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N-Phosphinyl Ureas: Synthesis, Characterization, X-Ray Structure, and In Vitro Evaluation of Antitumor Activity

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ABSTRACT: *A new series of N-phosphinylureas* **5b, 6a–7c** *was synthesized and characterized by* ^{1}H, ^{13}C, ^{31}P *NMR, IR, and elemental analysis. The three-dimensional structure of* **5b** *has been determined by X-ray crystallography. The crystal structure revealed the existence of four independent molecules. All structures form two chains with different arrangements and connect to each other via hydrogen bonds to produce two-dimensional polymeric chains. The cytotoxicity of cyclophosphamide (a standard antitumor compound) and its nine analogues with formula* $R^{1}C_{6}H_{4}NHC(O)NHP(O)XCH_{2}C(R^{2})_{2}CH_{2}Y(X = Y = NH, R^{2} = CH_{3}, R^{1} = H$ (**5a**), CH_{3} (**5b**), NO_{2} (**5c**), $X = O, Y = NH, R^{2} = H, R^{1} = H$ (**6a**, CH_{3} (**6b**), NO_{2} (**6c**), *and* $X = Y = O, R^{2} = CH_{3}, R^{1} = H$ (**7a**), CH_{3} (**7b**), NO_{2} (**7c**)) *as well as phenyl urea were evaluated in vitro against three human tumor cell lines K562, MDA-MB-231, and HepG2. The results showed that most of the compounds have significant activity against the selected cell lines. Also, HepG2 cells were more sensitive to all the tested compounds than other cell lines.* © 2011 Wiley Periodicals, Inc. Heteroatom Chem 23:74–83, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20754

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INTRODUCTION

N-phosphinylureas are some important instances of phosphoramidates that little attention has been given to their biological properties [1,2] and structural studies [1,3]. These compounds can cause attractive biological activities due to having urea and peptide moieties. Recently, we evaluated the cytotoxic effects of several phosphoramidates with the general formula $RP(O)[NHCH_{2}C(CH_{3})_{2}CH_{2}NH]$ against human leukemia K562 [4]. Results revealed that *N*-phosphinylureas are good candidates for antitumor activity. On the other hand, several studies are concentrated on the development of six-membered P-heterocycles, owing to their biological activities and unique conformational and stereochemical aspects [5–7]. Cyclophosphamide is one of the phosphorus heterocycles that is used in the treatment of human cancers [8–11]. Nowadays, modifications in cyclophosphamides have led to the design and synthesis of numerous cyclic analogues [12] to find an antitumor agent with fewer side effects. In this study, following our previous research, the novel derivatives were synthesized and characterized by ^{1}H, ^{13}C, ^{31}P NMR, and infrared (IR) spectroscopy and elemental analysis. X-ray crystallography confirms the existence of four conformers in crystalline lattice. Since few crystal structures of these types of molecules were reported on [1,3], we believe that it is the first example of *N*-phosphinylureas with four

conformers. Furthermore, we would like to report on the structural modifications of phosphorus heterocycles with the aim of examining their cytotoxic effects. However, the antitumor activity of cyclophosphamide, its nine analogues, and phenylurea were determined against K562, MDA-MB-231, and HepG2 cell lines by applying the MTT colorimetric assay.

EXPERIMENTAL

Material and Methods

All reactions were carried out in an argon atmosphere. All chemicals and solvents were purchased from Merck (Tehran, Iran) and used without further purification. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker (Avance DRS) 500-MHz spectrometer. ^{1}H and ^{13}C chemical shifts were determined relative to TMS, ^{31}P chemical shifts relative to 85% H_3PO_4 as an external standard. IR spectra were obtained using KBr pellets on a Shimadzu IR-60 model spectrometer. Elemental analysis was performed using a Heraeus CHN-O-RAPID apparatus. Melting points were determined on an electrothermal apparatus.

X-Ray Measurements

X-ray data of compound **5b** were collected on a Bruker SMART 1000 CCD [13] single-crystal diffractometer with graphite monochromated Mo Kα radiation ($k = 0.71073$ Å). The structure was refined with SHELXL-97 [14] by full-matrix least-squares on F^2. The positions of hydrogen atoms were obtained from the difference Fourier map. Routine Lorentz and polarization corrections were applied, and an absorption correction was performed using the SADABS program for the title structure [15].

