Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2023

Synthesis, nanostructuring and *in silico* studies of a new imine bond containing macroheterocycle as a promising PBP-2a non-β-lactam inhibitor

Alakbar Huseynzada*[a,c,e,p], Mirjavid Aghayev[d], Sarvinaz Hajiyeva[a,q], Aygun Israyilova[b,c,f,o,p], Koray Sayin[g], Eldar Gasimov[h], Fuad Rzayev[i], Ulviyya Hasanova[a,c,p], Goncha Eyvazova[j], Vagif Abbasov[k], Zarema Gakhramanova[c], Sanam Huseynova[o], Parvana Huseynova[l], Lala Huseynova[m], Nigar Salimova[n]

[a]ICRL, Baku State University, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan.

[b]Laboratory of Microbiology and Virology, Baku State University, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan.

[c]GPOGC SRI, Azerbaijan State Oil and Industry University, Baku, AZ 1010, Azerbaijan.

[d]Department of Pharmaceutical Sciences, Northeast Ohio Medical University, 4209 St, OH-44, Rootstown, OH 44272, USA

[e]Chemistry Department, Azerbaijan Engineers Union, Bashir Safaroglu 118, Baku, AZ 1009, Azerbaijan

[f]Research Institute of Crop Husbandry, Ministry of Agriculture, Baku, AZ 1098, Azerbaijan

[g]Chemistry Department, Faculty of Science, Sivas Cumhuriyet University, Sivas, 58140, Türkiye

[h]Department of Cytology, Embryology and Histology, Azerbaijan Medical University, 163 A Samad Vurgun, Baku AZ1078, Azerbaijan

[i]Laboratory of Electron Microscopy of the SRC, Azerbaijan Medical University, 163 A Samad Vurgun, Baku AZ1078, Azerbaijan

[j]Nanoresearch Center, Baku State University, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan

[k]Institute of Petrochemical Processes, K. Avenue 30, Baku, AZ 1005, Azerbaijan

[l]Chemistry Department, Ganja State University, H. Aliyev 429, Ganja, AZ 2001, Azerbaijan

[m]Industrial Safety and Labor Protection Department, Azerbaijan State Oil and Industry University, Baku, AZ 1010, Azerbaijan

[n]Petrochemical Technology and Industrial Ecology Department, Azerbaijan State Oil and Industry University, Baku, AZ 1010, Azerbaijan

[o]Department of Molecular Biology and Biotechnology, Baku State University, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan

[p]ICESCO Biomedical Materials Department, Baku State University, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan

[q]Physics Department, Kent State University, 800 E. Summit St., Kent, OH 44242, USA

