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Der Pharma Chemica, 2011, 3 (4):377-388
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and anti-cancer evaluation of cyclotriphosphazene hydrazone derivatives

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ABSTRACT

Hexasubstituted cyclotriphosphazene hydrazones [N_3P_3(-OC_6H_4-p-CH=N-NH-C(O)-C_6H_4-p-X)$_6$] were prepared by the reaction of hexachlorocyclotriphosphazene with N'-(4-hydroxybenzylidene)-4-substituted-benzohydrazides [HO-C_6H_4-p-CH=N-NH-C(O)-C_6H_4-p-X]. The structures were confirmed by elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectrometry. These cyclic model systems bearing hydrolysable hydrazone linkages were evaluated for their antiproliferative activity in vitro against the human liver carcinoma cell line (HepG2) and Human cervix carcinoma cell line (HeLa).

Keywords: Hydrazide; Hydrazone; Cyclotriphosphazene; HepG2; HeLa.

INTRODUCTION

Phosphazenes are an important family of inorganic molecules in which the phosphorus and nitrogen repeat units can form cyclic compounds or chains [1, 2]. The rich nucleophilic substitution chemistry of the P–Cl bonds of chlorocyclophosphazenes and the relative robustness of the cyclophosphazene framework have allowed the use of these inorganic P-N heterocyclic ring systems as synthetic and structural models for the reactions of the analogous high molecular weight polyphosphazenes [3-5]. Cyclophosphazenes are also versatile staring materials for the syntheses of star-shaped polymers [6, 7] and dendrimers [8, 9]. Cyclotriphosphazenes have continued to attract the increased attention of researchers in recent years for variety of biomedical [10] and material applications [11] resulting from the diversity of the products that can be obtained by simple halogen-substitution reactions. A great deal of research has been devoted to the design of cyclophosphazene derivatives as anticancer reagents [12-14]. Cyclotriphosphazene derivatives are used as effective drug carriers for anticancer therapy [15]. Nanoparticulate amphiphilic cyclotriphosphazene-drug conjugates are tested for anticancer activity [16]. Recently it is reported that platinum drugs physically encapsulated by the cyclotriphosphazene micelles exhibits outstanding pharmacokinetics along with excellent tumor selectivity [17].

Hydrazides and hydrazones are important synthons for several transformations and have gained importance due to their diverse biological and clinical applications [18]. Hydrazone linkage provides a suitable system for pH-dependent release of anticancer drugs from drug-conjugates [19]. Several studies have been devoted to the anticancer activity of aroylhydrazone derivatives [20, 21]. Hydrazone derivatives containing an azomethine (–CONHN=CH–) group have been shown to exhibit antiproliferative activities and act as cytotoxic agents with the ability to prevent cell progression in cancerous cells through different mechanisms [22].

Prompted by above observations it seemed worthwhile to investigate if the presence of hydrazone moieties on the cyclotriphosphazene skeleton can also give rise to a new class of biologically active compounds. Some cyclophosphazene hydrazones have been obtained by the condensation of aldehydes with hexamethylhydrazinocyclotriphosphazene [23, 24]. We have recently reported the synthesis of cyclophosphazene hydrazones by the reaction of aromatic hydrazides with hexakis(4-formylphenoxy)-cyclotriphosphazene [25]. In the present work we report the synthesis of similar hydrazones by a different methodology. Cyclophosphazene hydrazones described in this article were prepared by the reaction of hexachlorocyclotriphosphazene (HCCP) (**1**) with N'-(4-hydroxybenzylidene)-4-substituted-benzohydrazides. The symbiosis that exists between cyclophosphazenes and the corresponding polymeric systems will ensure that small molecule developments will be readily translated to the more complex macromolecules [26].

Cyclophosphazene hydrazone derivatives were evaluated for *in vitro* anticancer efficacy against human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa). The compounds reported here are prototypes only since the use of aryloxy cosubstituent groups precludes the possibility of total biodegradation of these cyclophosphazene hydrazones. In future work we hope to extend these studies to systems that possess more biologically compatible spacer groups and cosubstituent units.