In Vitro Cytotoxicity Assay

The cytotoxic activity of the synthesized compounds was determined on three human cancer cell lines: Human leukemia K562, Human breast MDA-MB-231, and HepG2 hepatoma by MTT assay using cyclophosphamide as a conventional anticancer agent. For a typical screening experiment, cells are inoculated into 96-well-microtiter plates in 100 μL at plating densities ranging from 5000 to 8000 cells per well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity for 24 h. After 24 h, exponentially growing cells were exposed to various concentrations of several tested compounds. Although all tested compounds were dissolved in DMSO and the final concentration of DMSO was 2%, the solvent showed no activity in these assays at the level that was used for screening. These compounds were stored in a freeze prior to use. An MTT assay was performed at 24, 48, and 72 h after adding all compounds to assess cell viability. MTT was added (20 μL of 5 mg/mL MTT in phosphate buffered saline (PBS)), and the plates were incubated at 37°C for an additional 3–4 h. MTT can be reduced to a blue formazan dye and thereby assesses cell viability by measuring mitochondrial function. DMSO was then added to dissolve the resultant crystals, and the optical absorbance was measured at 570 nm on an Eliza reader. The results were compared with those of a control reference plate fixed on the treatment day, and the growth inhibition percentage was calculated for each drug contact period. Each assay was set up in triplicate wells and repeated one to three times. Graph Pad PRISM version 5.0 [16] was used to fit the data. Also, to study the degree of selectivity in the cytotoxic activity of the compounds, separation of lymphocyte was down according to the following procedure [17]: Defibrinated or anticoagulant-treated blood was diluted with an equal volume of PBS and layered carefully over Ficoll–Paque PLUS (without intermixing) in a centrifuge tube. After a short centrifugation at room temperature (typically at 400 g_{av} for 30–40 min), lymphocytes, together with monocytes and platelets, are harvested from the interface between the Ficoll–Paque PLUS and sample layers. This material was then centrifuged twice in a balanced salt solution to wash the lymphocytes and to remove the platelets.

Synthesis

*5,5-Dimethyl-2-(N-4-methylphenylureido)-1,3,2-diazaphosphorinane-2-oxide (***5b***).* A solution of 2,2-dimethyl-1,3-diaminopropane (1.06 g, 10.4 mmol) in dry diethylether (15 mL) was treated dropwise to suspension of *N*-4-methylphenylureidophosphoryl dichloride (**3**) (1.38 g, 5.2 mmol) in dry diethylether (15 mL) with a 2:1 molar ratio and stirred at 0°C. After stirring for 5 h, the products were filtered off and then washed with H_2O.

Yield: 73%. mp 202–203°C. Anal. Calcd. for $C_{13}H_{21}N_4O_2P$ (%): C, 52.70; H, 7.14; N, 18.91. Found: C, 52.63; H, 7.20; N, 18.79. IR (KBr): ν_{max} = 3185 (N—H), 2925, 1686 (C=O), 1600, 1545, 1507, 1441, 1325, 1256, 1180 (P=O), 1106, 1043, 950, 910, 810, 761, 590 cm^{-1}. ^{1}H NMR (500.13 MHz, d_6-DMSO): $\delta = 0.79$ (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.57 (ddd, ^{2}J(H, H) = 12.0 Hz, ^{3}J(H, H) = 5.25 Hz, ^{3}J(P, H) = 24.31 Hz, 2H), 2.98 (dd, ^{2}J(H, H) = 12.0 Hz, ^{3}J(H, H) = 2.7 Hz, 2H), 4.65 (d, ^{2}J(P,

H) = 3.5 Hz, 2H, $NH_{endocyclic}$), 7.25 (d, ^3J(H, H) = 8.2 Hz, $2H_{aromatic}$), 7.05 (d, ^3J(H, H) = 8.2 Hz, $2H_{aromatic}$), 7.58 (d, ^2J(P, H) = 7.15 Hz, 1H, NHP), 9.22 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 20.22 (s, CH_3), 23.29 (s, CH_3), 24.84 (s, CH_3), 30.46 (d, ^3J(P, C) = 4.1 Hz), 52.32 (s), 118.16 (s), 129.09 (s), 130.75 (s), 136.75 (s), 153.33 (s). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = 3.85 (m).

General Procedure for the Synthesis of Oxazaphosphorinanes **6a–6c**. To a suspension, intermediates **2–4** (5.2 mmol) in acetonitrile (20 mL), 3-amino-1-propanol (0.78 g, 10.4 mmol) for **6a** or title amino alcohol (0.39 g, 5.2 mmol) and triethylamine (1.05 g, 10.4 mmol) for **6b** and **6c** at 0°C were added with stirring. The reaction mixture was stirred overnight at room temperature; the solvent was then partially evaporated off, was the oily residue dried after a few days and washed with cooled water, and the precipitate filtered off and dried at room temperature.

2-(N-Phenylureido)-1,3,2-oxazaphosphorinane-2-oxide (**6a**). Yield: 40%. mp 197–198°C. Anal. Calcd. for $C_{10}H_{14}N_3O_3P$ (%): C, 47.06; H, 5.53; N, 16.46. Found: C, 47.12; H, 5.56; N, 16.35. IR (KBr): ν_{max} = 3320 (N—H), 3185, 1679 (C=O), 1598, 1532, 1460, 1435, 1332, 1310, 1231 (P=O), 1204, 1125, 1105, 1048, 982, 942, 848, 767, 737, 466 cm^{-1}. ^{1}H NMR (500.13 MHz, d_6-DMSO): δ = 1.69 (d, ^2J(H, H) = 12.65 Hz, 1H), 1.80 (m, 1H), 3.11 (m, 1H), 3.30 (m, 1H), 4.25 (m, 1H), 4.36 (m, 1H), 5.24 (s, 1H, $NH_{endocyclic}$), 6.98 (t, ^3J(H, H) = 6.8 Hz, $1H_{aromatic}$), 7.12 (t, ^3J(H, H) = 7.15 Hz, $2H_{aromatic}$), 7.38 (d, ^3J(H, H) = 7.4 Hz, $2H_{aromatic}$), 7.97 (d, ^2J(P, H) = 7.2 Hz, 1H, NHP), 8.97 (s, 1H, PhNH).^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 25.43 (d, ^3J(P, C) = 7.3 Hz), 40.32 (s), 68.5 (d, ^2J(P, C) = 7.6 Hz), 118.33 (s), 122.36 (s), 128.68 (s), 138.97 (s), 152.77 (d, ^2J(P, C) = 2.69 Hz). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = −0.64 (m).