Supplementary Materials

Table of contents

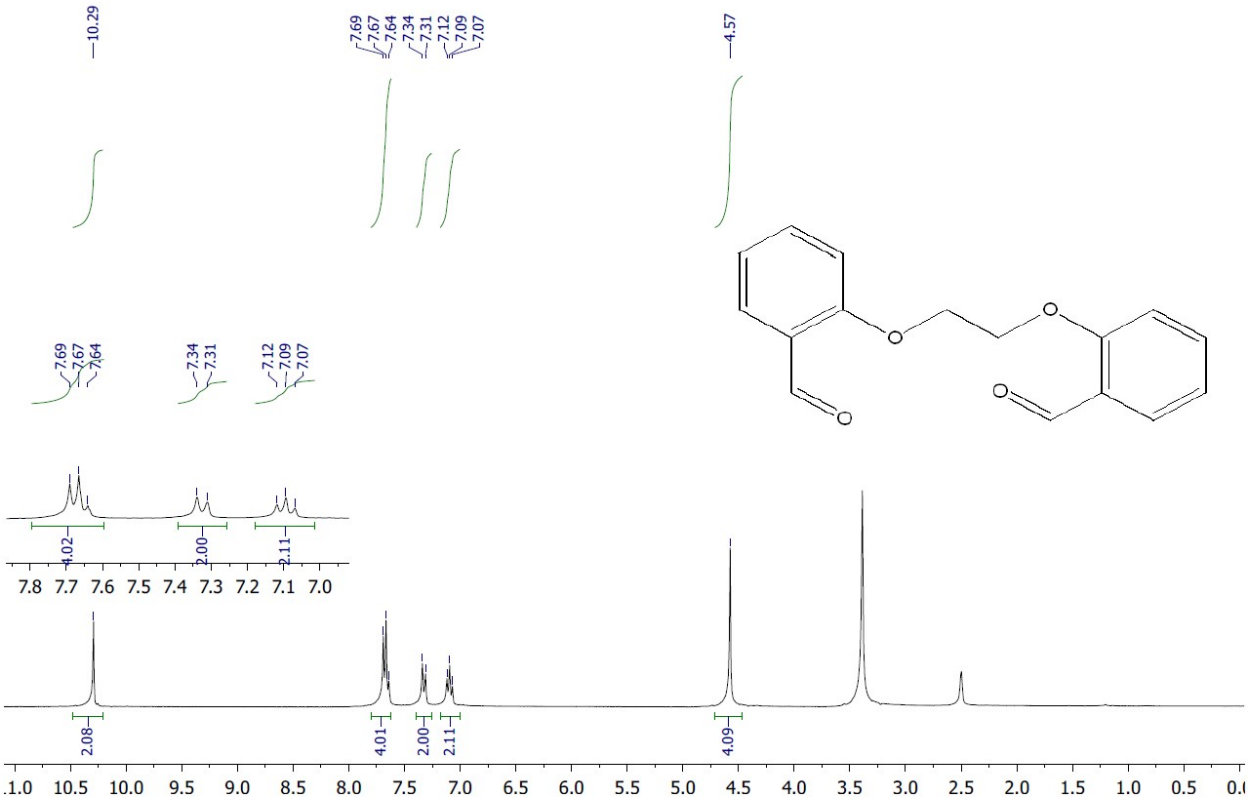

Figure 1S.[1]H NMR spectrum of synthesized dialdehyde **1**

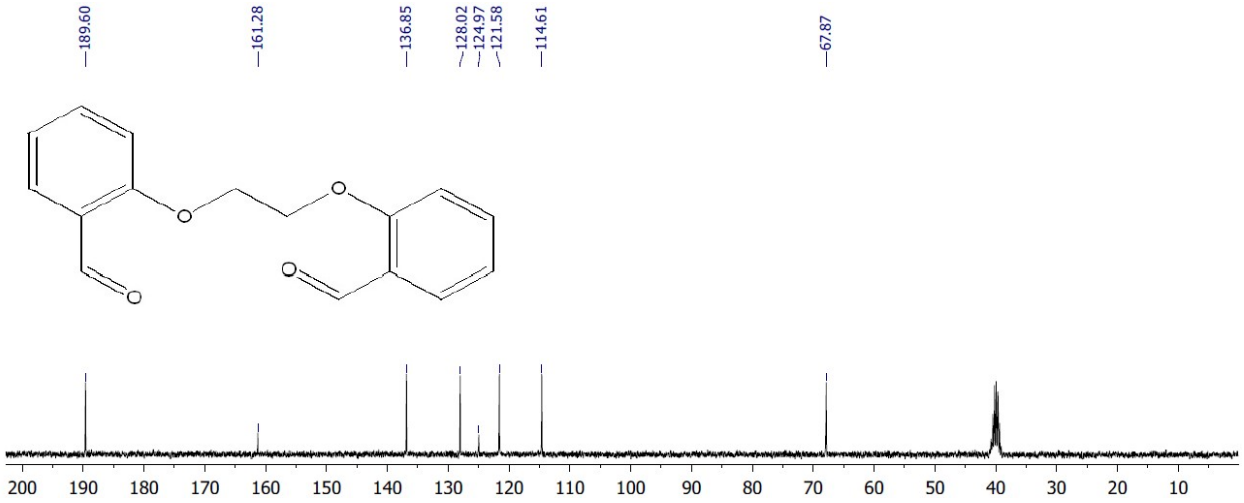

Figure 2S.[13]C NMR spectrum of synthesized dialdehyde **1**

