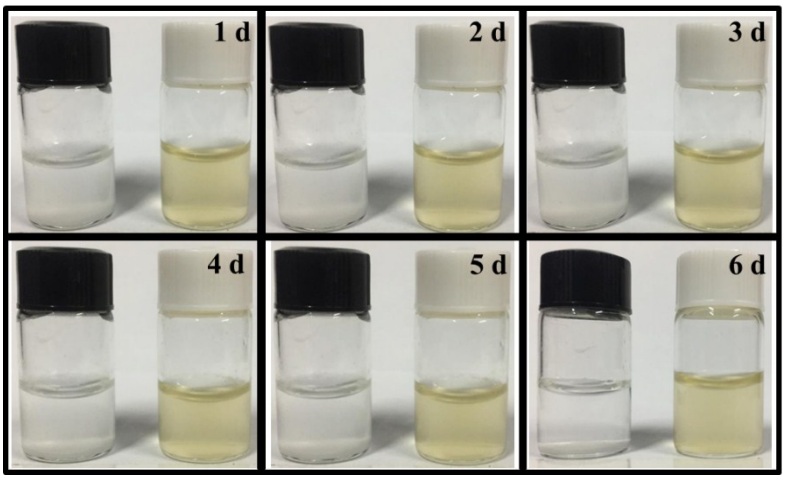

Fig. S2 The stability of the two kinds of nanoparticles(left: $RC_2@SiO_2$; right: $RC_2@SiO_2$–FA).

In vitro drug release

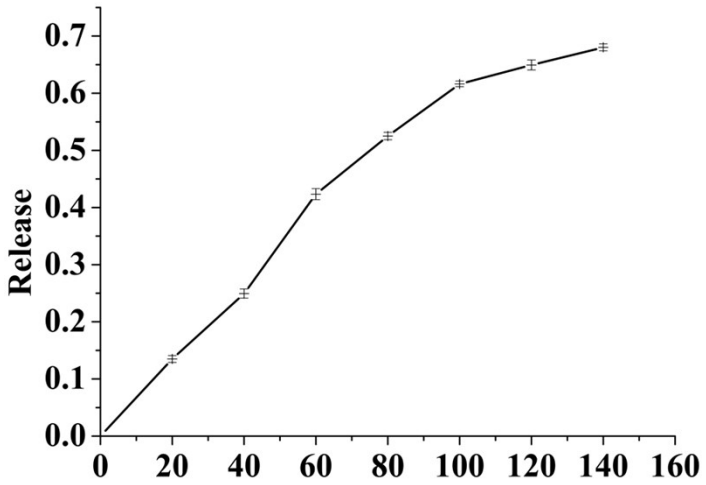

Fig. S3The release curve of $RC_2@SiO_2$–FA. The error bars in the graph represent standard deviations (n=3).

In vitro cytotoxicity studies

The drug release behavior of the nanoparticles was evaluated at 37 ^oC in PBS (pH=7.4). Nanoparticles which showed a slow release of RC_2 from $RC_2@SiO_2$–FA. As showed in Fig S3, about 13.5% of RC_2 was released in the first 20 h and roughly 61.63% released within 100 h. After that, the release becomes slow, the release rate up to 68.03% after140 hours.

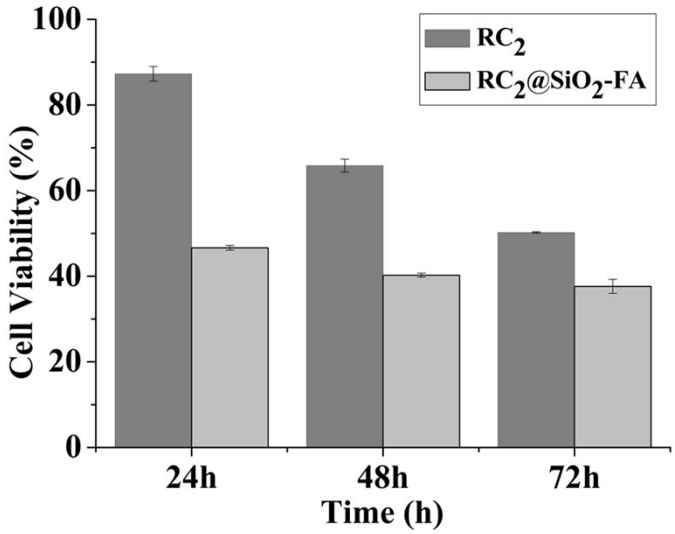

Fig. S4 Cell viability of HepG2 cells treated with RC_2@SiO_2–FA and free RC_2 after 24 h, 48 h and 72 h. Data represent mean ± SD (n=5).