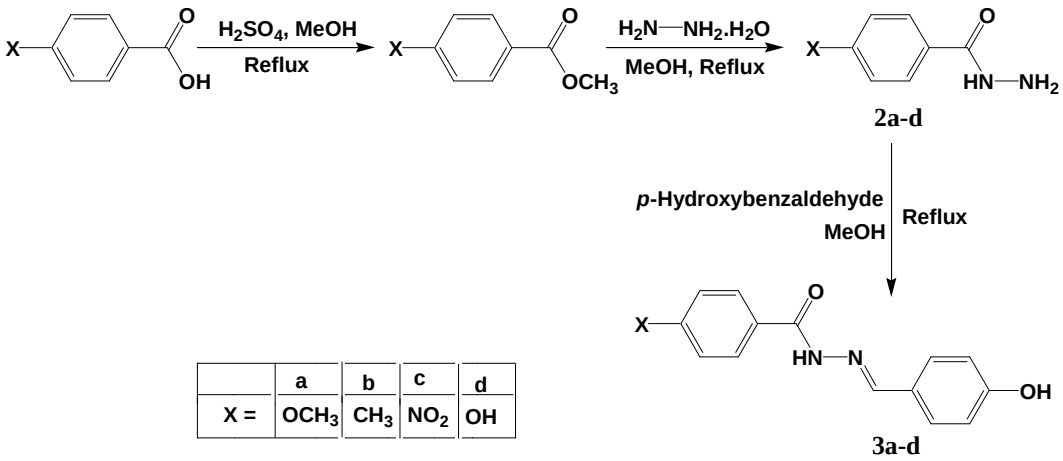
MATERIALS AND METHODS

Experimental

Materials and measurements

IR spectra were recorded in 4000–400 cm^{-1} range using an Impact-410 Nicolet (USA) FT-IR spectrometer in KBr matrix. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in DMSO-d_6 solvent on BRUKER AV-500 MHz High Resolution Multinuclear FT-NMR Spectrometer at room temperature. Chemical shifts were relative to tetramethylsilane as an internal standard at δ=0 ppm for ^{1}H and ^{13}C. The ^{31}P chemical shifts are reported in ppm relative to 85% H_3PO_4 as an external standard at 0 ppm. All heteronuclear NMR spectra were proton decoupled. Leco Model Truespec CHNS Analyser was used for elemental analyses (C, H, and N). The mass spectrum was obtained on Shimadzu GCMS-QP2010S and MALDI-TOF was measured with a Voyager-DE STR spectrometer with α-cyano-4-hydroxycinnamic acid as a matrix. Melting points were determined in an open capillary on a Gallenkamp melting point apparatus and are uncorrected.

All manipulations were carried out with standard high vacuum or dry nitrogen atmosphere techniques. Hexachlorocyclotriphosphazene (Aldrich) was re-crystallized from dry hexane. Solvents were purified by standard methods [27]. All other chemicals (sd fine chemicals, India) were used as received. All compounds were routinely checked by thin-layer chromatography on Merck aluminum-backed silica gel 60 F_{254} TLC plates.



Scheme 1. Synthesis of N'-(4-Hydroxybenzylidene)-4-substituted-benzohydrazides

Synthesis of p-substituted benzoic acid hydrazides

Methyl benzoates were synthesized from their respective *p*-substituted benzoic acids, using excess of dry methanol in presence of H_2SO_4. *para*-Substituted benzoic hydrazides **(2a-d)** were prepared by the reaction of corresponding methyl benzoates (10 mmol) with hydrazine hydrate 99% (50 mmol) in methanol under reflux for 4-6 h. The excess solvent was removed under vacuum and the residue was filtered under suction, washed with water, and dried. The spectral and analytical data of 4-methoxybenzoic hydrazide (**2a**) [28], 4-methylbenzoic hydrazide (**2b**) [29], 4-nitrobenzoic hydrazide (**2c**) [29] and 4-hydroxybenzoic hydrazide (**2d**) [30] are in good agreement with literature values.

Synthesis of N'-(4-Hydroxybenzylidene)-4-substituted-benzohydrazides

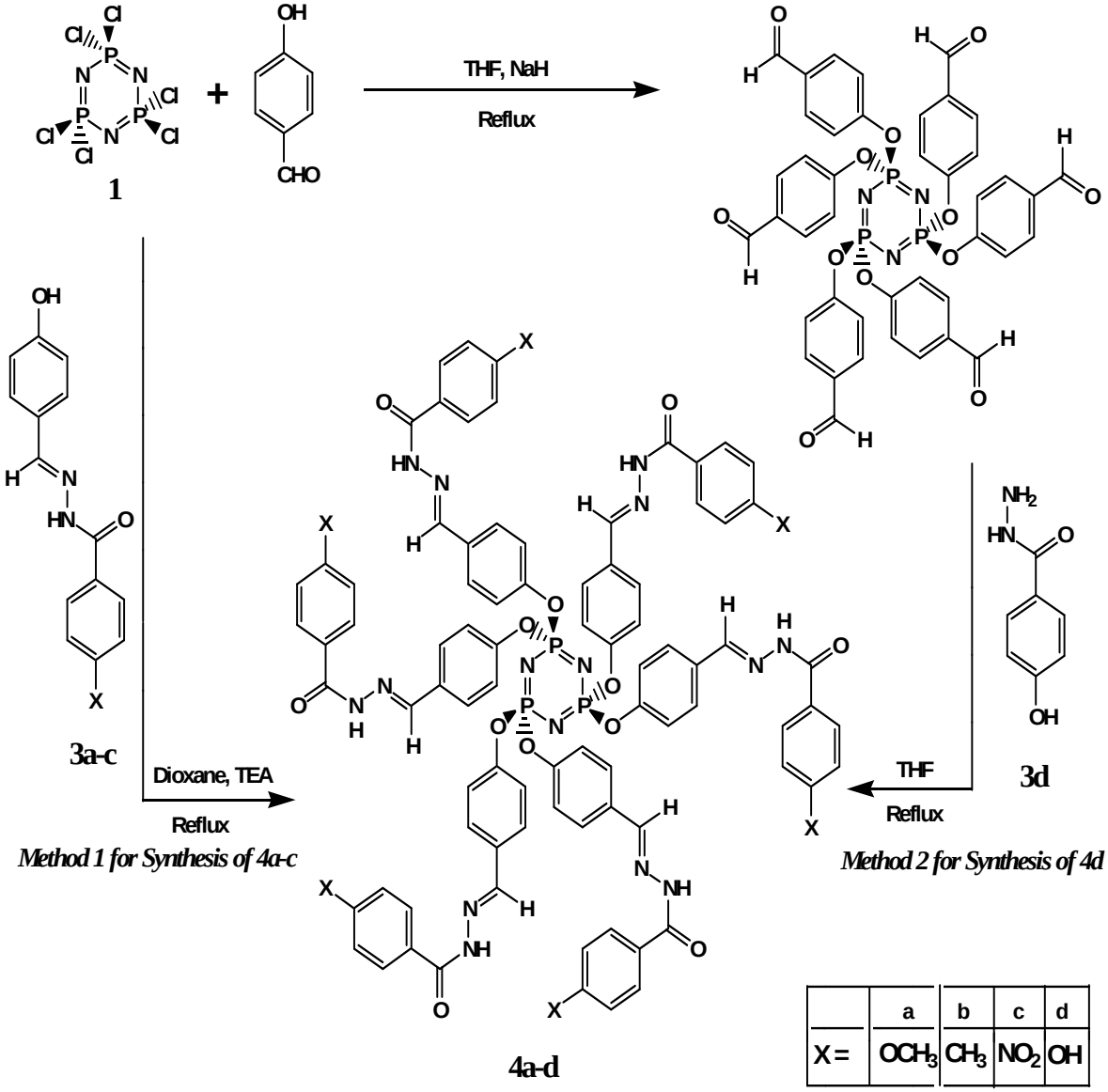
[HO-C_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-X]

