

Supporting Information

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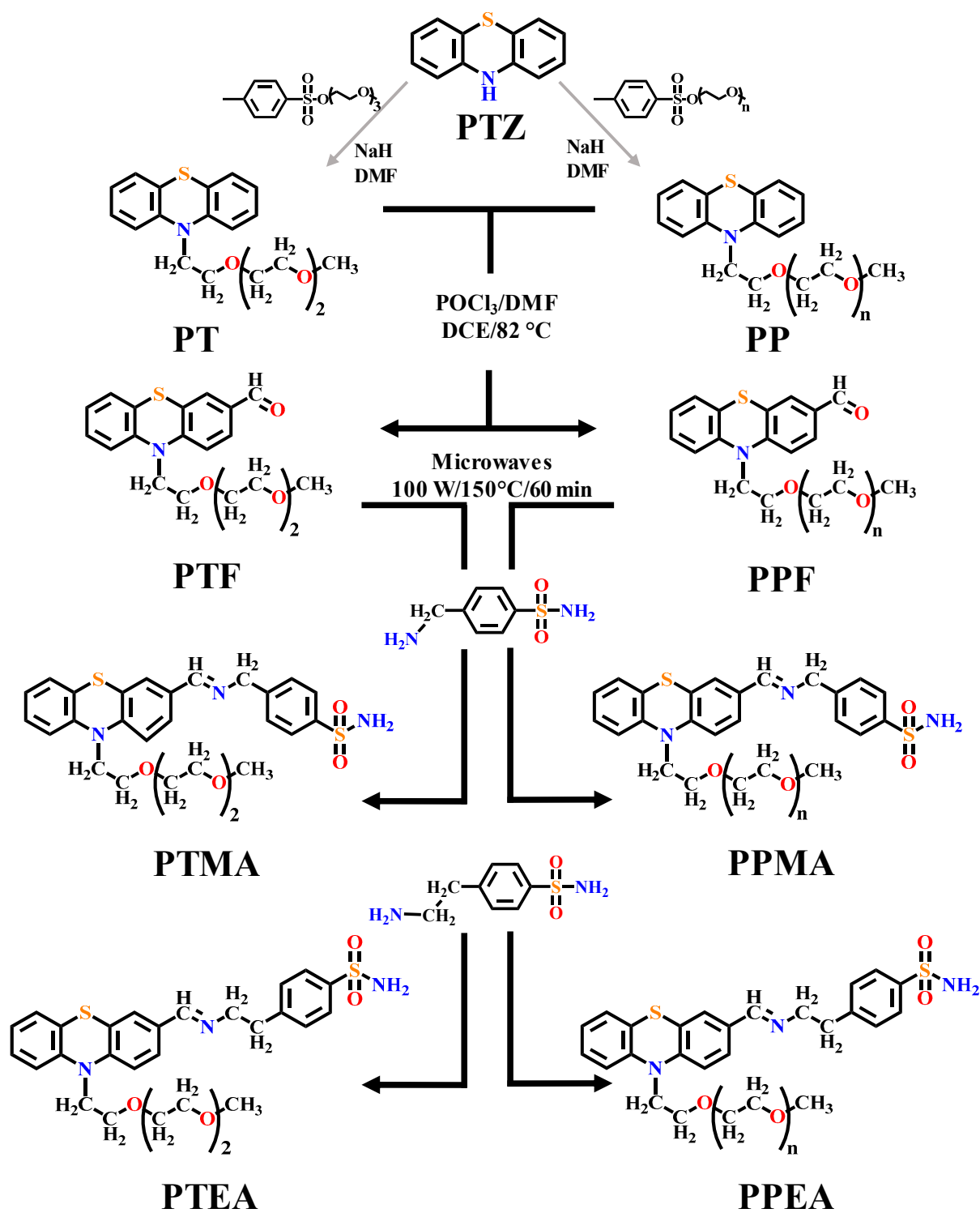
Antitumor Activity of PEGylated and TEGylated Phenothiazine

Derivatives: Structure–Activity Relationship

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Scheme S1. The synthesis pathway of the studied compounds

Table S1. Half maximal inhibition concentration (IC₅₀) of TEGylated and PEGylated derivatives on different tumor lines and a normal cell line. Cells were treated with various concentrations of PEGylated phenothiazines for 48 h. Cells viability was determined by MTS assay to obtain IC₅₀ values. The yellow marked values presented a higher concentration than the last used for experiment.