2-(N-4-methylphenylureido)-1,3,2-oxazaphosphorinane-2-oxide (**6b**). Yield: 40%. mp = 196–197°C. Anal. Calcd. for $C_{11}H_{16}N_3O_3P$ (%): C, 49.07; H, 5.99; N, 15.61. Found: C, 49.03; H, 6.05; N,15.65. IR (KBr): ν_{max} = 3310 (N—H), 3170, 1680 (C=O), 1596, 1527, 1458, 1238 (P=O), 1201, 1113, 1050, 981, 926, 845, 451 cm^{-1}. ^{1}H NMR (500.13 MHz, d_6-DMSO): δ = 1.70 (d, ^2J(H, H) = 13.4 Hz, 1H, CH_2), 1.81 (m, 1H, CH_2), 2.23 (s, 3H, CH_3), 3.08 (m, 1H), 3.29 (m, 1H), 4.23 (m, 1H), 4.35 (m, 1H), 5.19 (s, 1H, $NH_{endocyclic}$), 7.06 (d, ^3J(H, H) = 8.1 Hz, $2H_{aromatic}$), 7.25 (d, ^3J(H, H) = 8.2 Hz, $2H_{aromatic}$), 7.9 (d, ^2J(PNH) = 7.9 Hz, 1H, NHP), 8.87 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 20.25 (s), 25.4 (d, ^3J(P, C) = 7.4 Hz), 40.26 (d, ^2J(P, C) = 2.9 Hz), 68.4 (d, ^2J(P, C) = 7.61 Hz), 118.39 (s), 129.15 (s), 131.19 (s), 136.39 (s), 152.71 (d, ^2J(P, C) = 2.69 Hz). ^{31}P NMR (202.46, d_6-DMSO): δ = −0.58 (b).

2-(N-4-nitrophenylureido)-1,3,2-oxazaphosphorinane-2-oxide (**6c**). Yield: 35%. mp = 204–205°C. Anal. Calcd. for $C_{10}H_{13}N_4O_5P$ (%): C, 40.01; H, 4.36; N, 18.66. Found: C, 40.09; H, 4.38; N, 18.61. IR (KBr): ν_{max} = 3300 (N—H), 3080, 1704 (C=O), 1610, 1550, 1499, 1343 (NO_2), 1219, 1193 (P=O), 1112, 1062, 1009, 935, 848, 815, 744, 650, 529 cm^{-1}. ^{1}H NMR (500.13 MHz, d_6-DMSO): δ = 1.71 (d, ^2J(H, H) = 14.25 Hz, 1H, CH_2), 1.80 (m, 1H, CH_2), 3.09 (m, 1H), 3.17 (m, 1H), 4.27 (m, 1H), 4.38 (m, 1H), 5.34 (d, ^3J(H, H) = 3.1 Hz, 1H, $NH_{endocyclic}$), 6.69 (s, 1H, NHP), 7.63 (d, ^3J(H, H) = 9.05 Hz, $2H_{aromatic}$), 7.93 (d, ^3J(H, H) = 9.05 Hz, $2H_{aromatic}$), 9.6 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 25.31 (d, ^3J(P, C) = 7.45 Hz), 40.27 (d, ^2J(P, C) = 2.44 Hz), 68.69 (d, ^2J(P, C) = 7.73 Hz), 112.33 (s), 116.88 (s), 117.78 (s), 126.28 (s), 141.53 (s), 145.42 (s), 152.53 (d, ^2J(P, C) = 2.84 Hz). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = –1.38 (m).

General Procedure for the Synthesis of Dioxaphosphorinanes **7a–7c**. These compounds were synthesized from the reaction of (5.2 mmol) intermediates **2–4** suspended in 25 mL dichloromethane with (0.53 g, 5.2 mmol) 2,2-dimethyl-1,3-propandiol and (1.05 g, 10.4 mmol) triethylamine at 0°C. The solution was stirred overnight at room temperature and then evaporated in vacuum. The oily residue was washed with cooled water and the precipitate was filtered off and dried at room temperature.

