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Phosphorus-nitrogen compounds: Part 68. Synthesis, characterization, stereogenism, photophysical and bioactivity studies of novel unsymmetrical dispiro(N/N)cyclotriphosphazenes with carbazolyl and 4-chlorobenzyl pendant arms

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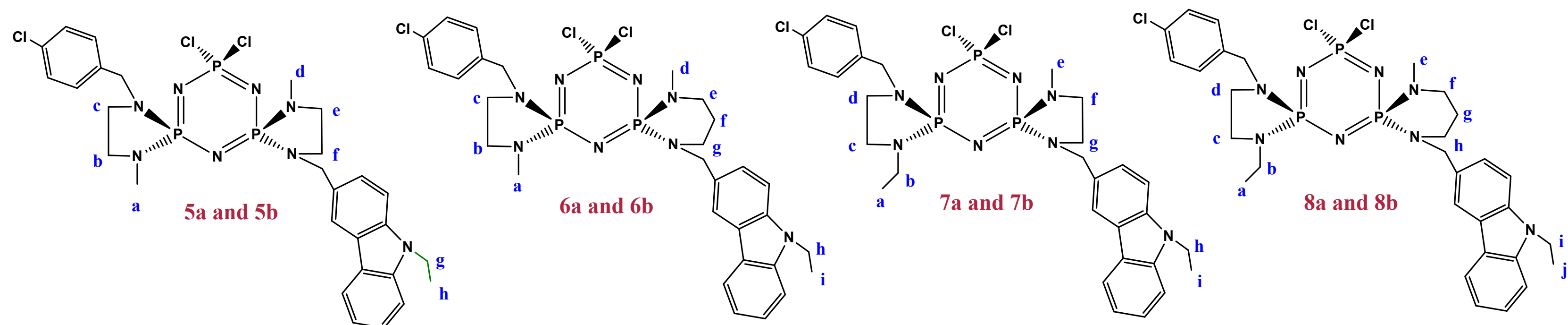
Supporting Information

Table S1 ^{13}C (decoupled) NMR spectral data of the phosphazenes. [Chemical shifts (δ) reported in ppm and *J* values in Hz].

	Ph$\underline{C}H_2$	Cz$\underline{C}H_2$	C1	C2	C3	C4	C1’	C2’	C3’	C4’	C5’	C6’	C7’	C8’	C9’	C10’	C11’	C12’
5a	48.14	49.12	136.32	129.48	128.66	133.14	127.72	126.21	120.56	139.46	122.92	108.47	140.21	122.70	108.47	118.80	125.68	120.32
			$^{3}J_{PC}$=6.9				$^{3}J_{PC}$=7.6											
5b	47.56	48.65	136.48	129.24	128.63	133.18	128.27	125.73	120.68	139.43	122.95	108.44	140.16	122.60	108.57	118.89	125.28	119.98
			$^{3}J_{PC}$=7.3				$^{3}J_{PC}$=7.5											
6a	48.40	50.93	136.39	129.43	128.65	133.09	127.98	126.39	120.60	139.48	122.90	108.37	140.21	122.70	108.45	118.81	125.64	120.46
	$^{2}J_{PC}$=6.1	$^{2}J_{PC}$=2.3	$^{3}J_{PC}$=6.1				$^{3}J_{PC}$=10.0											
6b	48.05	50.71	136.23	128.92	128.34	132.67	127.87	125.89	120.51	139.32	122.80	108.22	140.15	122.66	108.38	118.73	125.57	119.84
	$^{2}J_{PC}$=6.1		$^{3}J_{PC}$=6.1				$^{3}J_{PC}$=10.0											
7a	48.11	49.11	136.37	129.52	128.65	133.12	127.76	126.24	120.57	139.46	122.93	108.47	140.22	122.72	108.47	118.81	125.68	120.32
	$^{2}J_{PC}$=5.7	$^{2}J_{PC}$=5.8	$^{3}J_{PC}$=7.0				$^{3}J_{PC}$=7.0											
7b	47.83	49.02	136.20	129.29	128.44	132.85	127.76	125.87	120.54	139.34	122.85	108.39	140.17	122.67	108.43	118.77	125.63	119.89
	$^{2}J_{PC}$=6.4	$^{2}J_{PC}$=5.7	$^{3}J_{PC}$=6.4				$^{3}J_{PC}$=7.1											
8a	48.40	50.83	136.47	129.47	128.64	133.08	128.07	126.37	120.56	139.47	122.91	108.35	140.22	122.72	108.46	118.81	125.65	120.38
	$^{2}J_{PC}$=6.1		$^{3}J_{PC}$=6.8				$^{3}J_{PC}$=10.0											
8b	47.99	50.67	136.32	128.94	128.33	132.65	128.32	125.89	120.51	139.30	122.79	108.21	140.14	122.66	108.37	118.17	125.55	119.82
	$^{2}J_{PC}$=6.1																	