Cell viability was evaluated after incubation with 100 ug/mL of free RC_2 and RC_2@SiO_2–FA containing a corresponding RC_2 content for 24 h, 48 h and 72 h. After 24 h incubation, a comparison was made between RC_2@SiO_2–FA and free RC_2 at equivalent concentration. Fig S4 showed the difference was the cytotoxicity of free RC_2 is inferior to RC_2@SiO_2–FA at equal concentrations. This may be attributed to the different intracellular uptake rate of free RC_2 (the passive diffusion mechanism) and nanoparticles (endocytosis, EPR effect). After 48 h and 72 h incubation at 100 μg/mL concentration of free RC_2, the cytotoxicity of free RC_2 had an obvious raise, which based on the fact that more amounts of free RC_2 would transported into cells.

Table 2. Selected bond lengths (Å) and angles (°) for 1-5

		1	
Er(1)-O(8)A	2.255(7)	Er(1)-O(2W)	2.400(7)
Er(1)-O(4)	2.283(7)	Er(1)-O(1)	2.428(7)
Er(1)-O(7)	2.343(7)	Er(1)-O(2)	2.443(8)
Er(1)-O(1W)	2.366(7)	Er(1)-O(3W)	2.445(6)
O(8)A-Er(1)-O(4)	146.8(3)	O(8)A-Er(1)-O(1)	80.0(3)
O(8)A-Er(1)-O(7)	103.0(2)	O(4)-Er(1)-O(1)	76.4(2)
O(4)-Er(1)-O(7)	85.3(2)	O(7)-Er(1)-O(1)	147.4(2)
O(8)A-Er(1)-O(1W)	139.6(2)	O(1W)-Er(1)-O(1)	116.0(2)
O(4)-Er(1)-O(1W)	72.8(2)	O(2W)-Er(1)-O(1)	75.0(2)
O(7)-Er(1)-O(1W)	83.0(2)	O(8)A-Er(1)-O(2)	83.7(3)
O(8)A-Er(1)-O(2W)	73.2(3)	O(4)-Er(1)-O(2)	100.2(3)
O(4)-Er(1)-O(2W)	78.2(2)	O(7)-Er(1)-O(2)	158.7(3)
O(7)-Er(1)-O(2W)	75.0(2)	O(1W)-Er(1)-O(2)	79.2(3)
O(1W)-Er(1)-O(2W)	144.7(2)	O(2W)-Er(1)-O(2)	126.2(3)
O(1)-Er(1)-O(2)	53.2(2)	O(1W)-Er(1)-O(3W)	68.9(2)
O(8)A-Er(1)-O(3W)	72.6(3)	O(2W)-Er(1)-O(3W)	132.2(2)
O(4)-Er(1)-O(3W)	140.6(2)	O(1)-Er(1)-O(3W)	129.2(2)
O(7)-Er(1)-O(3W)	81.4(3)	O(2)-Er(1)-O(3W)	81.4(2)
		2	
Ho(1)-O(8)A	2.245(7)	Ho(1)-O(2W)	2.386(7)
Ho(1)-O(4)	2.272(7)	Ho(1)-O(2)	2.416(7)
Ho(1)-O(1W)	2.348(7)	Ho(1)-O(1)	2.418(8)
Ho(1)-O(7)	2.355(7)	Ho(1)-O(3W)	2.456(7)