5,5 - Dimethyl-2-(N-phenylureido)-1,3,2-dioxaphosphorinane-2-oxide (**7a**). Yield: 70%, mp = 232–233°C. Anal. Calcd. for $C_{12}H_{17}N_2O_4P$ (%): C, 50.71; H, 6.03; N, 9.86. Found: C, 50.85; H, 6.14; N, 9.75. IR (KBr): ν_{max} = 3380 (N—H), 3250, 3140, 1700 (C=O), 1600, 1541, 1487, 1443, 1310, 1244 (P=O), 1046, 1010, 983, 750, 482 cm^{-1}. ^{1}H NMR (500.13 MHz, d_6-DMSO): δ = 0.89 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 3.97 (dd, ^2J(H, H) = 10.45 Hz, ^3J(P, H) = 18.91 Hz, 2H), 4.29 (dd, ^2J(H, H) = 10.35 Hz, ^3J(P, H) = 3.95 Hz, 2H), 7.015 (t, ^3J(H, H) = 7.15 Hz, $1H_{aromatic}$), 7.28 (t, ^3J(H, H) = 7.65 Hz, $2H_{aromatic}$), 7.38 (d, ^3J(H, H) = 7.9 Hz, $2H_{aromatic}$), 8.38 (d, ^2J(P, H) = 8.3 Hz, 1H, NHP), 8.74 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 19.82 (s), 21.22 (s), 31.58 (d, ^3J(P, C) = 6.7 Hz), 77.40 (d, ^2J(P, C) = 7.07 Hz), 118.70 (s), 122.80 (s), 138.48 (s), 152.50 (d, ^2J(P, C) = 3.53 Hz). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = −8.95 (m).

5,5 - Dimethyl - 2 - (N - 4 - methylphenylureido)-1,3,2-dioxaphosphorinane-2-oxide (**7b**). Yield: 65%, mp = 209–210°C. Anal. Calcd. for $C_{13}H_{19}N_2O_4P$ (%):

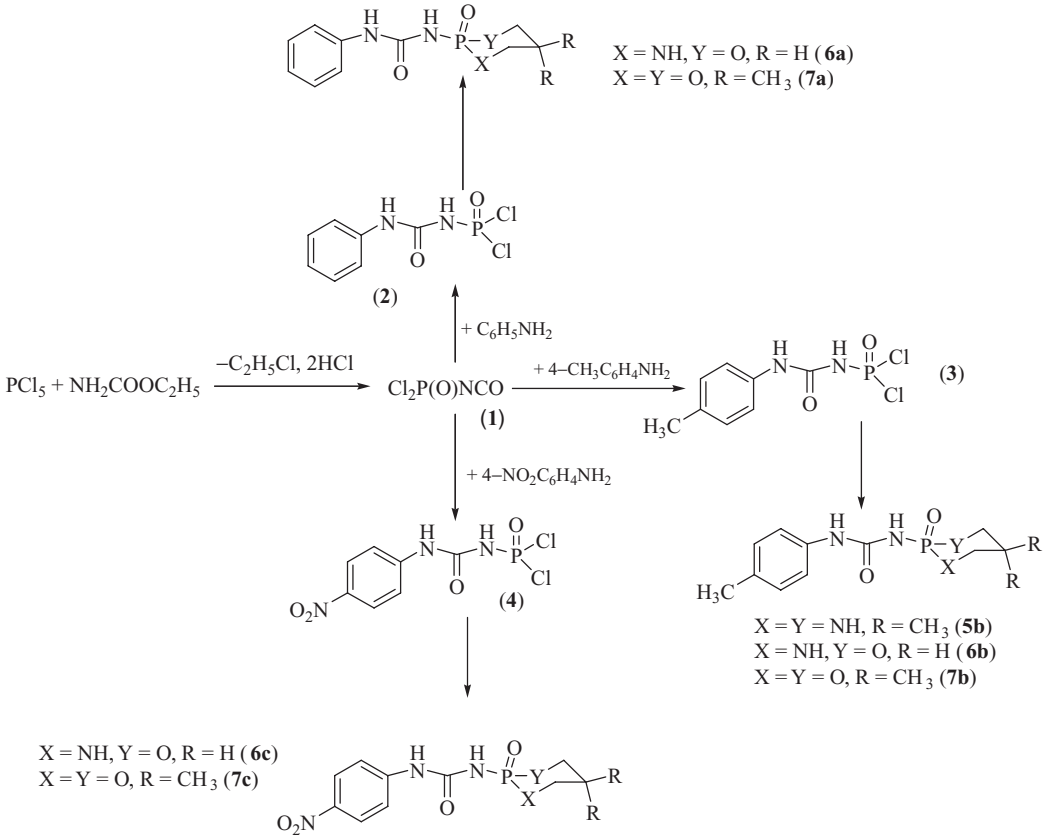
C, 52.36; H, 6.42; N, 9.39. Found: C, 52.27; H, 6.42; N, 9.45. IR (KBr): ν_{max} = 3380 (N—H), 3250, 1700 (C=O), 1600, 1541, 1487, 1443, 1310, 1244 (P=O), 1046, 1010, 983, 750, 462 cm^{-1}. ^1H NMR (500.13 MHz, d_6-DMSO): δ = 0.89 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.97 (dd, ^2J(H, H) = 10.45 Hz, ^3J(P, H) = 18.91 Hz, 2H), 4.29 (dd, ^2J(H, H) = 10.40 Hz, ^3J(P, H) = 4.40 Hz, 2H), 7.08 (d, ^3J(H, H) = 8.2 Hz, $2H_{aromatic}$), 7.26 (d, ^3J(H, H) = 8.3 Hz, $2H_{aromatic}$), 8.34 (d, ^2J(P, H) = 9.35 Hz, 1H, NHP), 8.64 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 19.83 (s), 20.28 (s), 21.22 (s), 31.58 (d, ^3J(P, C) = 7.11 Hz), 77.39 (d, ^2J(P, C) = 6.8 Hz), 118.77 (s), 129.17 (s), 131.73 (s), 135.91 (s), 152.03 (d, ^2J(P, C) = 2.88 Hz). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = −8.85 (m).