Synthesis of N'-(4-Hydroxybenzylidene)-4-methoxybenzohydrazide(**3a**), N'-(4-Hydroxybenzyli -dene)-4-methylbenzohydrazide(**3b**) [31], N'-(4-Hydroxybenzylidene)-4-nitrobenzohydrazide(**3c**) [32], N'-(4-Hydroxybenzylidene)-4-hydroxybenzohydrazide(**3d**) [33] are reported. Due to the non-availability of the IR and NMR spectral data in some of these reports, N'-(4-Hydroxybenzylidene)-4-substituted-benzohydrazides (**3a-d)** were synthesized by the following general procedure.

4-Hydroxylbenzaldehyde (2 mmol) was added to a solution of 4-substituted-benzoic hydrazide (2 mmol) in 100 ml of tetrahydrofuran. The mixture was stirred at refluxing temperature for 3 h and then concentrated under vacuum. The solid product was collected by filtration under suction, and dried. The spectral data of these compounds is given below.

[HO-C_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-OCH_3] (3a)

Yield: 86.83 %. IR (KBr) cm^{-1}: 3421 υ (O-H), 3210 υ (N-H), 1647 υ (C=O), 1606 υ (C=N). ^1H NMR (500 MHz, DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3), 6.82 (d, J=8.0 Hz, 2H, C^2H), 7.03 (d, J=7.8 Hz, 2H, C^9H), 7.53 (d, J=8.0 Hz, 2H, C^3H), 7.87 (d, J=7.8 Hz, 2H, C^8H), 8.53 (s, 1H, C^5H) 9.89 (s, 1H, OH), 11.49 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 55.9 (OCH_3), 114.40 (C^9), 116.00 (C^2), 123.97 (C^7), 126.4 (C^4), 128.5 (C^8), 129.43 (C^3), 147.10 (C^5), 159.10 (C^1), 160.46 (C^{10}), 162.40 (C^6). MS m/z: 270 (M^+). Anal. Calcd. For $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36%. Found: C, 66.53; H, 5.28; N, 10.30%.



Scheme 2. Synthetic pathway and chemical structure for cyclophosphazene hydrazones 4a-d.

[HO-C_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-CH_3] (3b)

Yield: 84.45 %. IR (KBr) cm^{-1}: 3430 υ (O-H), 3281 υ (N-H), 1650 υ (C=O), 1607 υ (C=N). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 6.84 (d, J=7.8 Hz, 2H, C^2H), 7.32 (d, J=7.1 Hz, 2H, C^9H), 7.56 (d, J=7.8 Hz, 2H, C^3H), 7.82 (d, J=7.1 Hz, 2H, C^8H), 8.36 (s, 1H, C^5H), 9.95 (s, 1H, OH), 11.61 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 20.92 (CH_3), 115.60 (C^2), 125.26 (C^4), 127.45 (C^8), 128.71 (C^9), 128.85 (C^3), 130.62 (C^7), 141.47 (C^{10}), 147.71 (C^5), 159.27 (C^1), 162.57 (C^6). MS m/z: 254 (M^+). Anal. Calcd. For $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02%. Found: C, 70.81; H, 5.59; N, 10.99%.

[HO-C_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-NO_2] (3c)

Yield: 81.18 %. IR (KBr) cm^{-1}: 3436 υ (O-H), 3328 υ (N-H), 1663 υ (C=O), 1597 υ (C=N). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 6.86 (d, J=8.4 Hz, 2H, C^2H), 7.59 (d, J=8.4 Hz, 2H, C^3H), 8.14 (d, J=8.2 Hz, 2H, C^8H), 8.37 (d, J=8.2 Hz, 2H, C^9H), 8.38 (s, 1H, C^5H), 9.99 (s, 1H, OH), 11.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 115.61 (C^2), 123.53 (C^9), 124.90 (C^4), 128.34 (C^8), 128.99 (C^3), 139.21 (C^7), 148.80 (C^{10}), 149.12 (C^5), 159.60 (C^1), 163.80 (C^6). MS m/z: 285 (M^+). Anal. Calcd. For $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73%. Found: C, 59.00; H, 3.88; N, 14.73%.