O(8)A-Ho(1)-O(4)	146.8(3)	O(8)A-Ho(1)-O(2)	85.4(3)
O(8)A-Ho(1)-O(1W)	140.4(3)	O(4)-Ho(1)-O(2)	99.4(3)
O(4)-Ho(1)-O(1W)	71.9(2)	O(1W)-Ho(1)-O(2)	76.7(3)
O(8)A-Ho(1)-O(7)	102.5(3)	O(7)-Ho(1)-O(2)	156.8(2)
O(4)-Ho(1)-O(7)	85.9(3)	O(2W)-Ho(1)-O(2)	127.6(2)
O(1W)-Ho(1)-O(7)	83.7(2)	O(8)A-Ho(1)-O(1)	79.8(3)
O(8)A-Ho(1)-O(2W)	72.4(3)	O(4)-Ho(1)-O(1)	76.9(3)
O(4)-Ho(1)-O(2W)	79.1(3)	O(1W)-Ho(1)-O(1)	115.0(3)
O(1W)-Ho(1)-O(2W)	145.2(2)	O(7)-Ho(1)-O(1)	148.2(3)
O(7)-Ho(1)-O(2W)	75.5(2)	O(2W)-Ho(1)-O(1)	75.1(2)
O(2)-Ho(1)-O(1)	54.2(2)	O(7)-Ho(1)-O(3W)	80.2(3)
O(8)A-Ho(1)-O(3W)	72.7(3)	O(2W)-Ho(1)-O(3W)	131.3(3)
O(4)-Ho(1)-O(3W)	140.5(3)	O(2)-Ho(1)-O(3W)	81.5(2)
O(1W)-Ho(1)-O(3W)	69.9(2)	O(1)-Ho(1)-O(3W)	129.3(3)
		3	
Sm(1)-O(4)	2.396(5)	Sm(1)-O(2)A	2.502(5)
Sm(1)-O(8)B	2.409(5)	Sm(1)-O(8)	2.512(5)
Sm(1)-O(5)B	2.414(5)	Sm(1)-O(7)	2.520(5)
Sm(1)-O(1)	2.450(5)	Sm(1)-O(1)A	2.548(5)
O(4)-Sm(1)-O(8)B	74.29(17)	O(4)-Sm(1)-O(8)	72.79(17)
O(4)-Sm(1)-O(5)B	138.10(16)	O(8)B-Sm(1)-O(8)	72.50(19)
O(8)B-Sm(1)-O(5)B	72.31(18)	O(5)B-Sm(1)-O(8)	73.58(16)
O(4)-Sm(1)-O(1W)	75.99(17)	O(1W)-Sm(1)-O(8)	121.02(17)
O(8)B-Sm(1)-O(1W)	141.09(18)	O(1)-Sm(1)-O(8)	104.73(16)
O(5)B-Sm(1)-O(1W)	144.31(18)	O(4)-Sm(1)-O(7)	74.70(19)
O(4)-Sm(1)-O(1)	142.05(18)	O(5)B-Sm(1)-O(7)	102.34(19)
O(5)B-Sm(1)-O(1)	71.16(17)	O(1W)-Sm(1)-O(7)	72.86(18)
O(1W)-Sm(1)-O(1)	73.49(18)	O(1)-Sm(1)-O(7)	75.15(16)
O(4)-Sm(1)-O(2)A	78.92(18)	O(2)A-Sm(1)-O(7)	143.66(19)
O(1W)-Sm(1)-O(2)A	76.8(2)	O(8)-Sm(1)-O(7)	51.30(15)
O(1)-Sm(1)-O(2)A	114.76(16)	O(8)B-Sm(1)-O(1)A	105.45(16)
		4	
Pr(1)B-O(1)	2.537(2)	Pr(1)A-O(5)	2.420(3)
Pr(1)-O(1)	2.594(2)	Pr(1)-O(7)	2.432(2)
Pr(1)-O(2)	2.542(3)	Pr(1)A-O(7)	2.621(2)
Pr(1)-O(4)	2.450(3)	Pr(1)A-O(8)	2.560(3)
Pr(1)-O(1W)	2.523(3)		
O(5)B-Pr(1)-O(7)	144.80(8)	O(1W)-Pr(1)-O(2)	74.07(9)
O(5)B-Pr(1)-O(4)	76.58(10)	O(5)B-Pr(1)-O(8)B	83.30(10)
O(7)-Pr(1)-O(4)	77.89(9)	O(7)-Pr(1)-O(8)B	102.57(8)
O(5)B-Pr(1)-O(1W)	141.09(9)	O(4)-Pr(1)-O(8)B	141.48(9)
O(7)-Pr(1)-O(1W)	71.87(8)	O(1W)-Pr(1)-O(8)B	72.64(9)
O(4)-Pr(1)-O(1W)	139.52(9)	O(1)A-Pr(1)-O(8)B	72.19(8)
O(5)B-Pr(1)-O(1)A	81.89(9)	O(2)-Pr(1)-O(8)B	145.29(9)
O(5)B-Pr(1)-O(2)	118.32(10)	O(7)-Pr(1)-O(1)	124.98(8)
O(7)-Pr(1)-O(2)	75.85(8)	O(4)-Pr(1)-O(1)	95.23(9)
O(4)-Pr(1)-O(2)	72.88(10)	O(1W)-Pr(1)-O(1)	81.18(9)
O(2)-Pr(1)-O(1)	50.60(8)	O(4)-Pr(1)-O(7)B	144.20(9)
		5	
Ce(1)-O(5)A	2.4546(16)	Ce(1)-O(1W)	2.5860(18)
Ce(1)-O(8)A	2.4775(17)	Ce(1)-O(4)	2.6303(17)
Ce(1)-O(1)B	2.4871(16)	Ce(1)-O(2W)	2.6459(18)
Ce(1)-O(7)	2.5150(16)	Ce(1)-O(5)	2.7563(17)
Ce(1)-O(2)	2.5857(17)	Ce(1)-O(1)	2.8146(17)
O(5)A-Ce(1)-O(8)A	70.98(6)	O(1W)-Ce(1)-O(4)	69.85(6)