*5,5-Dimethyl-2-(N-4-nitrophenylureido)-1,3,2-dioxaphosphorinane-2-oxide (***7c**). Yield: 60%, mp = 233–235°C. Anal. Calcd. for $C_{12}H_{16}N_3O_6P$ (%): C, 43.78; H, 4.89; N, 12.76. Found: C, 43.69; H, 4.75; N, 12.88. IR (KBr): ν_{max} = 3350 (N—H), 3065, 1700 (C=O), 1611, 1552, 1476, 1339 (NO_2), 1261(P=O), 1064, 1012, 943, 856, 499 cm^{-1}. ^1H NMR (500.13 MHz, d_6-DMSO): δ = 0.89 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 3.99 (dd, ^2J(H, H) = 10.55 Hz, ^3J(P, H) = 19.21 Hz, 2H), 4.29 (dd, ^2J(H, H) = 10.50 Hz, ^3J(P, H) = 4.30 Hz, 2H), 7.65 (d, ^3J(H, H) = 9.2 Hz, $2H_{aromatic}$), 8.19 (d, ^3J(H, H) = 9.2 Hz, $2H_{aromatic}$), 8.63 (d, ^2J(P, H) = 9.1 Hz, 1H, NHP), 9.38 (s, 1H, PhNH). ^{13}C NMR (125.77 MHz, d_6-DMSO): δ = 19.78 (s), 21.19 (s), 31.65 (d, ^3J(P, C) = 6.97 Hz), 77.57 (d, ^2J(P, C) = 6.88 Hz), 118.18 (s), 125.0 (s), 141.88 (s), 145.00 (s), 151.99 (d, ^2J(P, C) = 3.34 Hz). ^{31}P NMR (202.46 MHz, d_6-DMSO): δ = −9.68 (m).

RESULTS AND DISCUSSION

Synthetic and Spectroscopic Aspects

As indicated in Scheme 1, compounds **5b** and **6a–7c** were prepared from phosphorus pentachloride as a starting material, which was treated with ethyl carbamate in ethylene chloride to give dichloroisocyanatophosphine oxide (**1**) [18]. Then, treatment of aniline derivatives with **1** afforded $RC_6H_4NHC(O)NHP(O)Cl_2$ (R = H (**2**), CH_3 (**3**), NO_2 (**4**)) [19]. Afterward, derivatives **5b** and **6a–7c** were obtained from the reaction of intermediates **2–4** with the corresponding diamine, amino alcohol, and diol in the presence of an HCl scavenger.



SCHEME 1

TABLE 1 Some Spectroscopic Data of Compounds **5a–7c**

Compound	δ (^{31}P) (ppm)	^{3}J(PNCH) (Hz)	^{3}J(POCH) (Hz)	$^{2}J(P,H)_{exocyclic}$ (Hz)	$^{2}J(P,C)_{exocyclic}$ (Hz)	$^{3}J(P,C)_{endocyclic}$ (Hz)	ν(P=O)	ν(C=O)
5a[a]	3.78	24.21	–	6.76	–	4.3	1184	1677
5b	3.85	24.01	–	7.15	–	4.1	1180	1686
5c[a]	3.23	24.47	–	7.59	–	4.53	1200	1699
6a	−0.64	m	m	7.2	2.69	7.3	1231	1679
6b	−0.54	b	b	7.9	2.69	7.4	1238	1680
6c	−1.38	b	b	9.0	2.84	7.45	1240	1704
7a	−8.95	–	18.91(eq), 3.95(ax)	8.3	3.53	6.7	1244	1700
7b	−8.85	–	18.91(eq), 4.4(ax)	9.35	2.88	7.11	1244	1700
7c	−9.68	–	19.21(eq), 4.3(ax)	9.1	3.34	6.97	1261	1700

[a]For compounds **5a** and **5c**, see Ref. [1].