[HO-C_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-OH] (3d)
Yield: 83.27 %. IR (KBr) cm^{-1}: 3426 υ (O-H), 3272 υ (N-H), 1640 υ (C=O), 1606 υ (C=N). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 6.84 (m, 4H, C^2H & C^9H), 7.54 (d, J=7.1 Hz, 2H, C^3H), 7.78 (d, J=7.3 Hz, 2H, C^8H), 8.32 (s, 1H, C^5H), 9.92 (s, 1H, OH), 10.11 (s, 1H, OH), 11.45 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 114.86 (C^9), 115.57 (C^2), 123.97 (C^4), 125.38 (C^7), 128.59 (C^8), 129.43 (C^3), 147.04 (C^5), 159.12 (C^1), 160.40 (C^{10}), 162.38 (C^6). MS m/z: 256 (M^+). Anal. Calcd. For $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93%. Found: C, 65.59; H, 4.70; N, 10.95%.

Synthesis of cyclophosphazene hydrazone
[N_3P_3(-OC_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-X)$_6$] X= OCH_3 (4a); CH_3 (4b); NO_2 (4c).
Method 1: Hexachlorocyclotriphosphazene (0.07 g, 0.2 mmol) was added to a mixture of N'-(4-Hydroxybenzylidene)-4-methoxybenzohydrazide (**3a)** (0.343 g, 1.27 mmol) and triethylamine (0.17 mL) in 100 mL of dioxane. The mixture was refluxed for 48-72 h and then concentrated under vacuum. The solid was filtered under suction and washed with water and then with minimum amount of warm THF. The resulting solid (**4a**) was dried in vacuo at 40°C for 4 h. The same general procedure was followed for the compounds **4b** and **4c.** The analytical and spectral data of these compounds are in good agreement with the literature values for similar compounds reported by us earlier [25].

Method 2: **[N_3P_3(-OC_6H_4-*p*-CH=N-NH-C(O)-C_6H_4-*p*-OH)$_6$] (4d)**
This compound was synthesised by the reaction of *p*-hydroxybenzoic hydrazide **2d** with hexakis(4-formylphenoxy)-cyclotriphosphazene as detailed in our earlier report [25].

Biological study
Under a sterile condition, human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa) were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). Cytotoxicity of compounds **4a-d** at 100 µM concentration was tested against HepG2 and HeLa cell lines using MTT assay [34, 35]. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO.
MTT test

The cells at approximately 80% confluence were selected for trypsinization. The cells were harvested by removing the medium and then 1 mL of trypsin-EDTA (200 mg/L for EDTA, 500 mg/L for trypsin in a ratio (1:250) is added and incubated at 37°C for about 5 minutes. The cells were detached from the plate and collected in a centrifuge tube and centrifuged at 1000 rpm for 5 minutes. Supernatant solution was removed and the cells were resuspended in 10mL RPMI-1640 culture medium. Cell number was determined using hemocytometer. The cell suspension was diluted to required concentration of $5x10^4$ cells/mL. 24-well microplates were seeded with 500µL of cell suspension and incubated at 37 °C and 5% CO_2 for 24 h.

After 24 h incubation, the cells were treated with the newly synthesized compounds and then incubated for further 24 h. 100 ml of PBS solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 5 mg/mL) was added to each well, and incubation was continued for another 4 h. The medium was discarded and the cells were washed with sterile PBS and then the resultant formazan blue crystals were dissolved in 500 µL DMSO and the optical density (OD) was measured at 570 nm (reference filter 690 nm) using an automatic microplate reader. Triplicate readings were obtained for each sample, and optical density values of the three wells were averaged for one sample. The results were recorded and expressed as % inhibition in cell

growth of Human liver carcinoma cell line (HepG2) and Human cervix carcinoma cell line (HeLa) compared with the control (Table 1).

Table 1 Effect of test compounds on growth of HepG2 and HeLa cells line incubated for 24 h *in vitro*

Test compounds	% Inhibition	
	HepG2	HeLa
4a	44.20	13.49
4b	55.60	6.77
4c	51.93	14.56
4d	1.83	24.06

RESULTS AND DISCUSSION

Synthesis of cyclophopshazene hydrazone

The synthetic procedures adopted are illustrated in Scheme 1 and 2. The initial reaction consists of the condensation of 4-hydroxybenzaldehyde with the benzoic acid hydrazides **2a**–**d** in tetrahydrofuran to yield the corresponding hydrazones **3a**–**d** (Scheme 1). The second step consists of the reaction of six equivalents of hydrazones **3a–c** bearing phenolic hydroxyl group with hexachlorocyclotriphosphazene in Dioxane in presence of triethylamine for 48-72 h at refluxing temperature to obtain hexafunctionalized cyclophosphazene hydrazones **4a-c** (Scheme 2)**.** The reaction of hexachlorocyclotriphosphazene with **3d** bearing two reactive hydroxyl functional groups lead to mixture of compounds which could not be separated by routine chromatographic techniques. **4d** was synthesized by condensation of six equivalents of *p*-hydroxybenzoic hydrazide **2d** with the aldehyde functional groups of hexakis(4-formylphenoxy)-cyclotriphosphazene as detailed in our earlier report [25]. The synthetic method adopted for the preparation of cyclophosphazene hydrazone derivatives requires high temperature and longer reaction time and is limited for the synthesis of hydrazone derivatives **4a-c** bearing *p*-substituents which do not compete with hydroxyl functional group of the benzylidene part of the hydrazones **3a-c**.