Code Cell Line	PTZ	PPF	PTF	PTMA	PTEA	PPMA	PPEA
NHDF	0.175	>0.300	0.150	0.200	0.107	0.150	0.108
CT-26	0.186	0.139	0.063	0.107	0.080	0.040	0.029
HeLa	0.240	>0.300	0.015	0.029	0.015	0.116	0.086
HepG2	0.073	0.106	0.050	0.083	0.085	0.054	0.040
HOS	>0.300	>0.300	>0.300	>0.300	>0.300	>0.300	>0.300
LN229	>0.300	>0.300	0.186	0.219	0.183	0.239	0.109
MCF-7	0.188	0.270	0.108	0.141	0.138	0.090	0.090
MeWo	>0.300	0.235	0.147	0.287	0.239	0.104	0.068
U-118	>0.300	>0.300	0.190	>0.300	0.170	0.092	0.100

Table S2. Selectivity index of TEGylated and PEGylated derivatives on tested tumor cell lines

Selectivity index (SI)							
	PTZ	PTF	PPF	PTMA	PTEA	PPMA	PPEA
CT-26	-	2.38	-	2	1.25	3.75	3.44
HeLa	-	10	-	6.89	6.66	1.36	1.25
HepG2	2.42	3	-	2.5	1.25	3	2.5
HOS	-	-	-	-	-	-	-
LN229	-	-	-	-	-	-	-
MCF7	-	1.5	-	1.42	-	1.66	1.11
MeWo	-	-	-	-	-	1.5	1.66
U118MG	-	-	-	-	-	1.66	-

Table S3. IC₅₀ values for different tumor cell lines, of two therapeutic antitumor drugs

Drug	Cell Line	IC₅₀ (μM)	Reference
Doxorubicin	HepG2	1.03	[74]
	HeLa	0.18	[75]
	MCF-7	0.07	
	CT-26	10	[76]
	MeWo	88	[77]
	NHDF	0.137	[78]
5-Fluorouracil	HepG2	253	[79]
	HeLa	676.9	
	MCF-7	12.7	[80]
	CT-26	300	[81]
	MeWo	61.5	[82]
	NHDF	25	[78]

Table S4. Half maximal inhibition concentration (IC₅₀) of other phenothiazine-based compounds determined on different tumor lines

Drug	Cell Line	IC ₅₀ (μ M)	Reference
10- {[1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl]methyl}- 2-{trifluoromethyl}-10H-phenothiazine	MCF-7	29.6	[80]
10- {[1-(3,4,5-Trimethoxybenzyl)-1H-1,2,3-triazol-4-yl]methyl}-10H-phenothiazine		0.8	
3-([1,2,4]Triazolo[4,3-a]pyridin-3-yl)-10-ethyl-7-(4-methoxyphenyl)-10H-phenothiazine		57.2	[25]
3-([1,2,4]Triazolo[4,3-a]pyridin-3-yl)-10-ethyl-7-phenyl-10H-phenothiazine		10.2	
Trifluoperazine		11.3	[83]
Thioridazine		12.9	
1-[(10H-Phenothiazin-3-yl)carbonyl]-3-acetyl-7-methylindolizine		>10	[84]
1-[(10H-Phenothiazin-3-yl)carbonyl]-3-acetyl-6,8-dimethylindolizine		>10	
1-[(10H-Phenothiazin-3-yl)carbonyl]-3-acetyl-7-methoxyindolizine		>10	
Thioridazine	U87-MG	12.4	[85]
Prochlorperazine		0.97	[86]
Perphenazine		6.8	
Fluphenazine	CBM8401	10.0	[87]
thioridazine	HepG2	45.5	
10-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-10Hphenothiazine		1.3	
10-((1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-10Hphenothiazine (7f)		15.0	[88]
1-Phenethylamino-3-(10H-phenothiazin-10-yl)-2-propanol	HeLa	10.0	
N-alkyl-3-formylphenothiazine		57.69	[90]
Chlorpromazine	Colo205	10.0	[91]
Fluphenazine		7.18	[92]