Phosphorus–hydrogen and phosphorus–carbon coupling constants and $\delta(^{31}P)$ data of compounds **5a–7c** are summarized in Table 1. The ^{31}P NMR spectra of these compounds reveal the substituent effect on the $\delta(^{31}P)$ so that the phosphorus atom in derivatives containing NO_2 shows a high upfield shift compared to the CH_3 group. Substitution of the NH group in a diazaphosphorinane ring with an oxygen atom causes extremely great changes in $\delta(^{31}P)$, $^{2}J(PNH_{exocyclic})$, $^{2}J(PNH_{endocyclic})$, and ^{3}J(P, H) in oxaza and dioxaphosphorinanes. The ^{31}P NMR spectra for dioxaphosphorinanes **7a–7c** demonstrate large shifts to the upper field compared to those of their related oxaza and diazaphosphorinanes. The ^{1}H and ^{13}C NMR spectra of molecules **5a–5c** and **7a–7c** show two separate signals for CH_3 groups on diaza and dioxaphosphorinane rings. The $H_{equatorial}$ and H_{axial} atoms of CH_2 groups in these compounds appear at different chemical shifts in ^{1}H NMR spectra. As shown in Table 1, ^{3}J(PNCH) and ^{3}J(POCH) coupling constants for $H_{equatorial}$ are about 24.5 and 19.0 Hz. The H_{axial} atom has the largest coupling with the phosphor atom from 3.95 to 4.40 Hz in dioxaphosphorinanes **7a–7c**. Furthermore, the $^{2}J(PNH_{endocyclic})$ coupling constant in oxazaphosphorinanes decreases to zero, whereas it ranges from 2.7 to 3.5 Hz for diazaphosphorinanes **5a–5c**. It can be deduced that the oxygen atom decreases ring strain and hence diminishes the $^{2}J(PNH_{endocyclic})$ coupling constant. Besides, $^{2}J(PNH_{exocyclic})$ and $^{2}J(P, C_{exocyclic})$ coupling constants in dioxaphosphorinans are larger than oxaza and diazaphosphorinanes, respectively. The IR spectra for these compounds show the absorption stretching band values in the range of 1261–1180 cm^{-1} and 1704–1677 cm^{-1} for P=O and C=O groups, respectively. Higher values of stretching absorption bands are observed for the compounds with electron-withdrawing substituents.

X-Ray Crystallography

Colorless crystals of **5b** suitable for X-ray diffraction analysis were grown from an ethanol/chloroform mixture after slow evaporation at room temperature. Compound crystallizes in the triclinic crystal system with space group $P\bar{1}$. The crystal data and the details of the X-ray analysis are presented in Table 2, and selected bond lengths and angles are summarized in Table S1 (in the Supporting Information). Molecular structures (ORTEP view) and hydrogen-bonding chains are shown in Figs. 1 and 2, respectively. Compound contains four conformers **5b (1), 5b (2), 5b (3)**, and **5b (4)** in the solid state due to different spatial orientations of the four conformers relative to each other that cause different torsion angles (see Table S1). Four types of hydrogen bonds are established among these conformers. Hydrogen bonding data of these structures are presented in Table 3. There are intramolecular P=O···H–NPh, intermolecular C=O···NHP, C=O···NH, and P=O···NH hydrogen bonds. Each pair conformer (**5b (1), 5b (3)**, and **5b (2), 5b (4)**) produces a centrosymmetric dimer via C(O)···H–N hydrogen bonds. These conformers create two types of chains with different arrangements in the crystal lattice of **5b**. Linking of these chains by hydrogen bonding leads to the formation of a two-dimensional polymeric chain in the crystal lattice.

The mean P=O distances fall in the range of 1.4803–1.4845 Å, which are slightly longer than the normal P=O bond length (1.45 Å). The phosphoryl oxygen atoms (O(2), O(22), O(23), and O(24)) occupy a pseudoaxial position, and the 4-methylphenylureido groups adopt a pseudoequatorial orientation. In all of the compounds, the P–N_{amide} bond length is longer than the P–N_{amine} bond lengths (Table 3), because of the electrostatic interaction of the N_{amide} lone pair with the π^* CO. All of these

TABLE 2 Crystallographic Data and Structure Refinement Results for **5b**

Empirical Formula	$C_{13}H_{21}N_4O_2P_1$
Formula weight	296.31
Temperature (K)	120(2)
Wavelength (Å)	0.71073
Crystal system, space group	Triclinic, $P\bar{1}$
Unit cell dimensions	
a (Å)	11.9433(10)
b (Å)	15.6496(13)
c (Å)	18.3524(16)
α (°)	111.866(3)
β (°)	108.349(2)
γ (°)	91.766(2)
V (Å^3)	2978.6(4)
Z, Calculated density (Mg m^{-3})	8, 1.322
Absorption coefficient (mm^{-1})	0.192
F(000)	1264
Crystal size (mm^3)	0.55 × 0.40 × 0.20
Θ Range for data collection (°)	1.82–27.00
Index ranges	$-15 \le h \le 15$, $-19 \le k \le 19$, $-23 \le l \le 23$
Reflections collected	28,818
Independent reflections	12,934 [R(int) = 0.0410]
Completeness to $\theta = 27.00°$	99.4%
Absorption correction	Semiempirical from equivalents
Maximum and minimum transmission	0.970 and 0.914
Refinement method	Full-matrix least squares on F^2
Data/restraints/parameters	12934/0/733
Goodness-of-fit on F^2	1.007
Final R indices [for 6883 reflections with $I > 2\sigma(I)$]	$R_1 = 0.0581$, $wR_2 = 0.1003$
R indices (all data)	$R_1 = 0.1068$, $wR_2 = 0.1120$
Largest difference peak and hole (e Å^{-3})	0.1068 and 0.1120

bonds are in the range of 1.608(2)–1.639(2) Å and thus are significantly shorter than a typical P–N single bond (1.77 Å) [20]. The phosphorus atoms have a slightly distorted tetrahedral configuration with the angles in the range of 102.66(12)–116.01(12)° (in **1**), 102.25(12)–115.58(12)° (in **2**), 102.06(12)–115.50(12)° (in **3**), and 105.52(12)–116.55(12)°(in **4**). It is noteworthy, similar to most of the phosphoramidates, that the P=O and C=O bonds are in antiposition with respect to each other [21–23].