All products were generally obtained in high yields. The synthesized cyclophosphazene hydrazones were characterized by elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{31}P NMR spectroscopy and MALDI-TOF mass spectrometry. The diagnostic IR bands and detailed assignment of ^{1}H and ^{13}C NMR resonances along with analytical data are presented in the experimental section.

IR spectral studies

The formation of hydrazones **3a**–**d** was confirmed by the appearance of imine C=N stretching frequencies in the 1597-1607 cm^{-1} region along with a broad band at 3210-3328 cm^{-1} ascribed to N-H stretching frequency of the amide (-NH-C=O) moiety. A strong band at 1640-1663 cm^{-1} is due to the amide carbonyl (C=O) stretching frequencies.

A broad band observed at 3421-3436 cm^{-1} region ascribed to ν (OH) observed in **3a-c** are absent in **4a-c**. Slight variations in the infrared vibrations were observed when the IR spectra of hydrazones **3a-d** and **4a-d** are compared. P=N stretching vibrations, characteristic of cyclophosphazenes, are observed between 1166 and 1211 cm^{-1} as sharp bands for **4a-d**. Furthermore, P–O–Ar stretching is observed as a distinct band at 952-957 cm^{-1}. Thus, the IR spectral data provide strong evidences for the formation of the cyclophosphazene hydrazones.

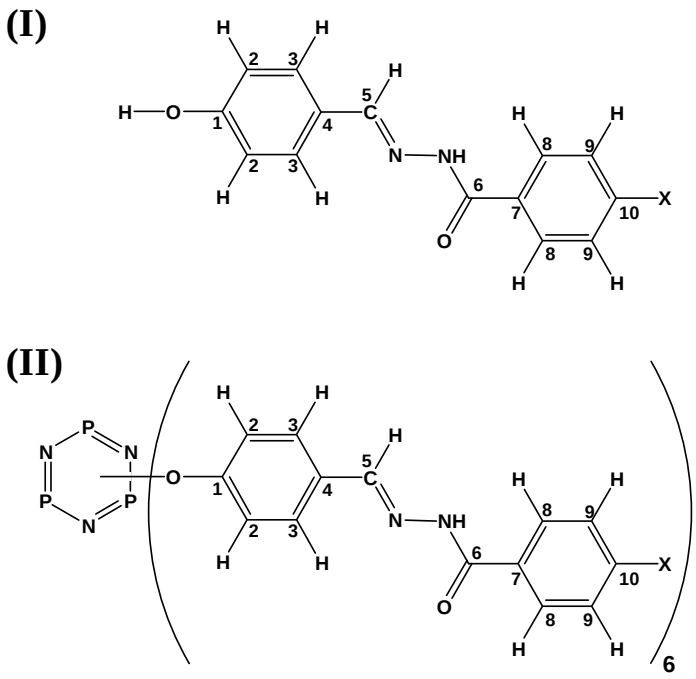


Figure 1. Numbering pattern for NMR assignments of (I) 3a-d and (II) 4a-d

NMR investigations

Detailed assignment of ^{1}H and ^{13}C NMR resonances is presented in experimental section was made based on the DEPT NMR analysis. The numbering used for NMR assiagnments is presented in Figure 1. According to the NMR spectral data, all the cyclophosphazene hydrazone (**4a-d**) molecules appear to have symmetric structures in solution.

The appearance of singlet peak for azomethine (-***H***C=N-N-) proton in the range 8.32-8.53 ppm supports the formation of hydrazones **3a-d**. This is further confirmed by the resonance for hydrazide (-CO-N***H***-N=C) proton as a broad singlet in the downfield region 11.45-11.98 ppm. The resonances due to aromatic protons appear in the range 6.82 to 8.37 ppm. A singlet at 3.83 ppm and 2.38 ppm for **3a** and **3b** accounts for protons of *p*-methoxy (-OCH_3) and *p*-methyl (-CH_3) groups respectively. In case of **3d** the two hydroxy (-OH) protons were observed as singlets at 9.92 and 10.11 ppm.

A singlet peak at 9.89–9.99 ppm assigned to the phenolic hydroxy group of **3a-d** is absent in **4a-d** indicating the formation of P–O–C linkage. This is further confirmed by a considerable change in the resonances of C ^{2}H protons. Other protons of **4a-d** have appeared in their usual pattern but with a slight variation in their chemical shift compared to the hydrazones **3a**–**d**. ^{1}H NMR spectra of **3b** and **4b** are given at Figure 2.