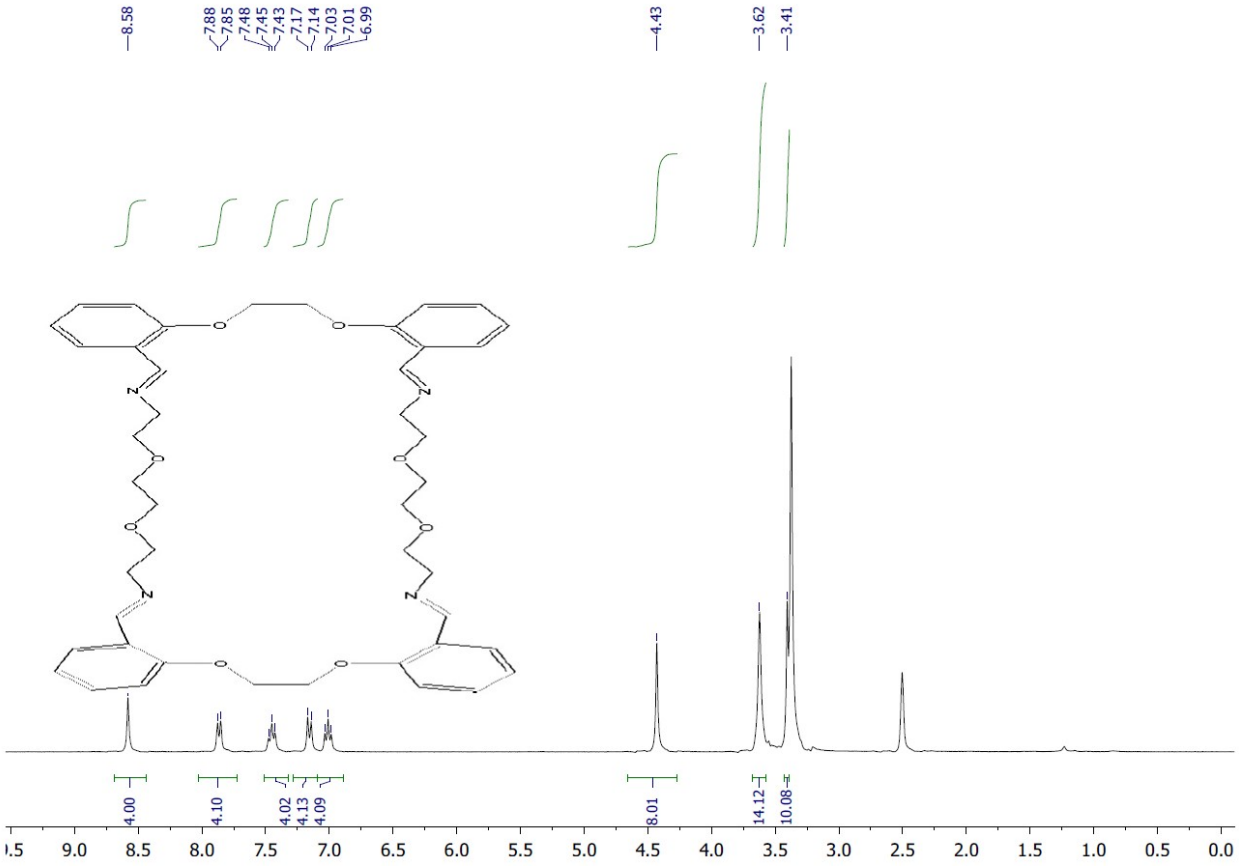

Figure 3S. [1]H NMR spectrum of synthesized macrocycle **3**

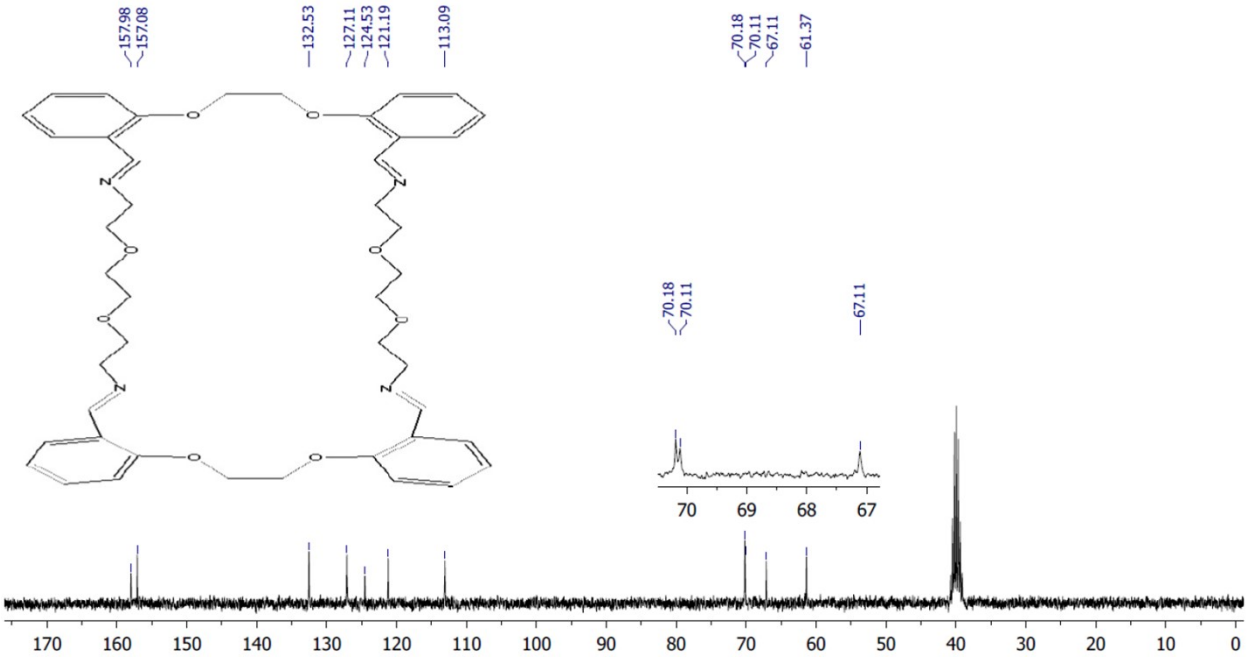

Figure 4S. [13]C NMR spectrum of synthesized macrocycle **3**

