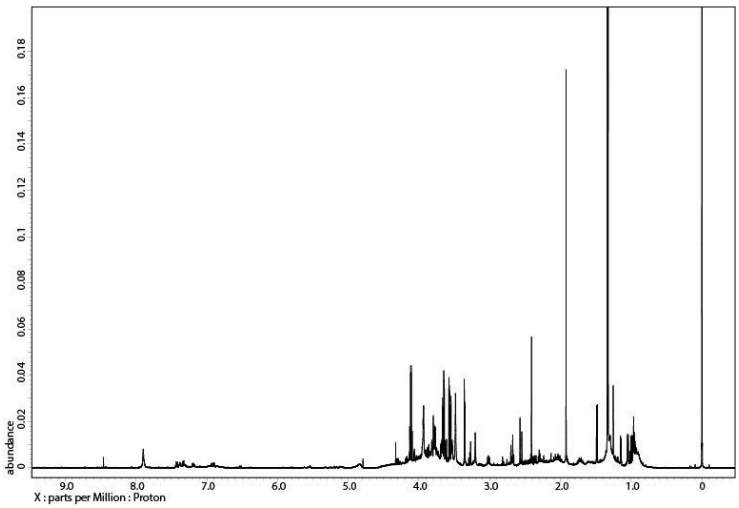
Supplementary material

Targeting protein aggregation using a cocoa-bean shell extract to reduce α-synuclein toxicity in models of Parkinson's disease

Supplementary Figure S1. (A) 600.17 MHz ^{1}H NMR spectrum of cocoa bean shell extract solubilized in D_2O containing 50 mM phosphate buffer pH7.4 and 0.4 mM TSP as internal standard. (B) MS spectra of the metabolites reported in Table 3.

A



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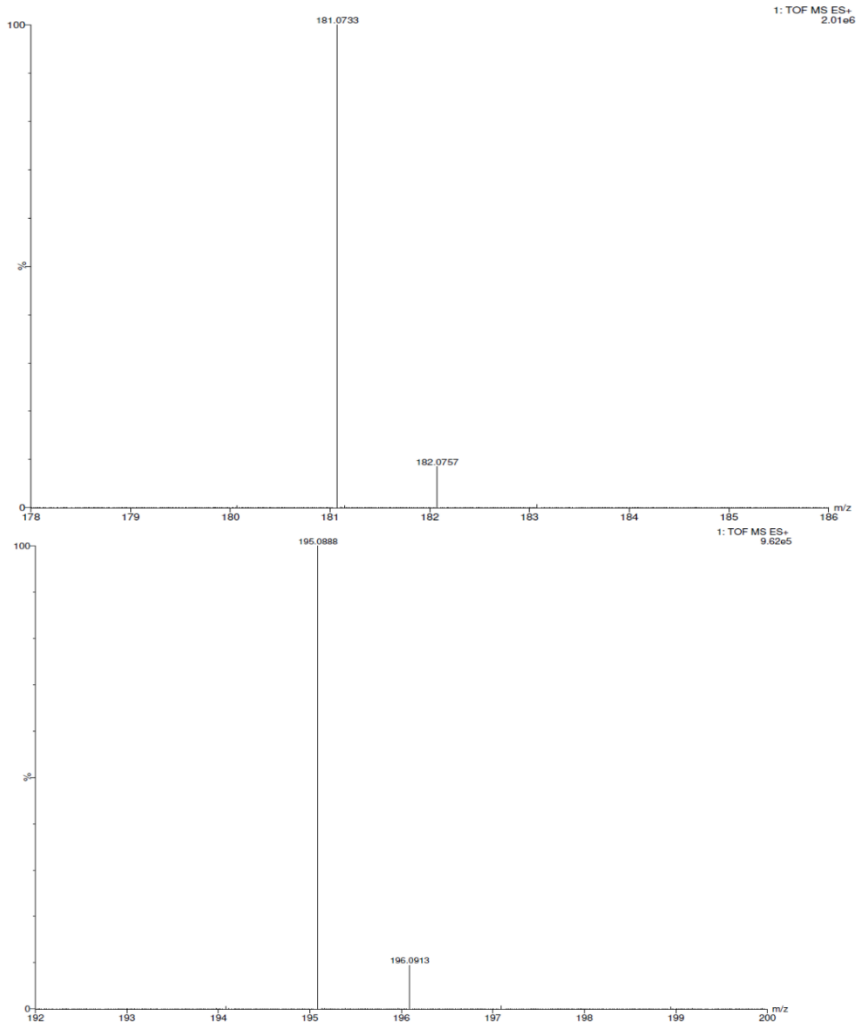