O(8)A-Ce(1)-O(7)	129.25(5)	O(1)B-Ce(1)-O(2W)	81.13(6)
O(8)A-Ce(1)-O(2)	72.31(6)	O(7)-Ce(1)-O(2W)	65.57(6)
O(7)-Ce(1)-O(2)	138.25(6)	O(2)-Ce(1)-O(2W)	74.82(6)
O(5)A-Ce(1)-O(1W)	135.56(6)	O(1W)-Ce(1)-O(2W)	133.41(5)
O(8)A-Ce(1)-O(1W)	66.18(6)	O(4)-Ce(1)-O(2W)	137.67(6)
O(7)-Ce(1)-O(1W)	141.71(6)	O(1)B-Ce(1)-O(5)	114.68(5)
O(2)-Ce(1)-O(1W)	76.76(6)	O(7)-Ce(1)-O(5)	65.89(5)
O(5)A-Ce(1)-O(4)	117.45(6)	O(2)-Ce(1)-O(5)	134.57(5)
O(8)A-Ce(1)-O(4)	84.26(6)	O(1W)-Ce(1)-O(5)	102.52(5)
O(7)-Ce(1)-O(4)	76.88(6)	O(4)-Ce(1)-O(5)	47.86(5)
O(2)-Ce(1)-O(4)	144.86(6)	O(2W)-Ce(1)-O(5)	123.71(5)
O(8)A-Ce(1)-O(1)	110.62(5)	O(1W)-Ce(1)-O(1)	68.68(5)
O(7)-Ce(1)-O(1)	119.05(5)	O(4)-Ce(1)-O(1)	124.03(5)
O(2)-Ce(1)-O(1)	47.82(5)	O(2W)-Ce(1)-O(1)	64.83(5)
O(5)-Ce(1)-O(1)	170.78(5)		

Symmetry transformations used to generate equivalent atoms in **1**: A -x+1,-y,-z ; **2**: A -x+1,-y,-z ;**3**: A -x,-y+2,-z+1 ; B -x+1,-y+2,-z+1 ; **4**: A x,-y+1/2,z-1/2 ; B x,-y+1/2,z+1/2 ;**5**: A -x,-y,-z+1 ; B -x,-y+1,-z+1.

Table 3. Bond lengths (Å) and angles (°) of hydrogen-bond for **1-5**.

D–H···A	***d(D–H)***	***d(H···A)***	***d(D···A)***	***∠DHA***
		1		
O1W-H1WA···O2	0.96	1.89	2.797(10)	156
O3W-H3WA···O3	0.96	2.08	3.032(10)	169
O3W-H3WA···O2	0.96	2.58	3.200(11)	122