^{13}C NMR spectra of hydrazones **3a**–**d** showed resonance in the range 147.04-149.12 ppm due to the carbon (C^5) of the azomethine (H***C***=N-N) function. The signal in the region 162.38–163.80 ppm is assigned to amide carbonyl (C^6). ^{13}C NMR resonances of **3a-d** in the range 159.10-160.40 ppm are assigned to C^1. The signals of C^2 were observed in the range 115.57-116.00 ppm. The C^{10} carbons show large variation in their chemical shift values depending upon the inductive and mesomeric effect of the substituent attached. In case of **3a**, carbons (C^{10}) bonded to the methoxy group are observed at 160.46 ppm. The signals due to the C^{10} attached to methyl group (**3b)** resonate at 141.47 ppm. The resonances arising from C^{10} attached to the nitro (**3c**) and hydroxy (**3d**) group are observed at 148.80 and 160.40 ppm respectively.

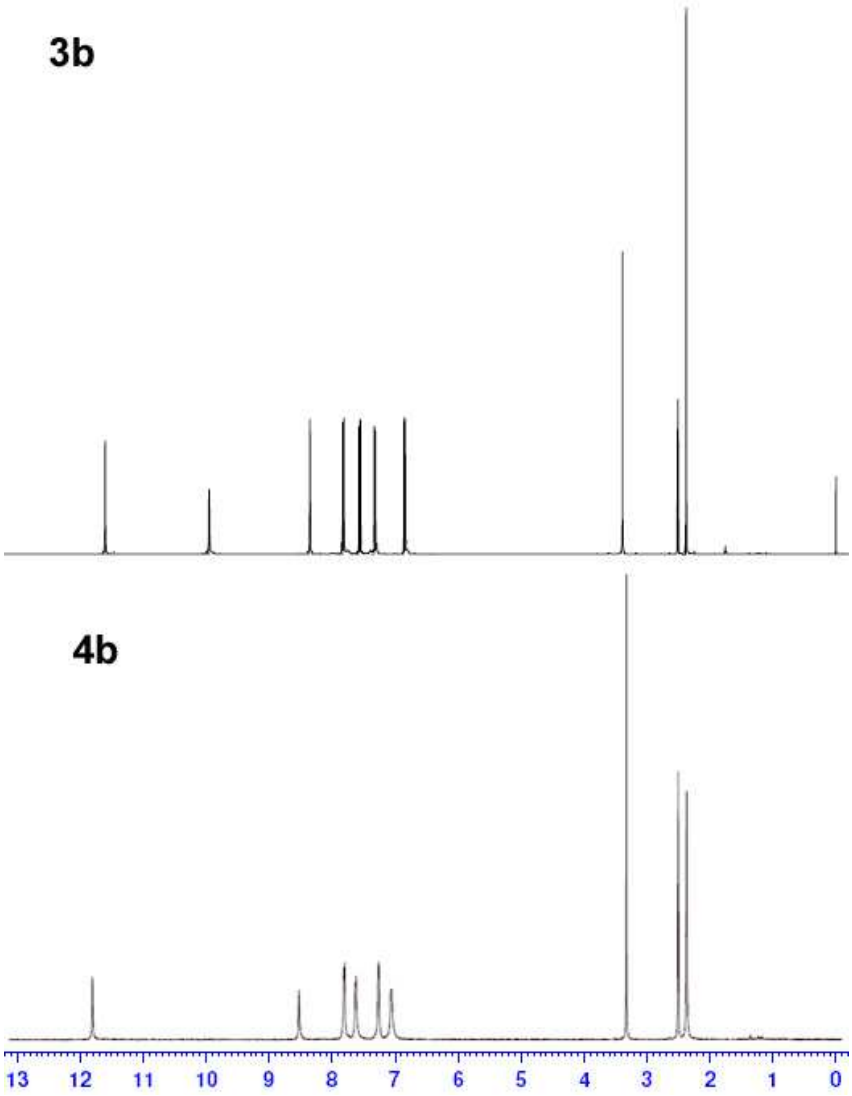


Figure 2. ^{1}H NMR spectra of 3b and 4b.

The resonances of C^{1} carbons of **4a-d** are observed at 151.06-151.32 ppm. These signals are shifted upfield compared to the corresponding signal at 159.10-160.40 ppm in **3a-d**. The C^{2} resonances of **3a-d** in 115.57-116.0 ppm range are shifted downfield and observed at 121.33-121.40 ppm in **4a-d**. These changes are attributed to the formation of P-O-C linkage. Resonances of other carbons of cyclophosphazene hydrazones exhibited slight variation in their chemical shift compared to the hydrazones **3a-d**. ^{13}C NMR spectrum of 3b and 4b are displayed in Figure 3.

The ^{1}H-decoupled ^{31}P NMR spectra of cyclophosphazene compounds **4a-d** bearing the six hydrazone arms exhibited a unique sharp singlet in the range 8.59 to 8.83 ppm indicating the symmetrically substituted phosphorus atoms in the cyclophosphazene ring. The para-substituents on the peripheral phenyl group are presumably too far from the cyclophosphazene center to influence the ^{31}P NMR shift to a larger extent. In the ^{31}P NMR spectrum of **3f** given as an example at Figure 4, singlet appears at 8.71 ppm.