Biological Activity

Preliminary antitumor activities of all the synthesized compounds, namely, diazaphosphorinane **5a–c**, oxazaphosphorinane **6a–c**, and dioxaphosphorinane **7a–c** and phenyl urea were assessed in vitro against three human tumor cell lines, that is, leukemia human cell line (K562), human breast cell line (MDA-MB-231), and hepatocellular carcinoma cell line (HepG2); and the cyclophosphamide (**CP**) was used as a control. The results are summarized in Table 4. The plots of IC_{50} values for all of the compounds are shown in Fig. 3. Cell viability was analyzed using the MTT colorimetric assay [24]. As shown in Table 4, the IC_{50} values of all the *N*-phosphinylureas, except derivative **7a**, were in the range of 153–202 nM in K562 cells. Similarly, in MDA-MB-231 and HepG2 cell lines, the IC_{50} values for all the compounds were found in the ranges of 57–260 and 63–339 nM, respectively (Table 4).

From the biological results of compounds **5a–c, 6a–c**, and **7a–c**, it is found that substituent on the phenyl ring significantly influences the activity against the title cell lines. Derivatives such as **6b** (the 4-CH_3 group) and **6c** (the 4-NO_2 group) showed higher anticancer activity (168 and 180 nm against K562 and 57, 60 against MDA-MB-231, respectively) than an unsubstituted molecule **6a** (198 nm against K562 and 260 nm against MDA-MB-231). Also, the activity of these molecules against the HepG2 cell line showed that replacement of H with NO_2 affords the more potent derivatives. The compound **5c** showed an IC_{50} value of 73 nM. In contrast, **5a** and **5b** exhibited IC_{50} values of 94 and 97 nM, respectively.

In an attempt to explore the influence of incorporating a heteroatom in the moiety of phosphorinane on the activity, the NH in **5a–c** was replaced with O to afford the corresponding compounds **6a–c** and **7a–c**. Biological results indicated that introduction of an O atom led to an increase in activity against the HepG2 cell line. For example, dioxaphosphorinane **7a** showed an IC_{50} value of 67 nM, whereas oxazaphosphorinane **6a** and diazaphosphorinane **5a** exhibited IC_{50} values of 78 and 94 nM against HepG2, respectively. In contrast, as shown in Table 4, replacement of NH with oxygen resulted in a decrease in K562 inhibitory activity, so that the IC_{50} value decreased from 179 (in compound **5a**) to 198 nM (in compound **6a**) and compound **7a** was nonactive.

Table 4 demonstrates that the derivatives **5b–c, 6b–c**, and **7b–c** exhibited antitumor activity on the MDA-MB-231 cell line with the average activity order of **6b–c** < **5b–c** < **7b–c**. The only exceptions were unsubstituted molecules **5**a, **6a**, and **7a**, which showed that the presence of dioxaphosphorinane ring (**7a**) afforded the more potent derivative of diaza (**5a**) and oxazaphosphorinane (**6a**), respectively.