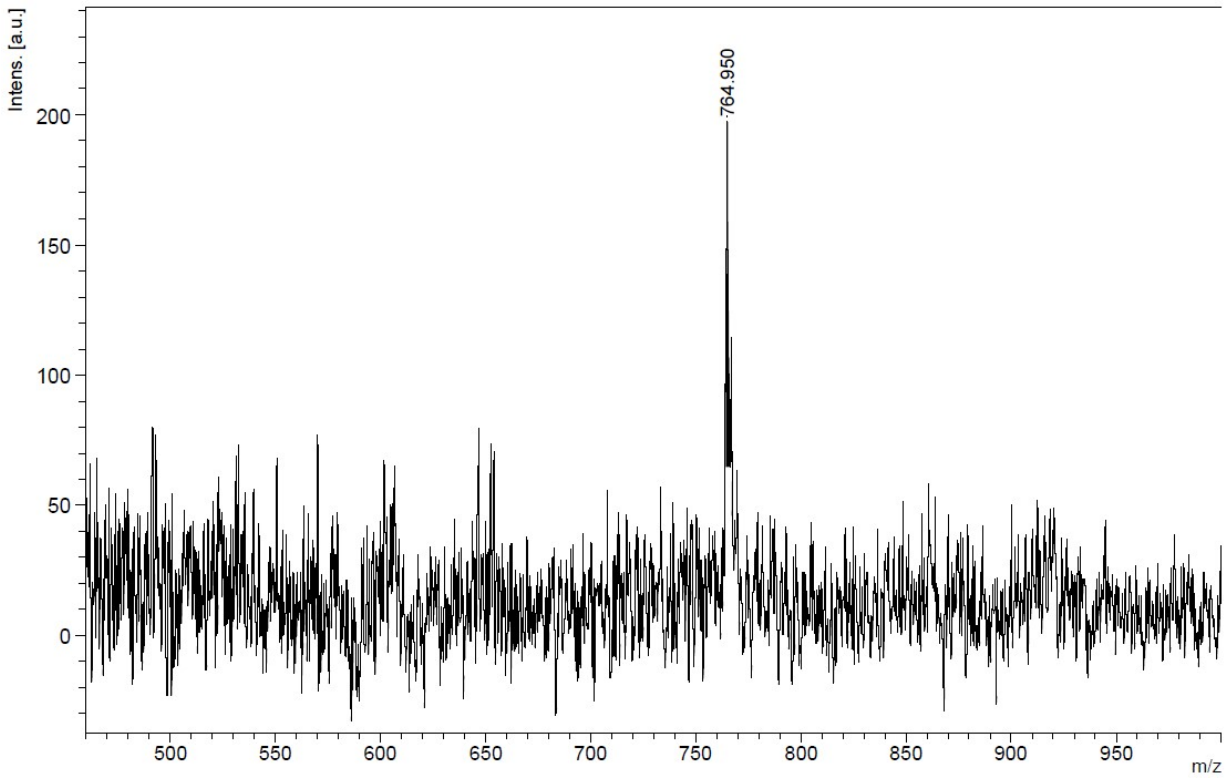

Figure 5S. MALDI-MS spectrum of synthesized macrocycle **3**

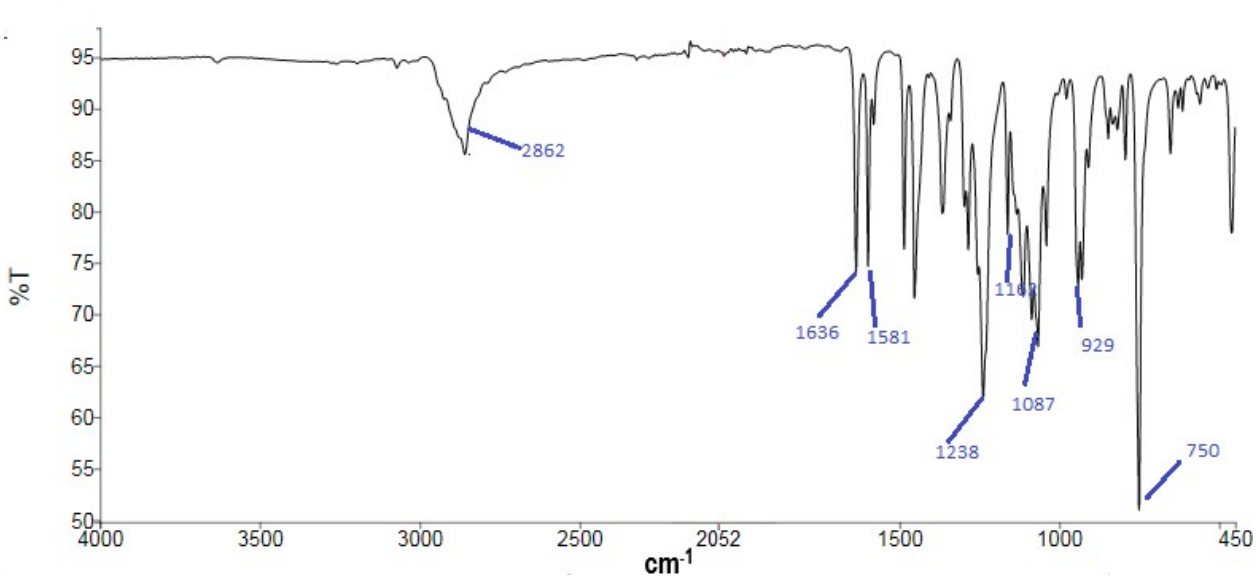

Figure 6S. FTIR spectrum of the MHC3

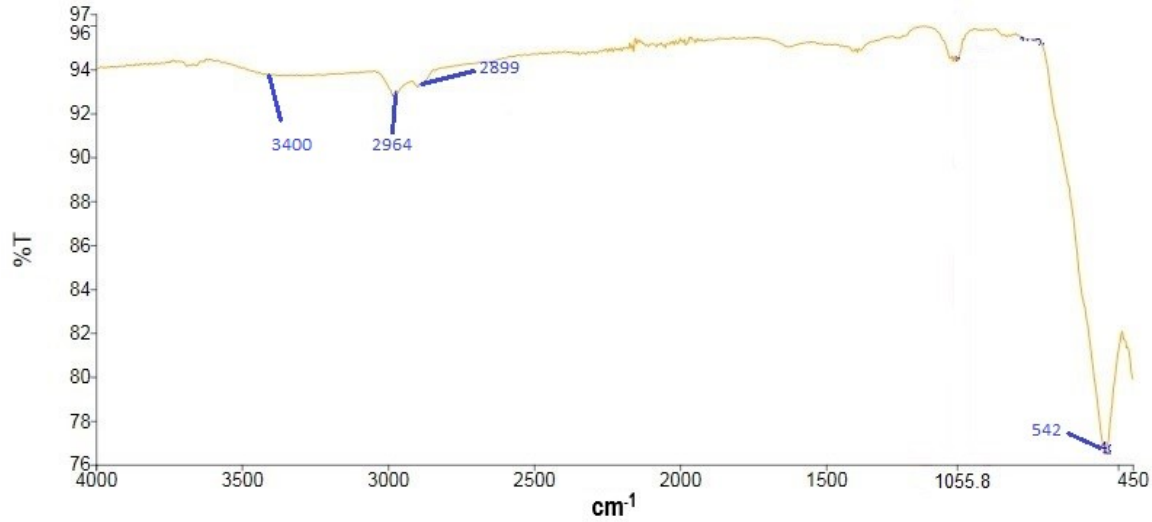