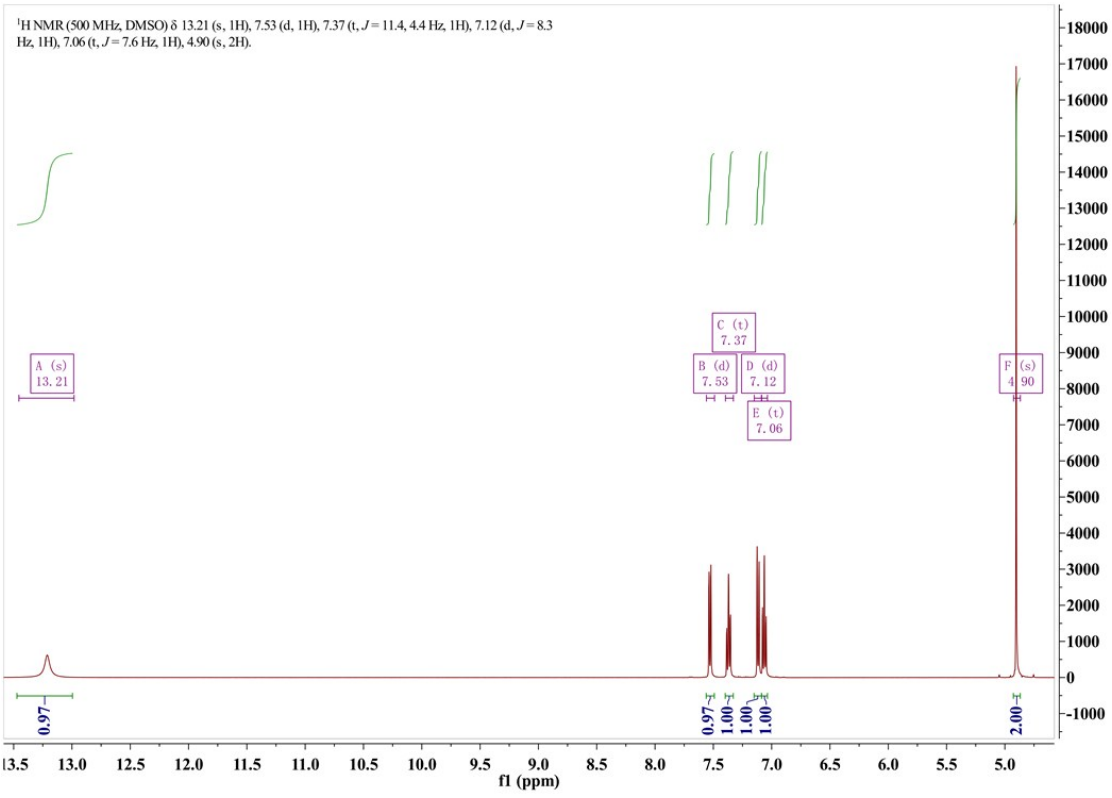

Fig. S5 ¹HNMR spectra of ***o*-HCPA**

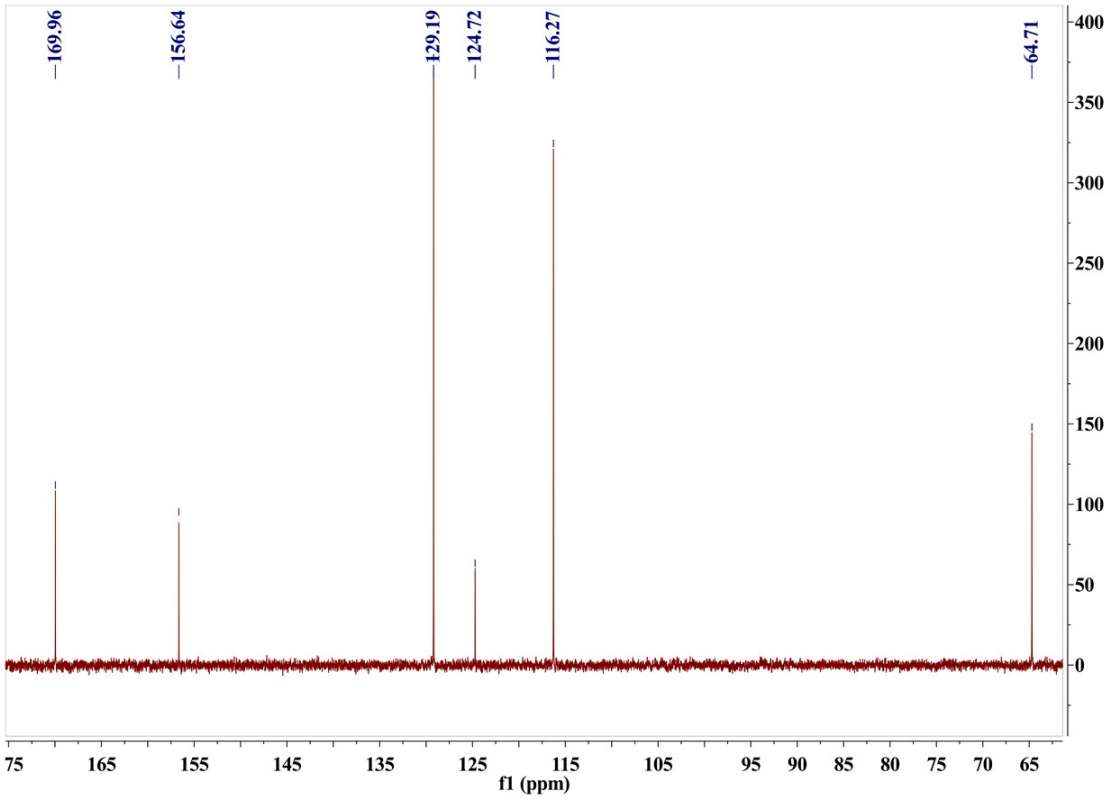