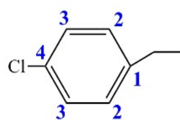


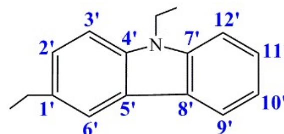
	Ca	Cb	Cc	Cd	Ce	Cf	Cg	Ch	Ci	Cj
5a	31.31	44.18 $^{2}J_{PC}$=13.0	47.21 $^{2}J_{PC}$=13.0	31.43 $^{2}J_{PC}$=1.5	43.91 $^{2}J_{PC}$=14.0	47.28 $^{2}J_{PC}$=12.3	37.59	13.83	-	-
5b	31.08	44.15 $^{2}J_{PC}$=11.0	47.06 $^{2}J_{PC}$=11.0	31.08	44.10 $^{2}J_{PC}$=14.2	47.12 $^{2}J_{PC}$=11.0	37.54	13.78	-	-
6a	35.59	44.26 $^{2}J_{PC}$=13.0	47.37 $^{2}J_{PC}$=12.3	31.76 $^{2}J_{PC}$=3.8	45.45	24.91 $^{3}J_{PC}$=1.6	50.47	37.59	13.84	-
6b	35.59	44.41 $^{2}J_{PC}$=13.9	47.35 $^{2}J_{PC}$=12.3	31.76 $^{2}J_{PC}$=3.8	45.39	24.84	50.58 $^{2}J_{PC}$=3.1	35.77	13.81	-
7a	13.74 $^{3}J_{PC}$=5.1	39.18 $^{2}J_{PC}$=4.5	43.96 $^{2}J_{PC}$=13.5	44.23 $^{2}J_{PC}$=13.6	31.31 $^{2}J_{PC}$=4.5	43.65 $^{2}J_{PC}$=12.9	47.12 $^{2}J_{PC}$=12.3	37.59	13.83	-
7b	13.74 $^{3}J_{PC}$=5.1	39.17 $^{2}J_{PC}$=4.5	44.24 $^{2}J_{PC}$=14.2	44.28 $^{2}J_{PC}$=13.5	31.26 $^{2}J_{PC}$=3.9	43.67 $^{2}J_{PC}$=13.0	47.31 $^{2}J_{PC}$=12.3	37.54	13.83	-
8a	13.68 $^{3}J_{PC}$=5.3	39.40 $^{2}J_{PC}$=4.6	43.70 $^{2}J_{PC}$=13.1	44.31 $^{2}J_{PC}$=13.9	35.68	45.36	24.74	50.50	37.60	13.84
8b	13.73	39.41 $^{2}J_{PC}$=4.6	43.75 $^{2}J_{PC}$=13.1	44.47 $^{2}J_{PC}$=13.8	35.70	45.37	24.66	50.51	37.53	13.80

Table S2 ^{1}H NMR spectral data of the phosphazenes. [Chemical shifts (δ) reported in ppm and *J* values in Hz]. [d: doublet, m: multiplet, q: guartet and dd: doublet of doublets]

	PhC$\underline{H}_2$	CzC$\underline{H}_2$	$\underline{H}$a	$\underline{H}$b	$\underline{H}$c	$\underline{H}$d	$\underline{H}$e	$\underline{H}$f	$\underline{H}$g	$\underline{H}$h	$\underline{H}$i	$\underline{H}$j
5a	4.27 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=7.6 4.24 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=7.6	4.07 1H, dd $^{2}J_{HH}$=13.2 $^{3}J_{PH}$=8.4 4.06 1H, dd $^{2}J_{HH}$=13.2 $^{3}J_{PH}$=8.4	2.58 3H, d $^{3}J_{PH}$=12.4	3.02-3.26 2H, m	3.02-3.26 2H, m	2.52 3H, d $^{3}J_{PH}$=12.8	3.02-3.26 2H, m	3.02-3.26 2H, m	4.36 2H, q $^{3}J_{HH}$=7.2	1.43 3H, t $^{3}J_{HH}$=7.2	-	-
5b	4.17 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=6.0 4.13 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=6.0	3.93 2H, d $^{3}J_{PH}$=9.2	2.72 3H, d $^{3}J_{PH}$=11.6	3.01-3.24 2H, m	3.01-3.24 2H, m	2.71 3H, d $^{3}J_{PH}$=11.6	3.01-3.24 2H, m	3.01-3.24 2H, m	4.32 2H, q $^{3}J_{HH}$=7.2	1.39 3H, t $^{3}J_{HH}$=7.2	-	-
6a	4.10 2H, d $^{3}J_{PH}$=8.0	4.32 1H, dd $^{2}J_{HH}$=14.2 $^{3}J_{PH}$=6.6 4.22 1H, dd $^{2}J_{HH}$=14.2 $^{3}J_{PH}$=6.6	2.57 3H, d $^{3}J_{PH}$=12.4	2.98-3.24 2H, m	2.98-3.24 2H, m	2.56 3H, d $^{3}J_{PH}$=13.2	2.98-3.24 2H, m	1.76-1.86 2H, m	2.98-3.24 2H, m	4.37 2H, q $^{3}J_{PH}$=7.2	1.44 3H, t $^{3}J_{PH}$=7.2	-
6b	3.92 2H, d $^{3}J_{PH}$=8.8	4.17 1H, dd $^{2}J_{HH}$=14.5 $^{3}J_{PH}$=6.6 4.02 1H, dd $^{2}J_{HH}$=14.5 $^{3}J_{PH}$=6.6	2.69 3H, d $^{3}J_{PH}$=12.8	2.96-3.27 2H, m	2.96-3.27 2H, m	2.69 3H, d $^{3}J_{PH}$=12.8	2.96-3.27 2H, m	1.72-1.86 2H, m	2.96-3.27 2H, m	4.32 2H, q $^{3}J_{HH}$=7.2	1.40 3H, t $^{3}J_{HH}$=7.2	-
7a	4.28 1H, dd $^{2}J_{HH}$=14.4 $^{3}J_{PH}$=6.6 4.21	4.06 2H, d $^{3}J_{PH}$=8.4	1.14 3H, t $^{3}J_{HH}$=7.2	2.94-3.04 2H, m	3.07-3.27 2H, m	3.07-3.27 2H, m	2.51 3H, d $^{3}J_{PH}$=11.6	3.01-3.26 2H, m	3.01-3.26 2H, m	4.36 2H, q $^{3}J_{HH}$=7.6	1.44 3H, t $^{3}J_{HH}$=7.6	-