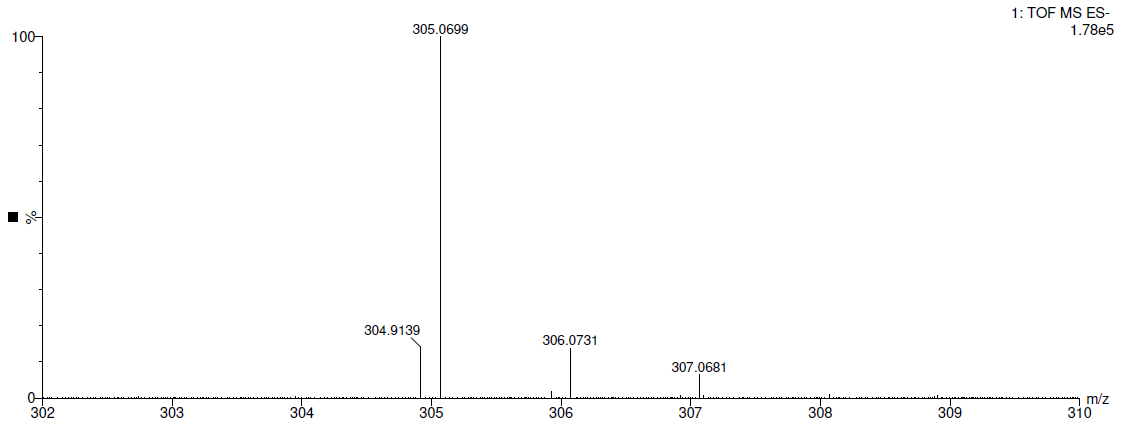
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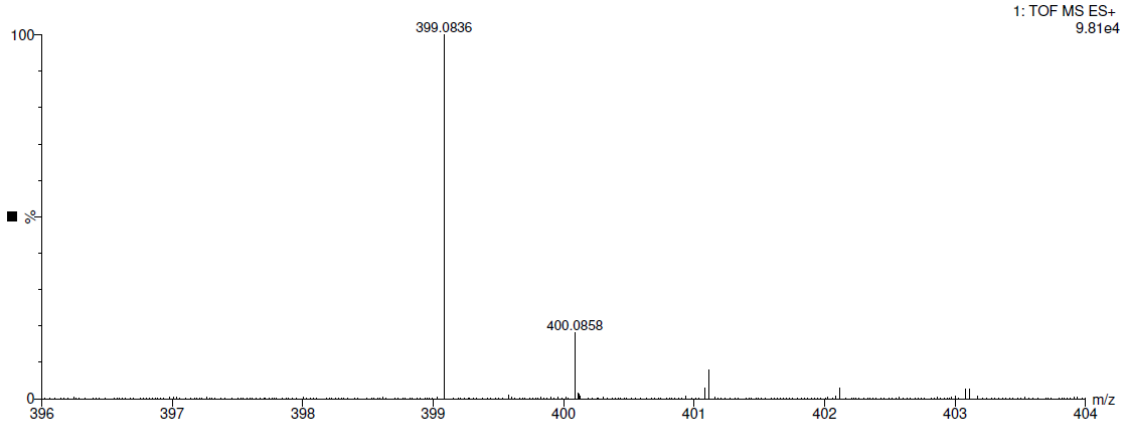
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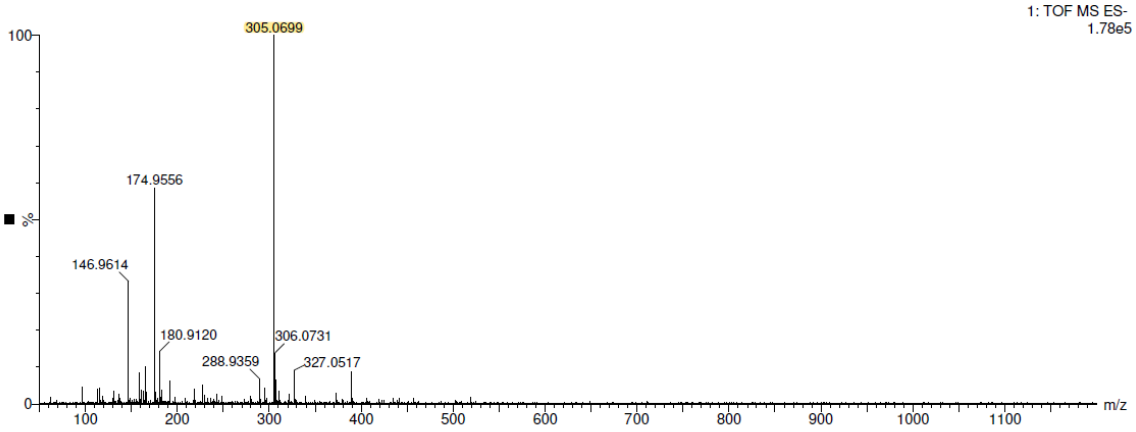