Figure 7S. FTIR spectrum of the magnetite nanoparticles

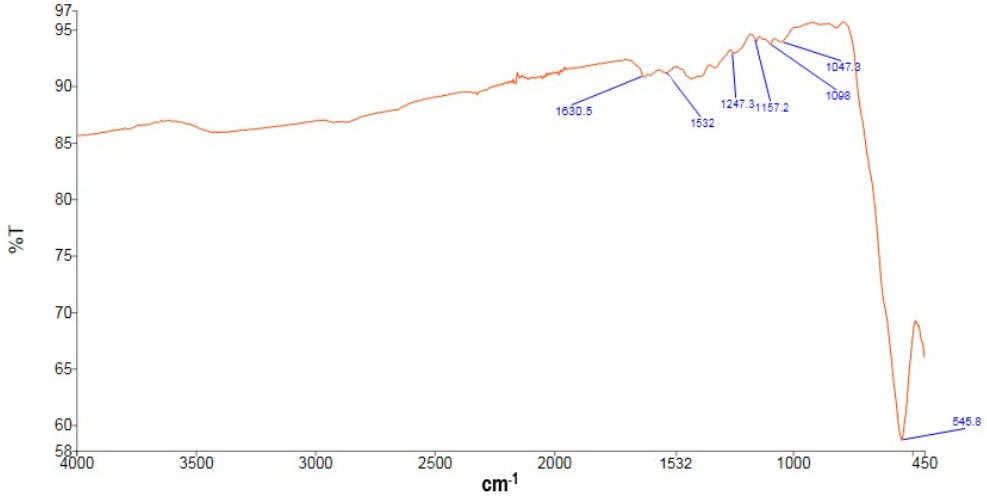

Figure 8S. FTIR spectrum of the MHC3@Fe3O4

Table 1S. Powder XRD qualitative results and peak list of the MHC3@Fe3O4

Phase name	Formula	Figure of merit	Phase reg. detail	DB card number
Magnetite	Fe_3O_4	0.789	ICDD (PDF-2/Release 2011 RDB)	00-001-1111
Phase name	Formula	Space group	Phase reg. detail	DB card number
Magnetite	Fe_3O_4	227 : Fd-3m,choice-2	ICDD (PDF-2/Release 2011 RDB)	00-001-1111