	1H, dd $^{2}J_{HH}$=14.4 $^{3}J_{PH}$=6.6											
7b	4.13 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=6.8 4.04 1H, dd $^{2}J_{HH}$=14.8 $^{3}J_{PH}$=6.8	3.90 2H, d $^{3}J_{PH}$=8.0	1.26 3H, t $^{3}J_{HH}$=7.2	3.22-3.28 2H, m	3.14-3.19 2H, m	3.14-3.19 2H, m	2.67 3H, d $^{3}J_{PH}$=11.6	2.99-3.12 2H, m	2.99-3.12 2H, m	4.33 2H, q $^{3}J_{HH}$=6.8	1.40 3H, t $^{3}J_{HH}$=6.8	-
8a	4.32 2H, d $^{3}J_{PH}$=8.0	4.19 1H, dd $^{2}J_{HH}$=14.4 $^{3}J_{PH}$=6.4 4.08 1H, dd $^{2}J_{HH}$=14.4 $^{3}J_{PH}$=6.4	1.06 3H, t $^{3}J_{HH}$=7.2	3.22-3.26 2H, m	3.06-3.16 2H, m	3.06-3.16 2H, m	2.56 3H, d $^{3}J_{PH}$=13.2	2.90-3.02 2H, m	1.73-1.83 2H, m	2.90-3.02 2H, m	4.37 2H, q $^{3}J_{HH}$=7.2	1.44 3H, t $^{3}J_{HH}$=7.2
8b	3.92 2H, d $^{3}J_{PH}$=8.4	4.19 1H, dd $^{2}J_{HH}$=14.6 $^{3}J_{PH}$=6.6 4.03 1H, dd $^{2}J_{HH}$=14.6 $^{3}J_{PH}$=6.6	1.24 3H, t $^{3}J_{HH}$=6.8	3.24-3.28 2H, m	2.97-3.18 2H, m	2.97-3.18 2H, m	2.69 3H, d $^{3}J_{PH}$=13.2	2.97-3.18 2H, m	1.76-1.85 2H, m	2.97-3.18 2H, m	4.32 2H, q $^{3}J_{HH}$=6.8	1.41 3H, t $^{3}J_{HH}$=6.8





	$\underline{H}_2$	$\underline{H}_3$	$\underline{H}_{2'}$	$\underline{H}_{3'}$	$\underline{H}_{6'}$	$\underline{H}_{9'}$	$\underline{H}_{10'}$	$\underline{H}_{11'}$	$\underline{H}_{12'}$
5a	7.42 2H, d $^3J_{HH}$=8.4	7.38 2H, d $^3J_{HH}$=8.4	7.58 1H, dd $^3J_{HH}$=8.2 $^4J_{HH}$=1.4	8.12 1H, d $^3J_{HH}$=8.2	7.46 1H, d $^4J_{HH}$=1.4	7.43 1H, d $^3J_{HH}$=7.8	7.24 1H, dd $^3J_{HH}$=8.2 $^3J_{HH}$=7.8	7.48 1H, d $^3J_{HH}$=8.2	8.14 1H, s
5b	7.38 2H, d $^3J_{HH}$=8.2	7.30 2H, d $^3J_{HH}$=8.0	7.47 1H, d $^3J_{HH}$=8.0	7.95 1H, d $^3J_{HH}$=8.0	7.44 1H, s	7.43 1H, d $^3J_{HH}$=8.0	7.24 1H, dd $^3J_{HH}$=8.0 $^3J_{HH}$=7.8	7.45 1H, dd $^3J_{HH}$=7.8 $^3J_{HH}$=8.0	8.12 1H, d $^3J_{HH}$=8.0
6a	7.42 2H, d $^3J_{HH}$=8.2	7.32 2H, d $^3J_{HH}$=8.2	7.55 1H, dd $^3J_{HH}$=8.0 J_{HH}=1.4	8.12 1H, d $^3J_{HH}$=8.0	7.45 1H, d $^4J_{HH}$=1.4	7.38 1H, d $^3J_{HH}$=8.2	7.23 1H, dd $^3J_{HH}$=8.2 $^3J_{HH}$=7.8	7.48 1H, d $^3J_{HH}$=7.8	8.12 1H, s
6b	7.38 2H, d $^3J_{HH}$=8.4	7.15 2H, d, $^3J_{HH}$=8.4	7.45 1H, s	7.90 1H, s	7.43 1H, s	7.29 1H, d $^3J_{HH}$=8.4	7.17 1H, dd $^3J_{HH}$=8.4 $^3J_{HH}$=6.8	7.20 1H, dd $^3J_{HH}$=6.8 $^3J_{HH}$=7.8	8.03 1H, d $^3J_{HH}$=7.8
7a	7.40, 2H, d $^3J_{HH}$=6.8	7.32 2H, d $^3J_{HH}$=6.8	7.57 1H, dd $^3J_{HH}$=7.7 $^4J_{HH}$=1.2	8.11 1H, d $^3J_{HH}$=7.7	7.40 1H, d $^4J_{HH}$=1.2	7.38 1H, d $^3J_{HH}$=8.0	7.24 1H, dd $^3J_{HH}$=8.0 $^3J_{HH}$=7.4	7.46 1H, d $^3J_{HH}$=7.4	8.13 1H, s
7b	7.27 2H, d $^3J_{HH}$=8.2	7.21 2H, d $^3J_{HH}$=8.2	7.46 1H, s	8.00 1H, s	7.39 1H, s	7.38 1H, d $^3J_{HH}$=8.0	7.15 1H, dd $^3J_{HH}$=8.8 $^3J_{HH}$=8.0	7.42 1H, dd $^3J_{HH}$=8.8 $^3J_{HH}$=8.2	8.08 1H, d $^3J_{HH}$=8.2
8a	7.41 2H, d $^3J_{HH}$=8.4	7.31 2H, d $^3J_{HH}$=8.4	7.53 1H, d $^3J_{HH}$=8.2	8.11 1H, d $^3J_{HH}$=8.2	7.40 1H, s	7.40 1H, d $^3J_{HH}$=8.0	7.23 1H, dd $^3J_{HH}$=8.0 $^3J_{HH}$=7.2	7.46 1H, d $^3J_{HH}$=7.2	8.12 1H, s
8b	7.38 2H, d $^3J_{HH}$=8.2	7.08 2H, d $^3J_{HH}$=8.2	7.47 1H, s	7.89 1H, s	7.43 1H, s	7.44 1H, d $^3J_{HH}$=8.4	7.17 1H, dd $^3J_{HH}$=8.4 $^3J_{HH}$=8.4	7.19 1H, dd $^3J_{HH}$=7.6 $^3J_{HH}$=8.4	8.03 1H, d $^3J_{HH}$=7.6

Cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) assay was performed according to ISO 10993-5 to determine the viability of L929 fibroblast and MCF-7 breast cancer cells, treated with synthesized compounds. MCF-7 breast cancer and L929 fibroblast cells were cultured in RPMI-1640 and DMEM, respectively, and were supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. All cells were incubated at 37°C for 24 hours and in a humidified atmosphere of 5% CO_2. Cells were inoculated into 96-well plates at a concentration of $1x10^4$ per well and were incubated at 37 ºC for 24 hours in a 5% CO_2-conditioned incubator. Then, synthesized compounds at various concentrations (between 2500 to 78 µM) were applied to cells and incubated for another 24 hours. For the control group, just RPMI-1640/DMEM mediums were used, and DMSO was used as a positive control. After incubation, the cell medium was discarded from the wells, and 50 µL of MTT solution (1mg/mL) was added to each. After 2-4 hours of incubation, the MTT solution was removed from the wells and 100 µL of isopropanol was added to each well and read at 570 nm wavelength in the microplate reader. Cell viability (%) was obtained by comparing the results from the plate reader with the control group and was calculated by the following formula:

$$Cell\,Viability\% = \frac{Compound\;OD}{Control\;OD}x100$$

OD: Optical density

Table S3 The IC_{50} values of the compounds.

Compounds	**IC_{50} Values (µM)**	
	L929	**MCF-7**
3	$2.0 \times 10^3 \pm 300$	$1.7 \times 10^3 \pm 30$
4	$2.3 \times 10^3 \pm 200$	$1.6 \times 10^3 \pm 200$
5a	$2.5 \times 10^3 \pm 200$	$2.6 \times 10^3 \pm 300$
5b	$2.9 \times 10^3 \pm 200$	$1.7 \times 10^3 \pm 40$
7b	$2.6 \times 10^3 \pm 300$	$1.9 \times 10^3 \pm 100$
8a	$2.7 \times 10^3 \pm 300$	$1.8 \times 10^3 \pm 100$
Cis-platin	$3.6 \times 10^1 \pm 3$	$2.1 \times 10^1 \pm 1$

Determinations of the DNA interactions with the compounds

Stock solutions of these compounds (**5a-8b**) were prepared in dimethyl sulfoxide (DMSO) at a concentration of 2500 µM and used within 1 h. After that, aliquots of decreasing concentrations of phosphazenes ranging from 2500 to 156 µM in Tris-EDTA buffer, and the 0.5 µg/µL pBR322 plasmid DNA (Thermo Scientific) in 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA was added to compounds and the mixtures were incubated at 37°C for 24 h in the dark. Compound/DNA mixtures were loaded onto agarose gel with a loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol). Agarose gel electrophoresis was performed under tris Acetate-EDTA (TAE) buffer (0.05 M Tris base, 0.05 M glacial acetic acid, and 1 mM ethylenediaminetetraacetic acid, EDTA, pH=8.0) for 1 h at 70 V. Eventually, the

gel was stained with ethidium bromide (0.5 µg/mL) and visualized under UV light using a transilluminator (BioDoc Analyzer, Biometra). The image was captured with a video camera as a TIFF file. Each experiment was repeated three times and the mean values were selected.

*BamH*I and *Hind*III digestion

Compound/DNA mixtures were incubated for 24 h, and then restricted with 1 U/µL *Hind*III in buffer *Hind*III (Thermo Scientific) and 1 U/µL *BamH*I in buffer *BamH*I (Thermo Scientific) enzymes for 1 h at 37 °C. The restricted DNA was run in 1% agarose gel electrophoresis for 2 h at 70 V in TAE buffer.[1] The gel was stained with ethidium bromide (0.5 µg/mL), and afterward, the gel was viewed with a transilluminator. The image was photographed with a video camera and saved as a Tagged Image File Format (TIFF) file.

*Bam*HI enzyme binds at the sequence 5'-G/GATCC-3'. Since pBR322 plasmid DNA contains a single sequence, it cleaves this sequence. After that, *Bam*HI converts supercoiled Form I and open circular Form II to linear form of linear DNA (Form III). *Hind*III recognizes the sequence 5'-A/AGCTT-3' and cleaves this sequence. Hereby, *Hind*III converts Form I and Form II to Form III, similar to *Bam*HI.