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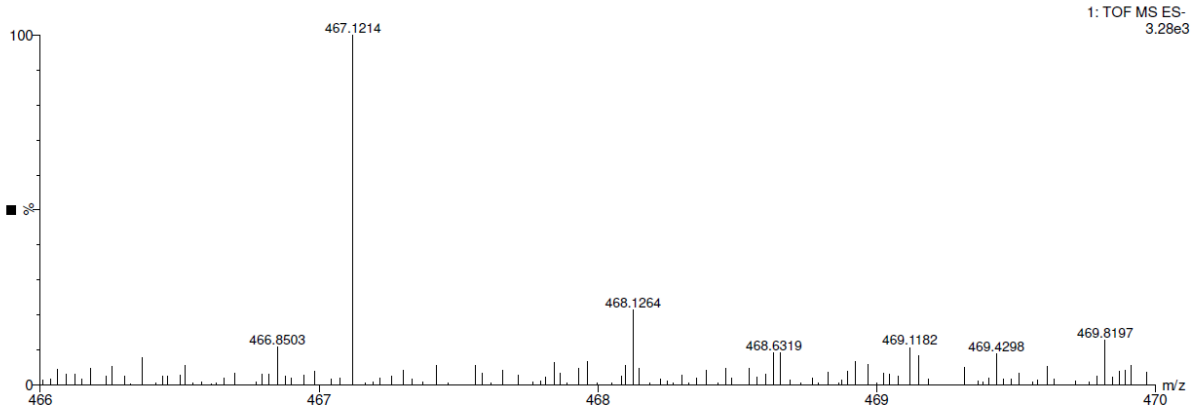
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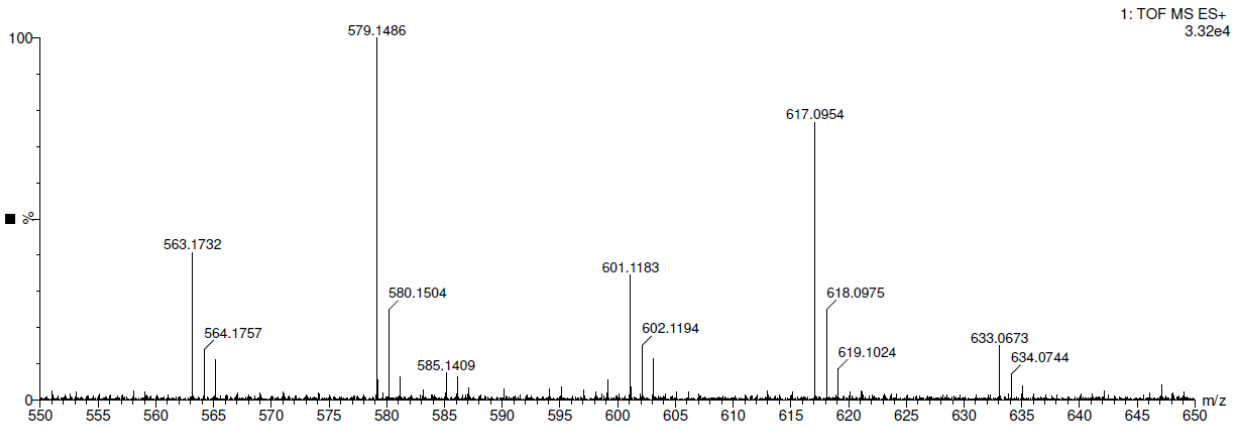
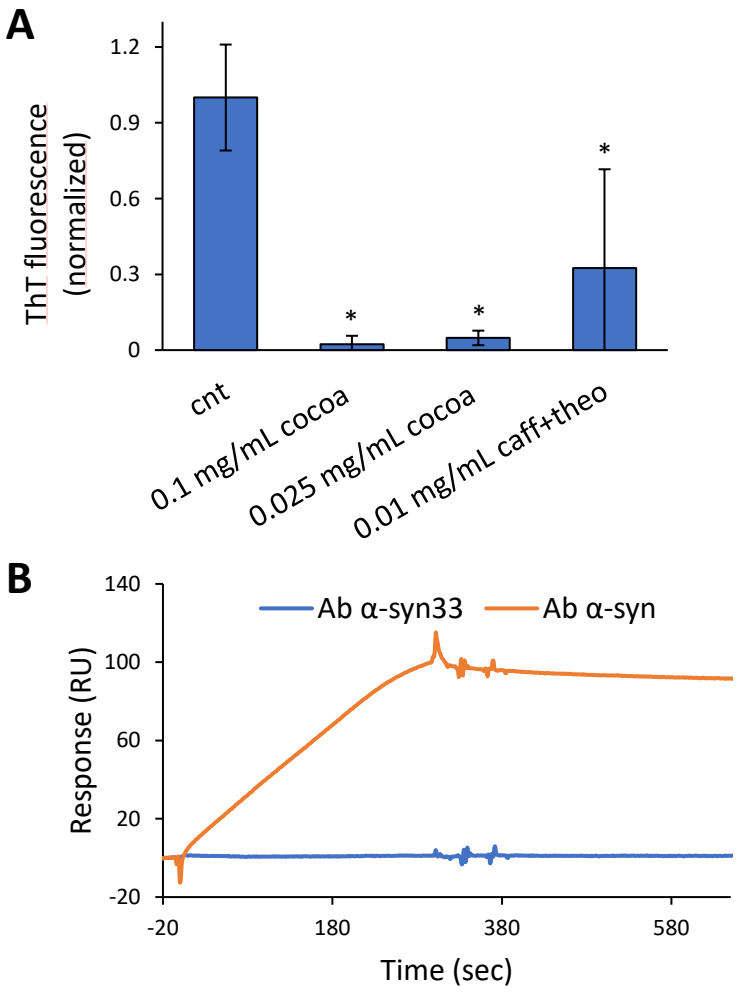