Fig. S6 ^{13}CNMR spectra of ***o*-HCPA**

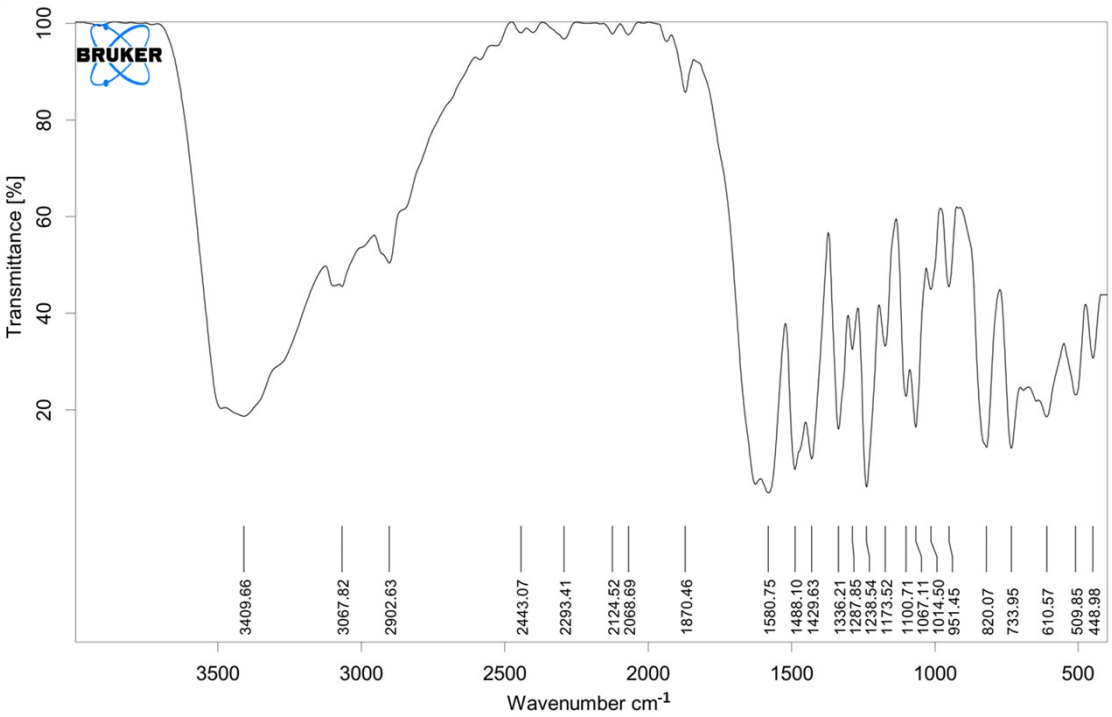

Fig. S7 IR spectra of compounds **1**.