Antimicrobial activity

For antimicrobial activity testing, a stock solution of the compounds was prepared in DMSO at a concentration of 2500 µM. For positive control, Ampicillin and Chloramphenicol were selected as the reference antibacterial agents, and Ketoconazole was used as a reference antifungal agent. They were the commercially available antibiotics, and were chosen as a control. These antimicrobial agents were dissolved in DMSO at a concentration of 2500 µM. Final concentration of DMSO in the tested cultures was 1%. The stock solutions of the compounds and the antimicrobial agents were stored at 4 °C. DMSO was used as negative control. Before the experiment, all compounds and antimicrobial agents were sterilized by passing through a syringe filter with a pore diameter of 0.2 µm.

The microorganisms were obtained from the collections of Gazi University Molecular Biology Culture Collection, Turkey. Eleven bacterial [six G(-), five G(+)] and three fungal strains were used for detection of antimicrobial activity of the new phosphazenes (**5a-8b**). G(-) bacterial strains were as follows; *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* RSKK 96029; G(+) bacterial strains were *Bacillus cereus* NRRL B-371, *Bacillus subtilis* ATCC 6633*,* *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, and fungal strains were *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* Y-12968. G(+) and G(-) bacteria were grown on nutrient agar (Merck, Germany) plates and incubated at 37 °C for 24 h. Whilst, the yeast strains were grown in Sabouraud dextrose agar (SDA) (Merck, Germany) medium and incubated at 30 °C for 48 h. After incubation, the inoculums of microorganisms were prepared from a single colony in the fresh cultures and suspensions in 0.9% NaCl were adjusted to 0.5 McFarland standard turbidity.

Determinations of the MIC and MBC/MFC values

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungucidal concentration (MFC) values of the compounds were measured by the broth microdilution method in 96-well microliter plates according to CLSI reference method M7-A7[2] for bacteria and M27-A3[3] for yeasts. For antimicrobial testing, 100 µL of medium (Mueller Hinton Broth for bacteria, Saboroud Dextrose Broth for fungi) (Merck, Germany) were added in each well. After adding the compounds (2500 µM), antimicrobial agents or DMSO to be tested individually to the first wells, serial two-fold dilutions were made in microtitre plates. Five microliters of microorganisms were added to all wells. The microtitre plates were then incubated for 24 h at 37 °C (for bacterial strains) and for 48 h at 30 °C (for fungal strains).

Furthermore, after incubation period, the minimum inhibitory concentration (MIC) values of the active phosphazenes were determined by lack visual turbidity. The minimum bactericidal concentration (MBC) and minimum fungucidal concentration (MFC) values of these compounds were designated using subculturing ten microliters volumes from non-turbid wells and spot inoculating onto agar plates [Tryptic Soy Agar (Merck, Germany) for bacteria and Saboroud Dextrose Agar (Merck, Germany) for fungi] and incubated for 24 h at 37 °C (for bacterial strains) and for 48 h at 30 °C (for fungal strains). After incubation, growth was recorded (visible colony formation) and MBCs/MFCs were defined as the lowest concentration without any microorganism growth. Also, carbazolyldiamines (**3** and **4**) were tested by inhibition zone diameters in mm. The assay was repeated at least three times and average MIC and MBC/MFC values were listed.

Table S4 Antimicrobial activities of **3**, **4** and **5a-8b** and positive controls against different bacterial and fungal strains. Inhibition zone diameters are expressed in mm. (Amp: Ampicillin, C: Chloramphenicol and Keto: Ketoconazole).

Test microorganisms	**Compounds**										**Positive Controls**	
	3	**4**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**8a**	**8b**	**Amp**	**C**	**Keto**
E. coli **ATCC 35218** ***G(-)***	12 ± 1	19 ± 0	11 ± 1	11 ± 0	-	-	-	-	10 ± 1	-	-	8 ± 0	NS
E. coli **ATCC 25922** ***G(-)***	15 ± 1	16 ± 1	-	-	-	-	11 ± 0	-	11 ± 1	12 ± 0	18 ± 0	25 ± 0	NS
B. cereus **NRRL B-3711** ***G(+)***	16 ± 1	22 ± 1	16 ± 1	16 ± 1	17 ± 1	17 ± 0	20 ± 1	18 ± 1	11 ± 1	11 ± 1	-	-	NS
B. subtilis **ATCC 6633** ***G(+)***	16 ± 1	19 ± 0	16 ± 2	17 ± 0	19 ± 0	16 ± 1	19 ± 1	20 ± 1	12 ± 1	13 ± 1	23 ± 1	21 ± 0	NS
S. aureus **ATCC 25923** ***G(+)***	15 ± 1	11 ± 0	12 ± 1	17 ± 2	15 ± 1	13 ± 2	18 ± 1	19 ± 1	12 ± 1	12 ± 1	44 ± 1	24 ± 1	NS
E. faecalis **ATCC 29212** ***G(+)***	14 ± 1	11 ± 1	19 ± 1	17 ± 1	17 ± 2	15 ± 2	17 ± 1	19 ± 1	11 ± 1	12 ± 0	27 ± 0	20 ± 0	NS
P. aeruginosa **ATCC 27853** ***G(-)***	-	-	-	-	-	-	-	-	13 ± 0	11 ± 1	60 ± 0	34 ± 0	NS
K. pneumaniae **ATCC 13883** ***G(-)***	15 ± 1	14 ± 1	-	10 ± 0	-	-	10 ± 0	10 ± 1	12 ± 1	13 ± 0	-	31 ± 1	NS
S. typhimurium **ATCC 14028** ***G(-)***	-	12 ± 2	-	-	-	-	11 ± 0	11 ± 0	14 ± 0	-	19 ± 1	38 ± 1	NS
E. hirae **ATCC 9790** ***G(+)***	15 ± 1	12 ± 1	17 ± 1	16 ± 1	15 ± 1	14 ± 1	-	15 ± 0	11 ± 0	-	9 ± 1	22 ± 1	NS
P. vulgaris **RSKK 96029** ***G(-)***	-	-	10 ± 1	10 ± 1	-	-	11 ± 1	11 ± 0	-	11 ± 1	-	32 ± 1	NS
C. albicans **ATCC 10231**	29 ± 1	33 ± 2	10 ± 1	10 ± 1	11 ± 1	-	-	11 ± 1	-	10 ± 1	NS	NS	11 ± 1
C. krusei **ATCC 6258**	37 ± 1	15 ± 2	-	-	-	11 ± 0	-	11 ± 1	-	-	NS	NS	18 ± 1
C. tropicalis **Y-12968**	36 ± 2	20 ± 0	15 ± 0	15 ± 1	15 ± 2	13 ± 1	14 ± 1	15 ± 1	-	12 ± 1	NS	NS	34 ± 2