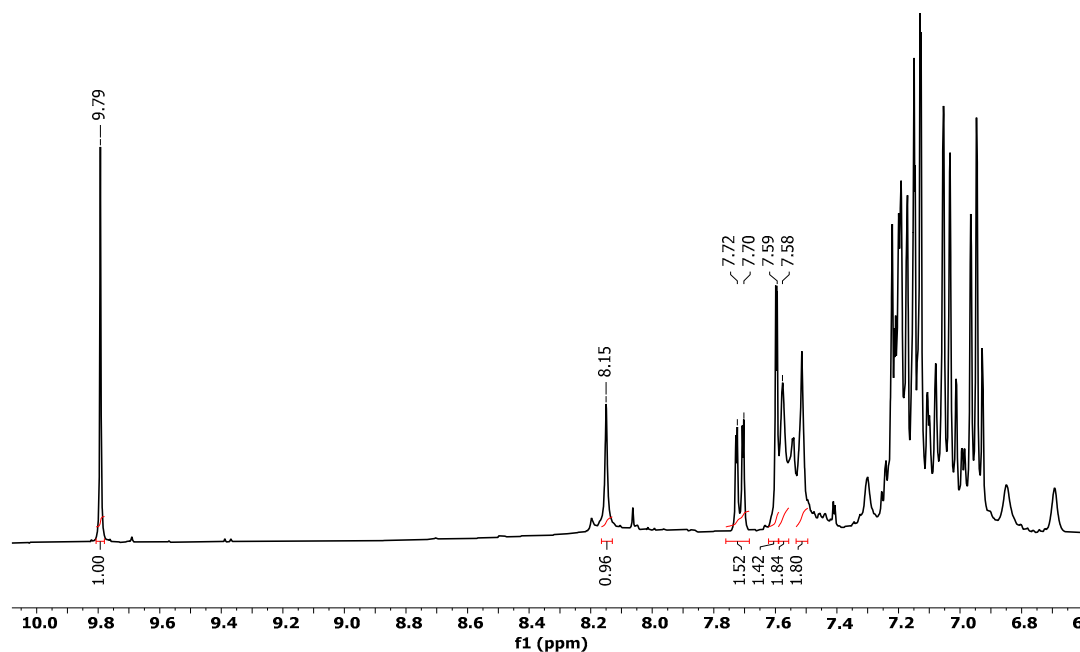


Figure S1. The ^1H -NMR spectrum for the PTF-glutamine mixture, recorded in DMSO-d_6 , at 600 MHz.