Supplementary Figure S2. (A) α-syn aggregation process, followed by ThT fluorescence, in the absence (cnt) or presence of the 0.01 mg/mL of caffeine and theobromine. (B) The bioactivity of the CM5 sensor chip was determined by injecting positive (Ab anti-α-syn) and negative (Ab anti-α-syn33) controls onto immobilised α-syn protein.



Supplementary Table S1. PM21-PM25 chemical compounds divided into functional groups. Drugs from plates PMs 21–25 divided into 6 groups, based on their structure and function: ions, cyclic compounds, organic compounds, chelators, antibiotics and nitrogen compounds. These 24 different chemical agents are presented in 4 different concentrations. The data presented for each drug concentration represent the ratio between the y-maximum value of cells grown with the cocoa-shell treatment and the untreated control.

Groups	Drugs	Concentration 1	Concentration 2	Concentration 3	Concentration 4
Ions	Sodium dichromate	0.568	0.624	7.381	2.483
	Sodium arsenate	0.841	0.441	0.263	0.553
	Sodium arsenite	0.623	0.603	3.281	0.355
	Sodium cyanate	0.211	0.416	0.846	0.712
	Sodium orthovanadate	0.628	8.260	3.682	0.995
	Sodium selenate	0.538	0.738	0.637	0.562
	Sodium selenite	0.655	0.655	0.698	0.990
	Sodium cyanide	0.440	4.112	1.891	14.762
	Sodium thiosulfate	1.605	0.276	0.281	1.838
	Sodium metasilicate	0.883	0.891	0.395	1.333
	Sodium metaborate tetrahydrate	0.695	0.842	4.864	24.808
	Sodium (meta)periodate	0.834	0.979	0.645	0.417
	Sodium metavanadate	0.827	10.249	2.406	0.795
	Sodium fluoride	0.504	0.580	0.350	0.384
	Potassium chromate	0.328	0.982	28.241	84.368
	Copper (II) sulfate	0.747	20.745	5.493	4.990
	Thallium(I) acetate	0.941	2.938	0.198	0.037
	Cadmium chloride hydrate	61.403	12.128	13.669	7.381
	Chromium(III) chloride	0.572	0.445	0.484	0.007
	Cobalt(II) chloride hexahydrate	0.842	0.775	1.138	24.255
	Cupric chloride dihydrate	0.885	0.709	3.532	0.475
	Manganese(II) chloride	0.833	0.780	1.465	2.444
	Magnesium chloride	1.027	0.854	0.836	0.804
	Nickel chloride	0.591	0.645	0.698	0.979
	Zinc chloride	15.230	0.056	0.231	0.019
	Benzethonium chloride	0.007	1.035	105.006	16.826
	Protamine sulfate	0.562	0.580	0.617	1.352
	Poly-L-lysine hydrochloride	0.611	3.562	3.156	0.527
	Cetylpyridinium chloride	0.425	86.487	135.200	18.796
	Domiphen bromide	0.820	0.906	112.782	93.627