Determination of antioxidant activities of the compounds

1,1 -Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

These compounds were dissolved in DMSO at a concentration of 2500 μM. 0.5 mL of the compound solutions mixed with a freshly prepared ethanolic solution of DPPH (Sigma-Aldrich) radical (0.1 mM), incubated 30 min at room temperature, and the absorbance was measured at 517 nm by a spectrophotometer (Shimadzu UV-1800, Japan) against a blank. The percentage of DPPH radicals inhibition was calculated using the equation $[(A_{control}-A_{sample})/A_{control}]x100$, where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of the compounds. Butylated hydroxytoluene (BHT) is a phenolic antioxidant and was used as a positive control in this study. BHT (Sigma-Aldrich) was dissolved in ethanol at a concentration of 2500 μM and used at this concentration for antioxidant activity assay.

Antioxidant activities of phosphazenes 5a-8b

The most widely used experimental method for the determination of antioxidant activities of natural or synthetic compounds is the evaluation of free radical scavenging activity with DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate).[4] Free radicals are very reactive groups and/or molecules with unpaired electrons formed during metabolic reactions within cells. Free radicals can induce cancers by altering the redox system, causing DNA damage and increasing the activation of pro-carcinogens. Excessive formation of free radicals in living things is among the causes of many chronic diseases and cancers.[5] When there is not enough antioxidant compound, the free radical/antioxidant balance is disrupted and oxidative stress causes many health problems such as cancer, diabetes, neurodegeneration, cardiovascular diseases, rheumatoid arthritis, kidney disease and eye disease occur in the body.[6,7]. However, antioxidant molecules can minimize free radicals and greatly reduce their damage. Therefore, interest in synthesizing new antioxidant compounds is increasing due to their protective role against oxidative stress in the body. Thereof, neutralizing free radicals is the primary and essential task in protecting human health through antioxidants. The activities of antioxidants are estimated by the DPPH test.[4] The results found to provide important evidence for the use of these molecules in medical applications.[8,9]

The antioxidant activities of the synthesized compounds (**5a**-**8b**) and butylated hydroxytoluene (BHT) in this study were calculated using the DPPH scavenging test. The experiment was repeated three times. Absorbance values at 517 nm were measured for the control group and compounds at concentrations of 2500 μM. The % radical scavenging activities of these compounds at 2500 μM concentration were listed in Table S5. Compound **5b** has the highest antioxidant capacity with a radical scavenging activity value of 35.20%, while **8b** has the lowest activity at 1.98%. However, antioxidant activities of all compounds are lower than synthetic antioxidant BHT as used for positive control. None of the compounds were high antioxidant activity up to BHT.

Table S5 DPPH radical scavenging activities of the compounds at a concentration of 2500 μM.

Compounds	Radical Scavenging Activity %
5a	17.18 ± 1.90
5b	35.20 ± 1.17
6a	2.96 ± 1.21
6b	12.08 ± 0.62
7a	11.59 ± 0.31
7b	18.28 ± 0.95
8a	4.35 ± 0.65
8b	1.98 ± 2.33
BHT	83.31 ± 2.05