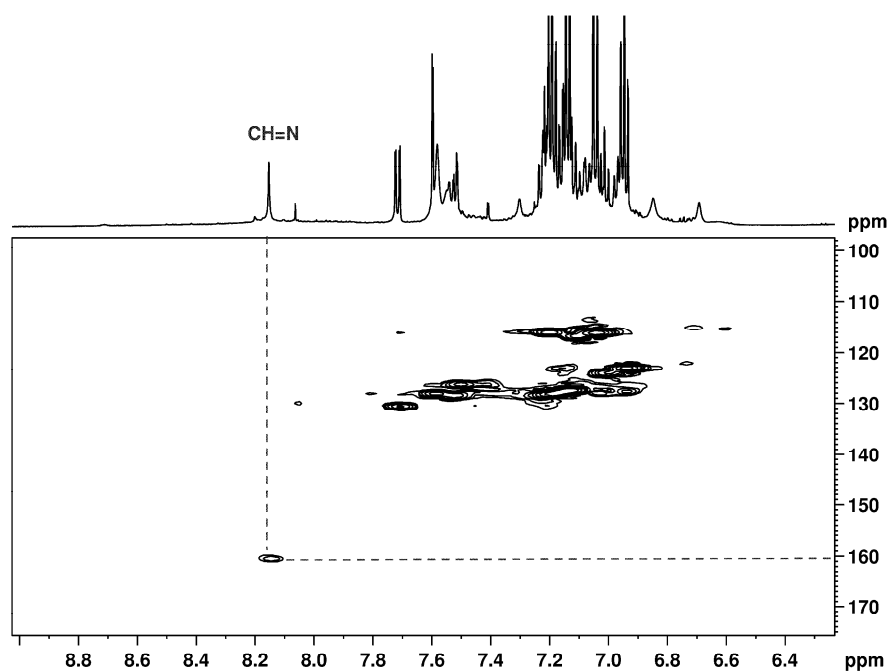


Figure S2. The ^1H , ^{13}C -HSQC NMR spectrum for the PTF-glutamine mixture, showing the direct bond correlation signal between proton from 8.15 ppm and carbon from 160.5 ppm, as prove for amine group formation.

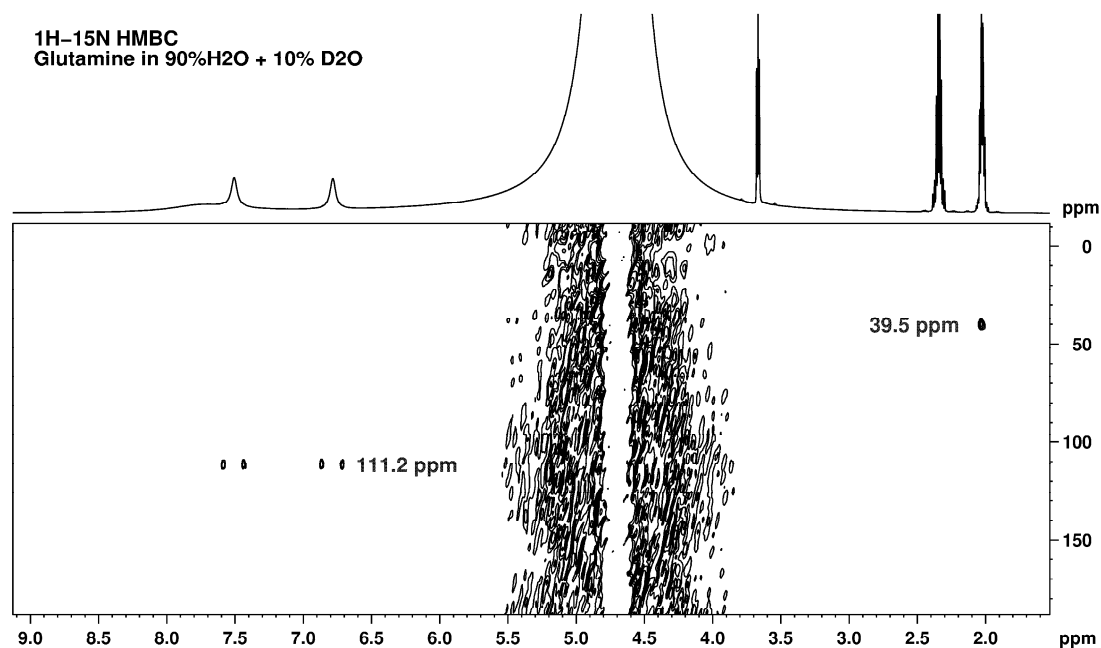


Figure S3. The ^1H , ^{15}N -HMBC NMR spectrum for glutamine, recorded in 9/1 $\text{H}_2\text{O}/\text{D}_2\text{O}$ mixture, showing proton-nitrogen correlation signals at 39.5 ppm (primary amine group) and 111.2 ppm (amide group).

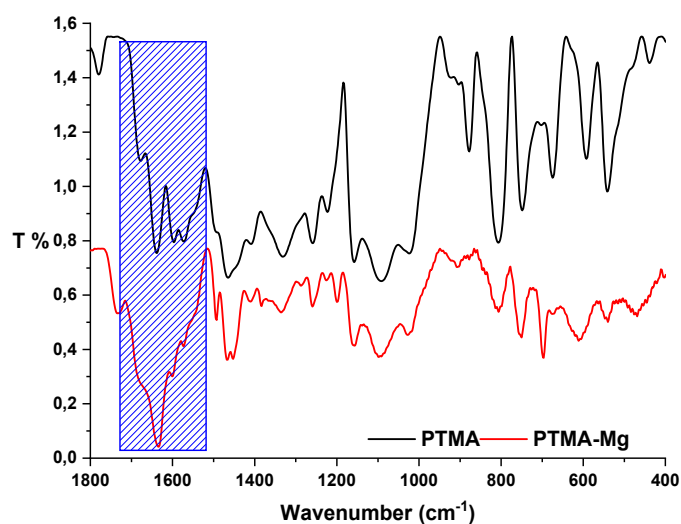


Figure S4. FTIR spectra of the PTMA incubated with magnesium salts compared to pristine derivatives