No.	2-theta(deg)	Interplanar spacing (*d* spacing) (A°)	Height(cps)	FWHM(deg)	Int. I(cps deg)	Int. W(deg)	Asym. factor	Miller indices (hkl)
1	12.6(3)	7.04(15)	246(20)	2.6(2)	691(97)	2.8(6)	0.9(4)	(111)
2	30.13(4)	2.964(4)	1221(45)	0.68(5)	1208(64)	0.99(9)	0.9(3)	(220)
3	35.536(13)	2.5242(9)	3873(80)	0.633(12)	3094(57)	0.80(3)	1.18(1	(311)
4	43.17(6)	2.094(3)	949(40)	0.74(8)	1164(59)	1.23(11)	1.1(5)	(400)
5	57.07(4)	1.6126(11)	1193(45)	0.72(4)	911(63)	0.76(8)	0.9(2)	(333) (511)
6	62.75(3)	1.4796(7)	1738(54)	0.79(3)	1676(62)	0.96(7)	1.1(2)	(440)

Table 2S. Crystallite size of the MHC3@Fe_3O_4

Data set name	Crystallite size(A)	Phase name	Crystallite size(A)
MHC3@Fe_3O_4	91(12)	Magnetite	91(12)

All the MIC experiments were done in triplicate (Table 1). The box and whisker plot does not demonstrate any variations in the results, because the MIC test done by the application of resazurin disodium salt is considered a precise method, which if performed appropriately and for individual compounds provides identical values for each compound in triplicate (Figure 1).

Table 3S. Results of triplicate 96-well microtiter assay of tested compounds on bacterial strains

Bacterial strains	**MIC, µg/mL**								
	MHC3	MHC3	MHC3	MHC3 @Fe_3O_4	MHC3 @Fe_3O_4	MHC3 @Fe_3O_4	Fe_3O_4 NPs	Ampicillin	Ampicillin
K. pneumoniae	512	512	512	>1024	>1024	>1024	-	512	512
P. aeruginosa	256	256	256	1024	1024	1024	-	512	512
S. aureus	32	32	32	0.5	0.5	0.5	-	16	16

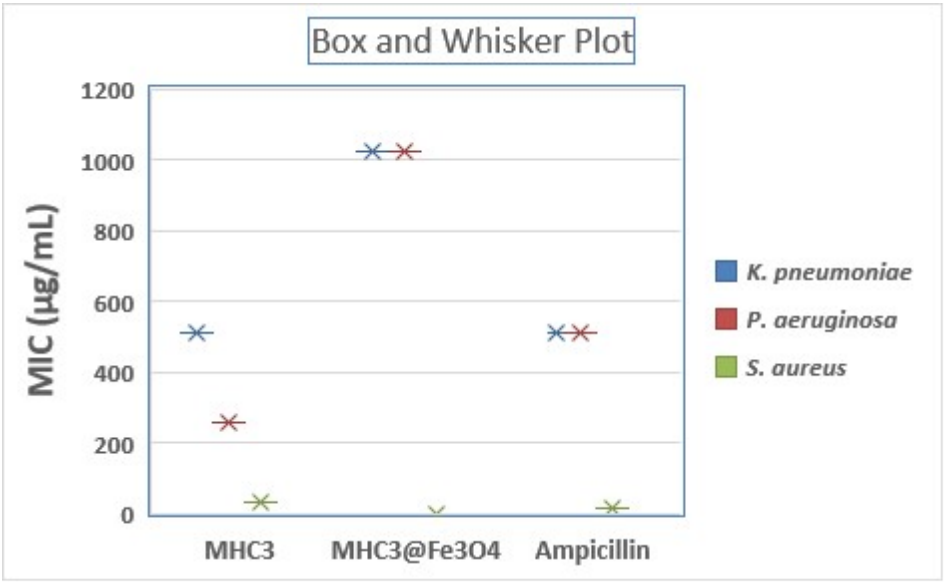

Figure 9S. The Box and Whisker plot for the results of the 96-well microtiter assay

The two-way and post hoc ANOVA were applied to the obtained results by using GraphPad Prism (version 9 forWindows, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Post hoc analysis by two-way ANOVA was applied as multiple comparisons in three families (Figure 2, Table 2). The antibacterial activity of tested compounds on three bacterial strains was compared with each other and ampicillin as a control. The adjusted P value is less than 5%, so the obtained results can be considered significantly different. The only not significant difference has been observed for ampicillin and MHC3 activity towards Klebsiella pneumoniae because values of MIC for both compounds were equal to 512 µg/mL.