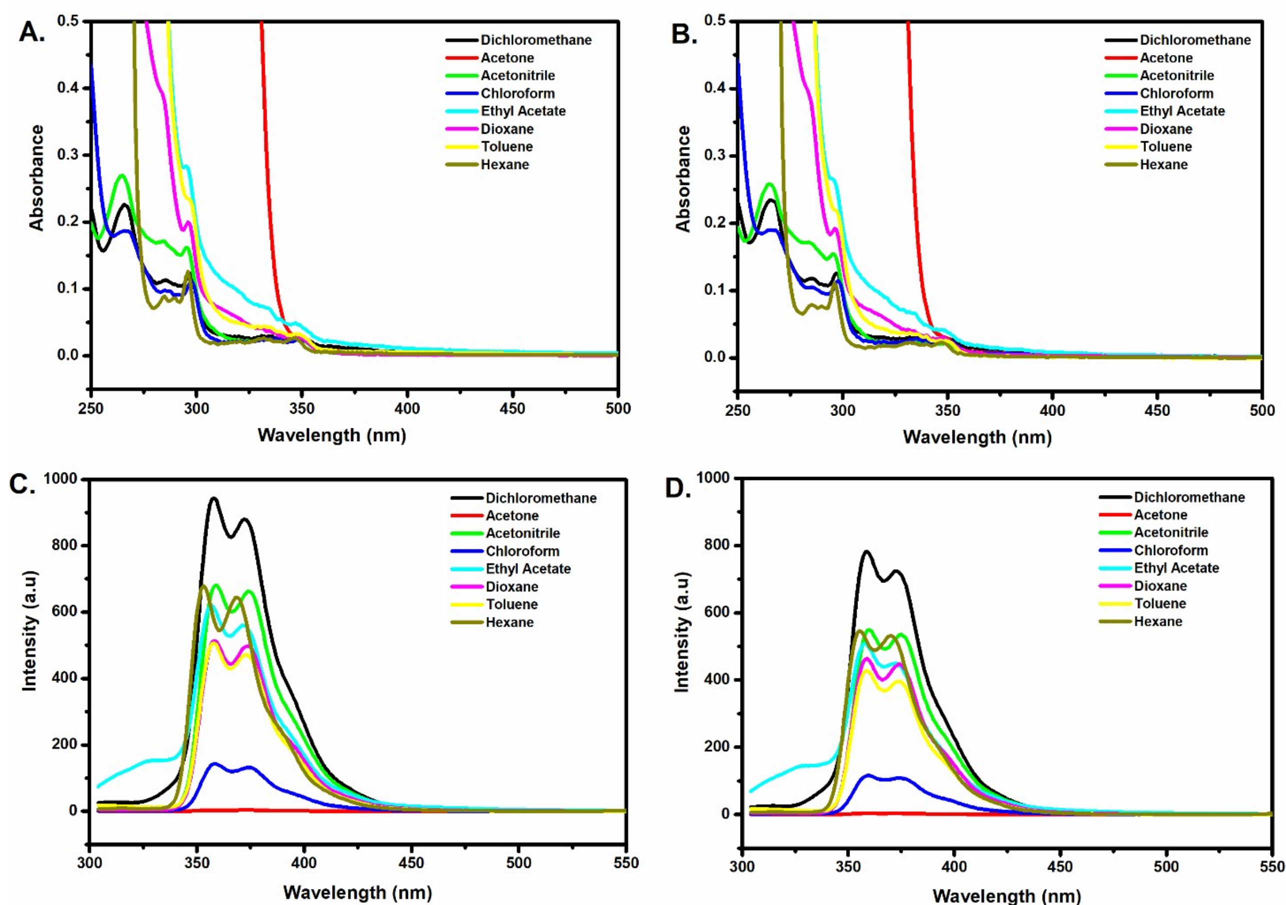


Fig S1 Optical absorption and fluorescence emission spectrum of **6a** (A, C) and **6b** (B, D) in solvents with various polarities ($5x10^{-6}$ M; ex: 290 nm).

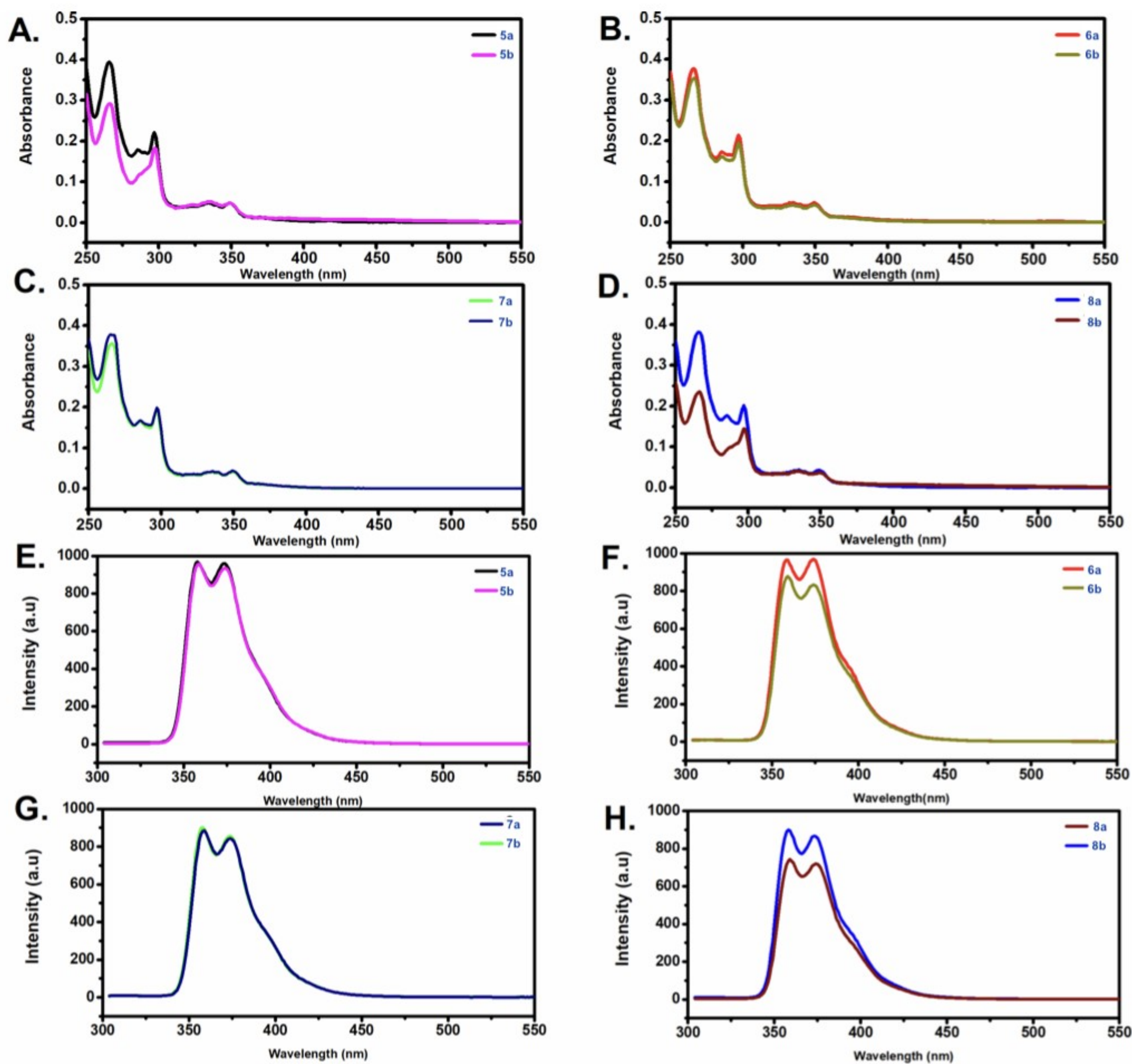


Fig. S2 Comparison of optical absorption (A-D) and fluorescence emission (E-H) spectra of trans **5a**, **6a**, **7a** and **8a** and cis **5b**, **6b**, **7b** and **8b** isomers in DCM ($5x10^{-6}$ M; ex: 290 nm).