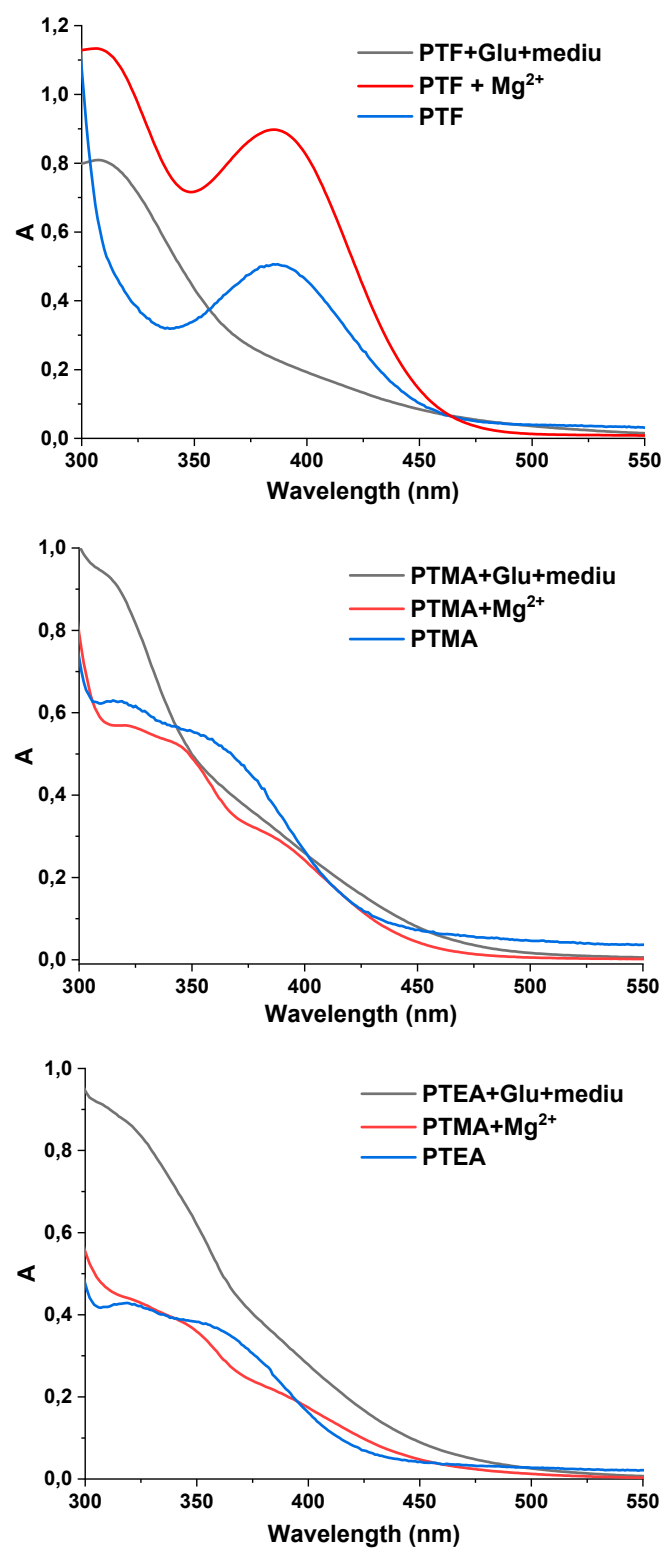


Figure S5. The comparative UV-vis spectra of the TEGylated derivatives with their mixture with glutamine and with Mg²⁺: a) PTF, b) PTMA, c) PTEA

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