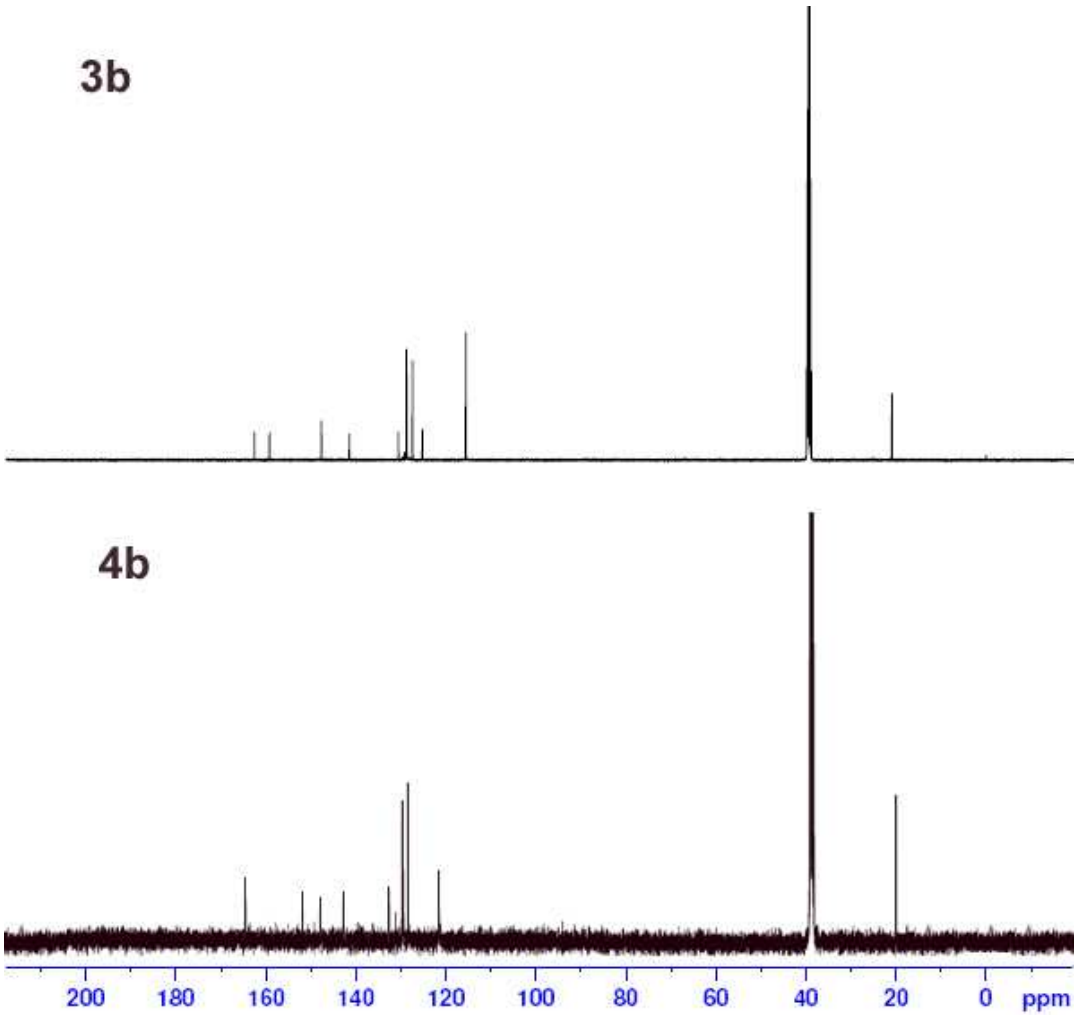


Figure 3. ^{13}C NMR spectra of 3b and 4b.

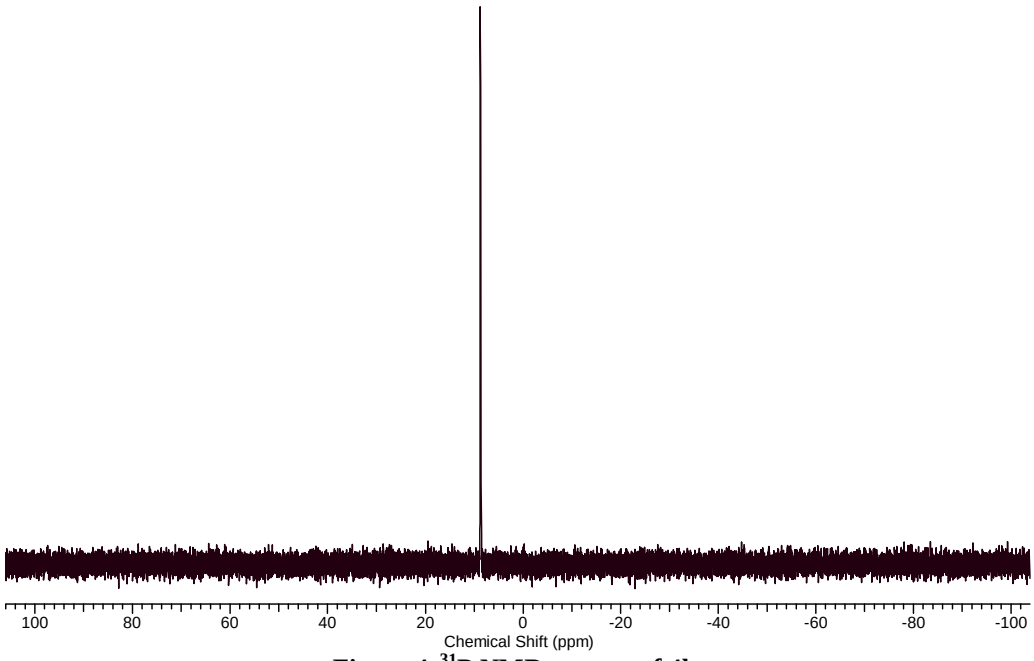


Figure 4. ^{31}P NMR spectra of 4b.