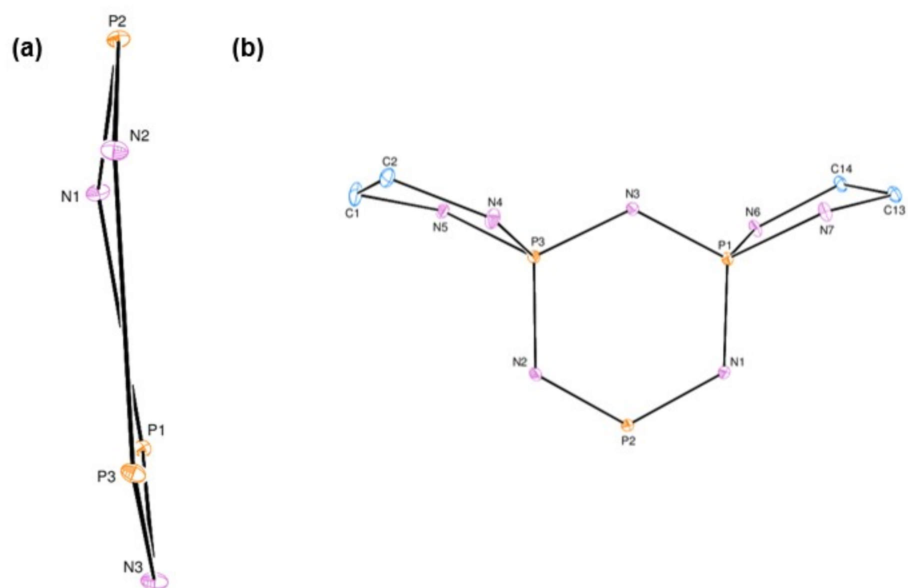


Fig. S3 The conformations of (a) the phosphazene ring and (b) the five-membered spiro-rings of **7a**.

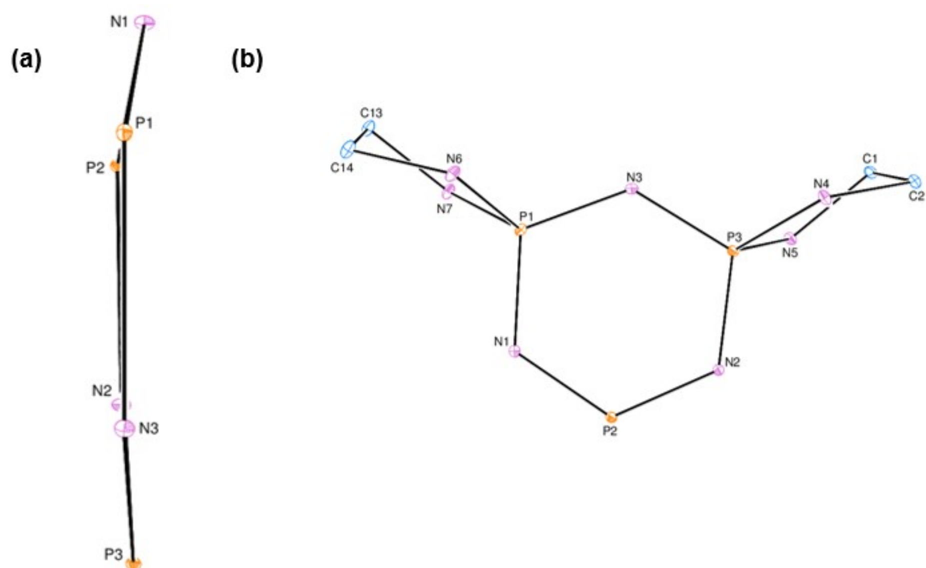


Fig. S4 The conformations of (a) the phosphazene ring and (b) the five-membered spiro-rings of **7b**.

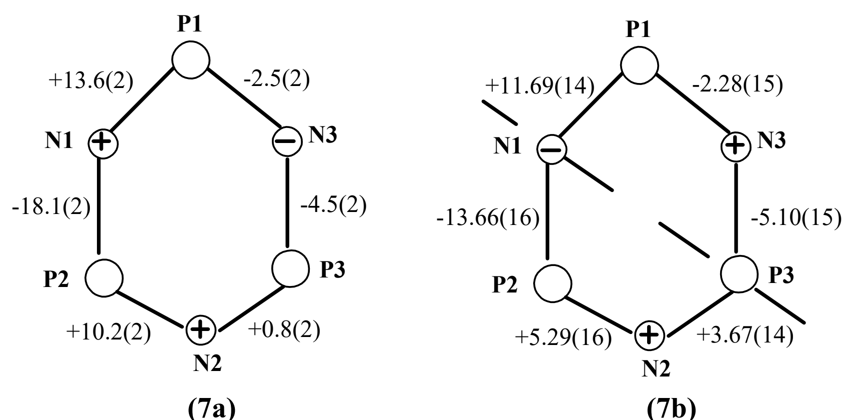


Fig. S5 The shapes of the phosphazene rings in trans-**7a** and cis-**7b** with torsion angles (deg).

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