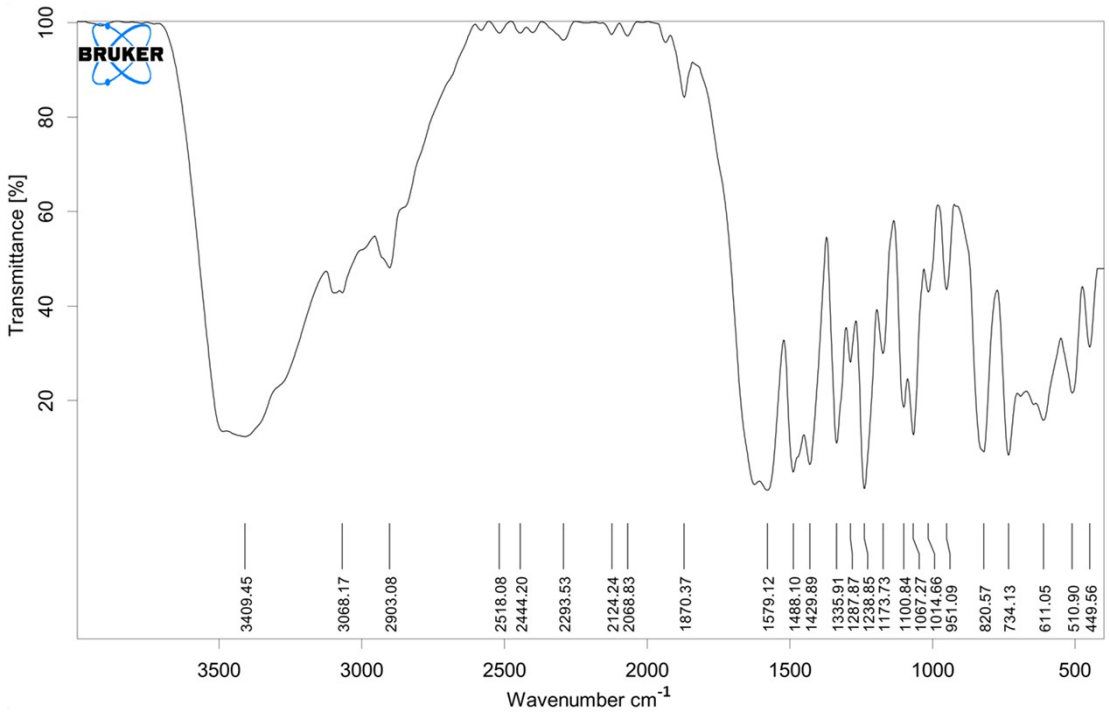

Fig. S8 IR spectra of compounds **2**.

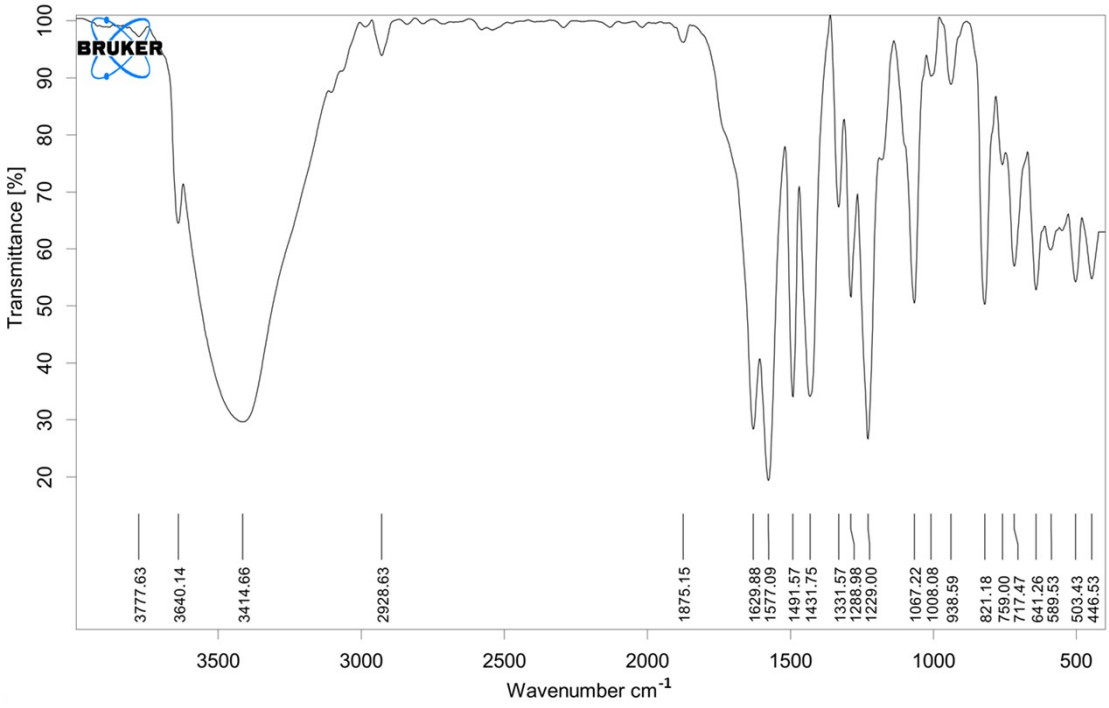

Fig. S9 IR spectra of compounds **3**.