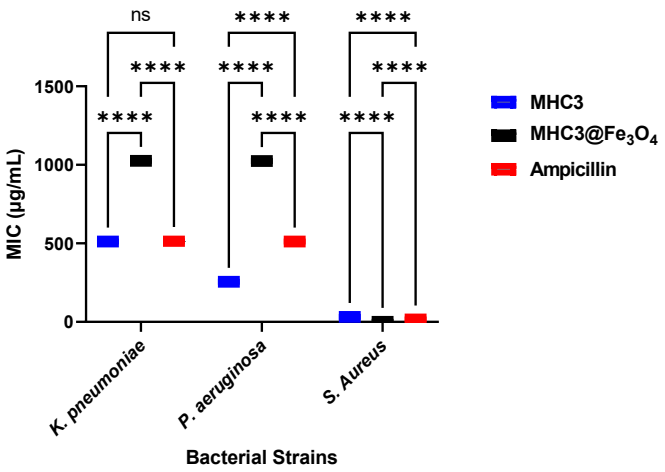

Figure 10S. ANOVA: Tukey's multiple comparisons results

Table 4S. ANOVA: Tukey's multiple comparisons results

Number of comparisons per family	**3**							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
K. pneumoniae								
MHC3 vs. MHC3@Fe_3O_4	-513.0	-514.2 to -511.8	Yes	****	<0.0001			
MHC3 vs. Ampicillin	-1.000	-2.203 to 0.2031	No	ns	0.1135			
MHC3@Fe_3O_4 vs. Ampicillin	512.0	510.8 to 513.2	Yes	****	<0.0001			
P. aeruginosa								
MHC3 vs. MHC3@Fe_3O_4	-768.0	-769.2 to -766.8	Yes	****	<0.0001			
MHC3 vs. Ampicillin	-256.0	-257.2 to -254.8	Yes	****	<0.0001			
MHC3@Fe_3O_4 vs. Ampicillin	512.0	510.8 to 513.2	Yes	****	<0.0001			
S. Aureus								
MHC3 vs. MHC3@Fe_3O_4	31.50	30.30 to 32.70	Yes	****	<0.0001			
MHC3 vs. Ampicillin	16.00	14.80 to 17.20	Yes	****	<0.0001			
MHC3@Fe_3O_4 vs. Ampicillin	-15.50	-16.70 to -14.30	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
K. pneumoniae								
MHC3 vs. MHC3@Fe_3O_4	512.0	1025	-513.0	0.4714	3	3	153	18.

							9	00
MHC3 vs. Ampicillin	512.0	513.0	-1.000	0.4714	3	3	3.000	18.00
MHC3@Fe_3O_4 vs. Ampicillin	1025	513.0	512.0	0.4714	3	3	1536	18.00
P. aeruginosa								
MHC3 vs. MHC3@Fe_3O_4	256.0	1024	-768.0	0.4714	3	3	2304	18.00
MHC3 vs. Ampicillin	256.0	512.0	-256.0	0.4714	3	3	768.0	18.00
MHC3@Fe_3O_4 vs. Ampicillin	1024	512.0	512.0	0.4714	3	3	1536	18.00
S. Aureus								
MHC3 vs. MHC3@Fe_3O_4	32.00	0.5000	31.50	0.4714	3	3	94.50	18.00
MHC3 vs. Ampicillin	32.00	16.00	16.00	0.4714	3	3	48.00	18.00
MHC3@Fe_3O_4 vs. Ampicillin	0.5000	16.00	-15.50	0.4714	3	3	46.50	18.00