	Ammonium sulfate	0.540	5.319	1.545	0.032
	Lithium chloride	0.943	0.747	30.688	12.906
	Aluminum sulfate	0.780	0.386	3.144	1.803
	Dequalinium chloride	1.332	100.927	85.825	131.658
	Methyl viologen dichloride hydrate	4.242	0.089	3.603	0.076
	Dodecyltrimethyl ammonium bromide	0.975	18.914	6.043	13.221
	Alexidine	0.652	71.948	20.141	26.238
	Palladium(II) chloride	0.566	1.386	0.302	0.783
Cyclic Compounds	Promethazine	0.577	6.096	1.007	0.028
	Chlorpromazine hydrochloride	712.891	63.116	51.796	1.000
	Cycloheximide	0.762	0.732	0.488	0.528
	Trifluoperazine	6.548	3.298	1.187	1.732
	Thioridazine hydrochloride	0.721	81.016	14.520	1.000
	Fluconazole	0.726	0.482	1.386	0.240
	Tamoxifen	0.680	3.988	1.103	2.973
	Miconazole nitrate	2.408	3.024	0.661	0.622
	Tetrazolium Violet	67.922	25.351	8.318	5.290
	Amitriptyline hydrochloride	94.348	16.094	1.000	7.009
	Zaragozic acid A	0.859	0.621	0.441	0.027
	Berberine chloride	0.535	0.534	0.768	2.818
Organic Compounds	L-Aspartic acid β-hydroxamate	0.527	0.272	1.930	0.192
	L-Glutamic acid g-hydroxamate	0.920	0.325	1.284	14.924
	L-Arginine hydroxamate	0.273	0.766	1.242	97.123
	Glycine hydroxamate	0.900	125.862	130.341	57.425
	D,L-Serine hydroxamate	0.710	4.095	0.980	0.779
	D-Serine	0.921	0.789	1.847	0.423
	Thialysine	0.641	0.379	0.094	0.616
	5-Fluorocytosine	22.995	9.564	8.840	10.070
	5-Fluorouracil	0.434	0.590	0.082	0.017
	Propiconazole	1.019	0.945	1.717	7.836
	Sodium benzoate	0.658	0.595	0.415	0.295
	Hydroxyurea	0.927	0.974	1.464	34.670
	Azaserine	0.994	26.104	11.241	8.007
	Urea hydrogen peroxide	0.882	0.707	0.835	0.647
	Succinic acid	0.589	0.749	4.131	6.484
	Malic acid	0.812	0.747	116.117	11.930
	Tartaric acid	0.716	0.863	1.350	0.473
	Fumaric acid	0.556	0.729	0.392	0.029