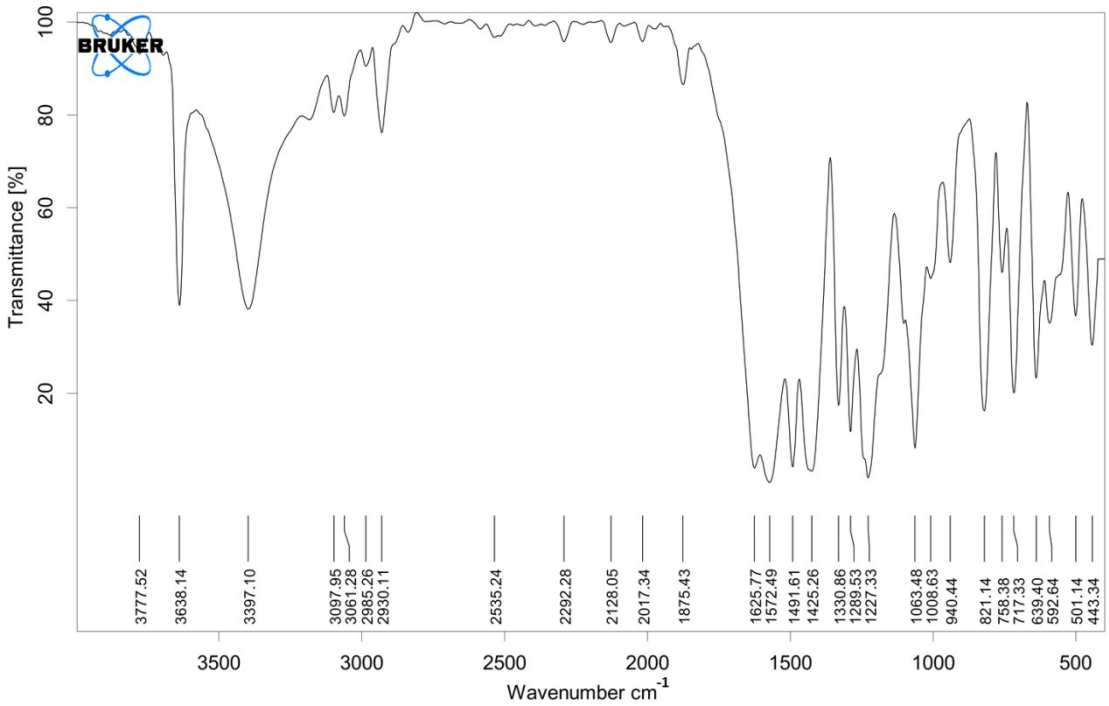

Fig. S10 IR spectra of compounds **4**.

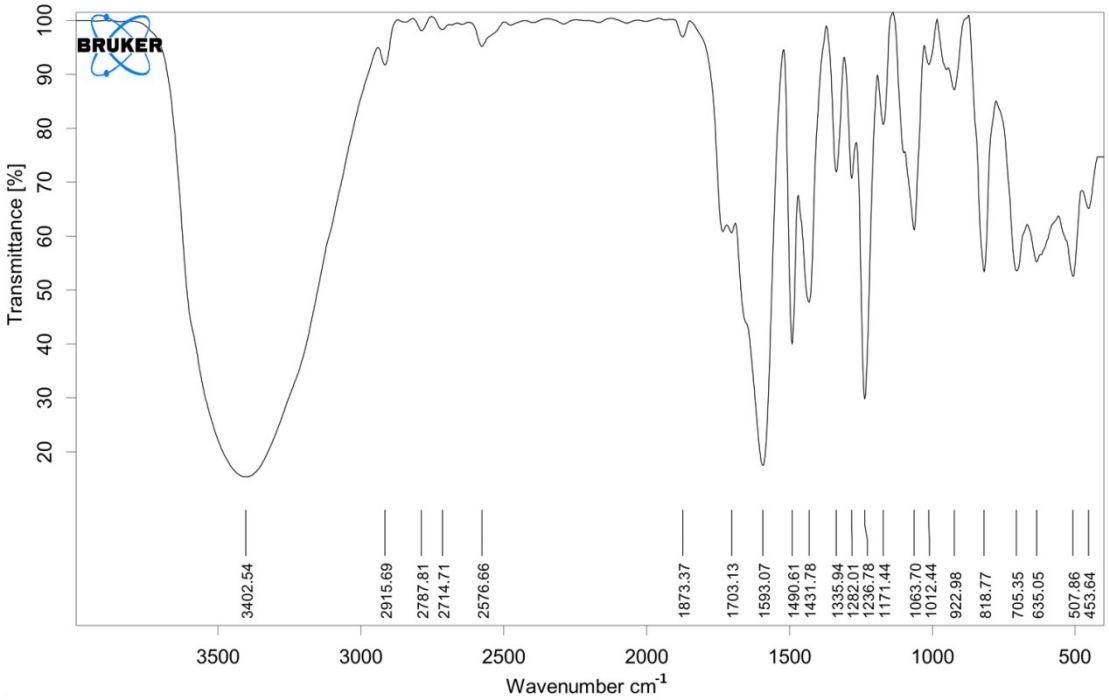

Fig. S11 IR spectra of compounds **5**.