Mass spectrometry

The single molecular nature of the hydrazone cyclophosphazenes **4a-d** was also checked by MALDI-TOF mass spectrometry, which confirmed the expected chemical structures with m/z values corresponding to $(M+Na)^+$ ion.

Biology

Antiproliferative activity *in vitro*

The compounds synthesized were tested for their antiproliferative activity *in vitro* against human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa) cell lines. The result presented in figure 5 indicates that the compounds exhibited moderate antiproliferative activity against the cell lines tested. The compounds behaved differently in relation to the different cell lines. In the case of the HepG2 cells **4b** bearing *para*-methyl substituents exhibited highest activity followed by compounds **4c** and **4a** with *p*-nitro and *p*-methoxy groups respectively. Cyclophosphazene hydrazone derivatives were not sufficiently effective against the HeLa cell lines. It is interesting to note that the compound **4d** with *para*-hydroxy substituent exhibited least activity against HepG2 cells but highest activity against the HeLa cells.

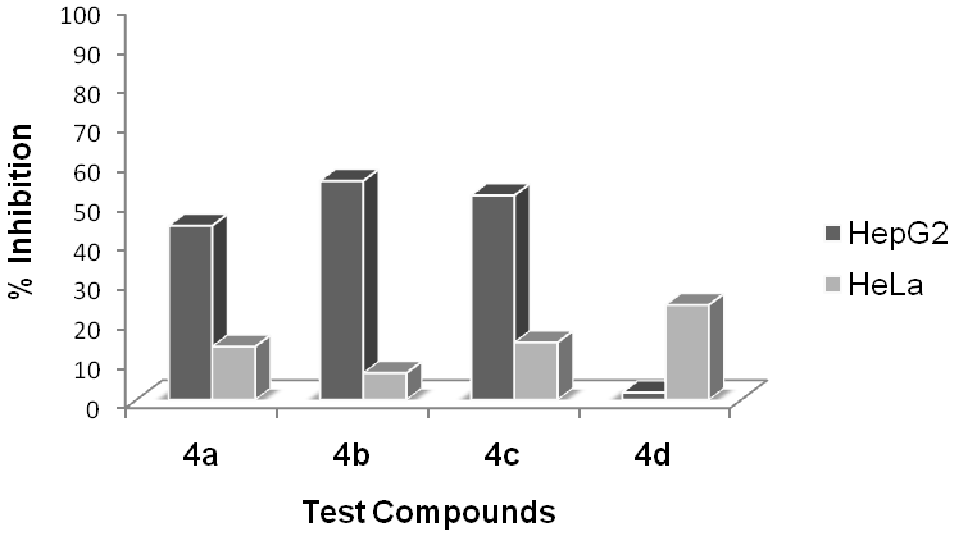


Figure 5. Effect of test compounds on cell growth of human liver carcinoma cell line (HepG2) and human cervix carcinoma cell line (HeLa) incubated for 24 h *in vitro*.

CONCLUSION

The condensation of 4-hydroxybenzaldehyde with the 4-substituted-benzoic hydrazides lead to aromatic hydrazones **3a-d**. Hexa-arm star shaped cyclophosphazene hydrazones are prepared by reaction of HCCP with N'-(4-hydroxybenzylidene)-4-substituted-benzohydrazides. Formation of the hydrazones was corroborated by IR, ^{1}H and ^{13}C NMR spectra. Mass spectrometry and microanalysis also confirmed the expected chemical structures. Based on ^{31}P NMR spectrum analysis compounds **4a-i** have been identified as fully substituted symmetric cyclophosphazenes and are important as synthetic and structural models for the reactions and molecular structure of the analogous high-polymeric phosphazenes and dendrimers. The present compounds exhibited lower activity against HeLa cell lines but reasonably moderate *in vitro* cytotoxicity against HepG2 cell lines.

The cyclophosphazene hydrazone derivatives reported here are prototypes only since these are not designed as water soluble facile biodegradable species. In future work we hope to extend

these studies to systems that possess more biologically compatible spacer groups and cosubstituent units. Efforts on the synthesis and characterization of higher generation hydrazones are in progress and will be reported in future communications.

Acknowledgements
Authors are thankful to the NMR Research Center, Indian Institute of Science, Bangalore, for NMR facility. Fellowship for B. R. Patil and S. S. Machakanur by the University Grants Commission under 'Research Fellowship in Sciences for Meritorious Students' (UGC-RFSMS) is gratefully acknowledged. K. B. Gudasi is thankful to the Association of Commonwealth Universities, for Commonwealth fellowship.

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