	Cinnamic acid	0.728	0.603	0.821	0.256
	Fluorodeoxyuridine	0.739	0.884	0.645	0.026
	Sodium caprylate	0.669	0.000	0.785	0.380
	Sodium salicylate	0.871	1.452	1.116	1.159
	Glycine hydrochloride	0.305	0.768	6.428	1.666
	Caffeine	0.581	0.585	-0.028	0.407
	Chloroalanine hydrochloride	0.639	1.207	1.000	24.164
	2-Deoxy-D-glucose	0.934	0.049	0.694	0.026
	Clomiphene citrate	0.744	10.841	1.258	0.524
	Ibuprofen	0.575	0.527	0.009	0.009
	Chloroquine	0.904	1.952	95.179	1.149
	Guanidine hydrochloride	0.561	0.472	16.468	15.944
	Myclobutanil	0.881	0.671	0.911	0.120
	Niaproof	0.204	0.327	0.013	1.221
	Polymyxin B	0.681	0.808	131.563	31.240
	Miltefosine	0.764	0.537	70.027	80.424
	FCCP	0.771	0.722	0.610	0.017
	CCCP	0.536	0.699	1.534	2.951
	Triclosan	0.536	19.741	1.574	14.318
Chelators	1-Hydroxypyridine-2-thione	0.659	0.714	0.873	0.327
	EDTA	0.060	139.272	131.186	59.347
	BAPTA	0.694	0.755	108.205	2.685
	EGTA	3.324	5.524	1.920	7.977
	Sodium pyrophosphate decahydrate	0.898	0.005	0.038	0.501
	Benzamidine	0.430	14.517	13.875	1.269
	2,2`-Dipyridyl	0.936	0.806	0.614	17.780
Antibiotics	Nystatin	0.601	0.428	17.862	12.708
	D-Cycloserine	8.931	3.427	1.049	1.153
	Apramycin sulfate	0.322	0.680	0.980	1.703
	Blasticidin hydrochloride	0.185	0.982	0.617	0.186
	Chlortetracycline hydrochloride	0.570	0.962	3.965	0.878
	Kanamycin monosulfate	0.804	0.394	0.177	1.119
	Paromomycin	9.092	3.369	2.545	1.473
	Isoniazid	0.664	2.075	10.869	1.815
	Bleomycin	0.859	128.058	60.042	3.171
	Doxycycline hyclate	0.748	0.129	0.921	2.946
	Tobramycin	0.652	34.116	24.032	3.491
	Hygromycin B	1.575	20.553	20.219	28.204
	Neomycin	38.855	117.128	36.588	14.900
	Pentamidine isethionate	0.770	1.049	0.030	2.279

Nitrogen Compounds	6-Azauracil	0.786	0.629	0.935	1.873
	Diamide	0.419	0.623	0.000	2.044
	Thiourea	0.088	0.604	12.648	0.425
	4-Aminopyridine	0.131	0.353	29.848	37.486
	Sodium azide	0.477	0.853	0.278	25.055
	Sodium nitrite	0.748	0.898	1.132	1.314
	Cisplatin	8.866	9.519	4.374	11.550
	Aminacrine	1.193	7.287	1.707	2.750
	3-Amino-1,2,4-triazole	0.588	0.632	143.026	39.586
	4-Nitroquinoline-N-oxide	0.860	0.737	0.725	0.984
	Hydroxylamine hydrochloride	0.861	0.698	1.060	0.001
	Compound 48/80	0.386	2.525	136.429	61.358

Supplementary Table S2. Compounds towards which cocoa-shell extract alters sensitivity, reported in Fig. 5. Compounds were selected when the fold change (ratio between y-maximum value of CBSE treated vs control cells) was >5 or <0.2 in at least three concentrations. Drugs are categorised according to their structure and function, and their specific effects are described alongside the pertinent reference.

Groups	**Drugs**	**Effects**	**References**
Ions	Dodecyltrimethyl ammonium bromide	ability to promote disaggregation of the fibrillar assembly (*in vitro*)	(Pandey et al., 2013)
	Cadmium chloride hydrate	autophagy inducer (in hepatocytes)	(Niture et al., 2021)
	Cetylpyridinium chloride	antimicrobial effect (drug dose-dependently inhibit mitochondrial complex 1 and potent and dose-dependent AMPK inducer)	(Allen et al., 2020)
	Dequalinium chloride	act as an inducer and/or stabilizer of the protofibrils of α-synuclein (*in vitro*)	(Lee et al., 2006)
	Alexidine	antimicrobial effect	(Kim et al., 2013)

Chelators	EDTA	autophagy inducer (in *A. fumigatus*)	(Richie et al., 2007)
organic compounds	Glycine hydroxamate	autophagy inhibitor (in neuron through the AMPK pathway both *in vitro* and *in vivo*)	(Cai et al., 2019)
	Azaserine	antioxidant effect	(Rajapakse et al., 2009)
Cyclic Compounds	5-Fluorocytosine	autophagy inducer (in BGC-823 cells)	(X.-X. He et al., 2018)
	Tetrazolium Violet	apoptotic inducer (antitumor effect in Human Lung Cancer A549 cells)	(Zhang et al., 2012)
	Amitriptyline hydrochloride	autophagy inhibitor (not block the beginning of autophagy, block its turnover, via inhibiting autophagosome maturation in neuronal cells)	(Kwon et al., 2020)
antibiotics	Hygromycin B	inhibit growth (in *S. cerevisiae*)	(Kaster et al., 1984)
	Bleomycin	autophagy inducer (in idiopathic pulmonary fibrosis)	(Yue et al., 2022)
	Neomycin	autophagy inducer (in both cochlear HCs and HEIOC-1 cells after neomycin injury)	(Z. He et al., 2017)

Supplementary References

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