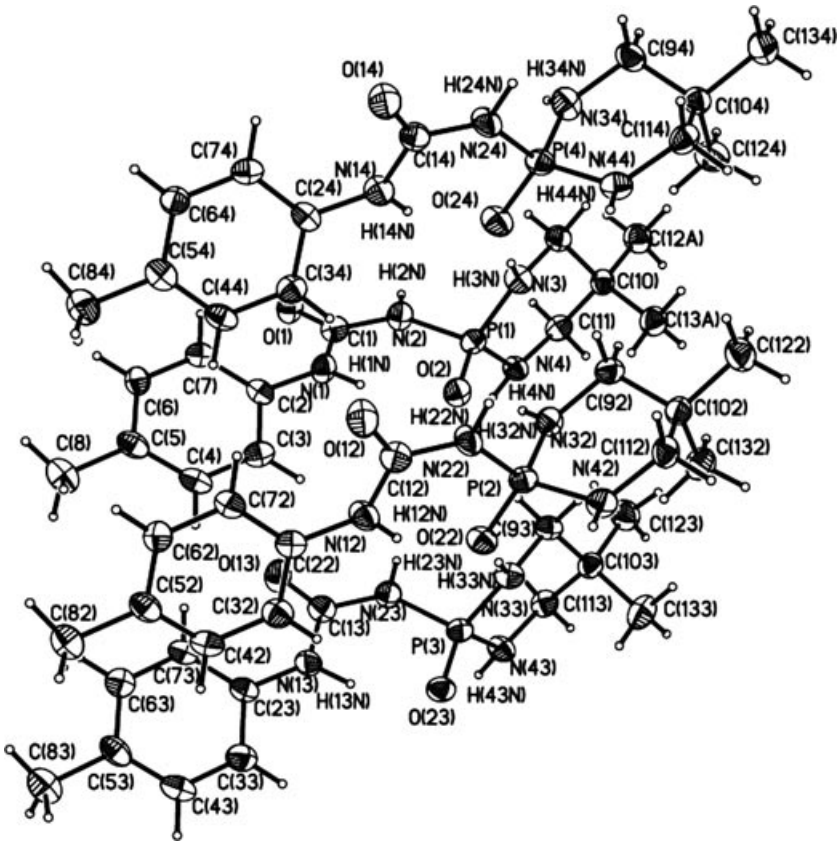


FIGURE 1 Thermal ellipsoids (at the 50% probability level) of four conformers.

TABLE 3 Data of the Hydrogen Bonds in the Crystalline Compound

D—H···A	*d(D—H)*	*d(H···A)*	*d(D···A)*	*<(DHA)*
N(1)—H(1N)···O(2)#1	0.85	1.91	2.685(3)	152
N(2)—H(2N)···O(13)#2	0.85	2.01	2.844(3)	167
N(3)—H(3N)···O(24)#1	0.79	2.11	2.893(3)	176
N(4)—H(4N)···O(1)#2	0.91	2.53	3.362(3)	152
N(12)—H(12N)···O(22)#1	0.85	1.89	2.675(3)	153
N(13)—H(13N)···O(23)#1	0.96	1.77	2.669(3)	155
N(14)—H(14N)···O(24)#1	0.82	1.94	2.698(3)	154
N(22)—H(22N)···O(14)#3	0.88	2.05	2.927(3)	172
N(23)—H(23N)···O(1)#2	0.89	2.04	2.930(3)	178
N(24)—H(24N)···O(12)#3	0.91	1.96	2.870(3)	178
N(32)—H(32N)···O(2)#1	0.78	2.09	2.859(3)	173
N(33)—H(33N)···O(22)#1	0.86	2.03	2.883(3)	172
N(34)—H(34N)···O(23)#4	0.83	2.03	2.853(3)	169
N(44)—H(44N)···O(14)#3	0.84	2.58	3.370(3)	156

Furthermore, the insertion of a phosphorinane ring at the terminal position of phenyl urea led to formation of compounds **5a, 6a**, and **7a**, with better cytotoxic activity compared to the parent molecule in HepG2 cells (Table 4). All novel synthesized compounds also exhibit an enhanced activity at nanomolar concentrations compared to the reported urea derivatives against the title cell lines [9,25–27]. These results indicate that the presence of —C(O)NHP(O)— moiety and phosphorinane ring may be increased the antiproliferative activities of the new compounds.

Comparison of potencies of the tested molecules and conventional anticancer agent, cyclophosphamide, demonstrate that all of the derivatives were more potent than the standard antitumor

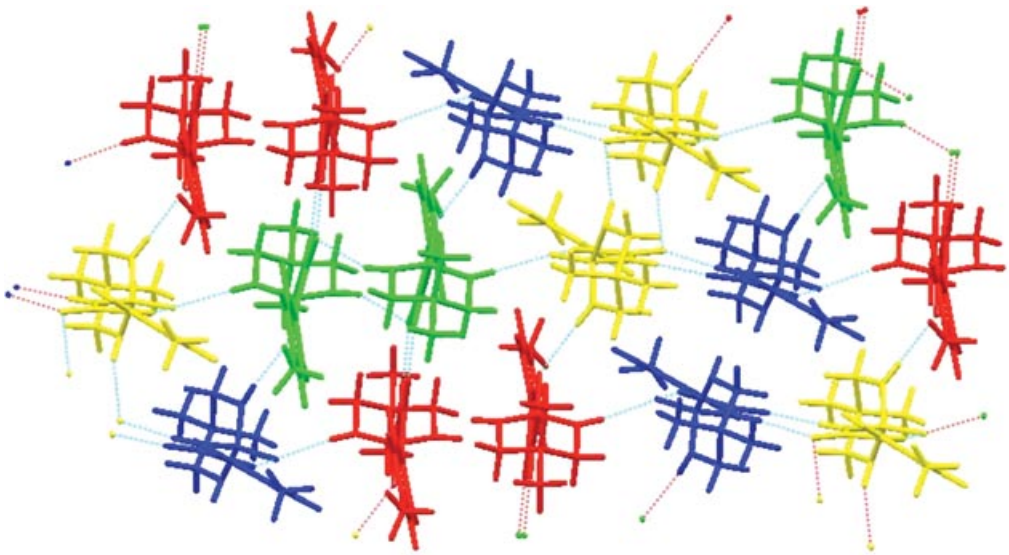


FIGURE 2 A two-dimensional polymeric chain produced by strong and weak hydrogen bonds in the crystalline lattice of compound **5b**.

compound in both the HepG2 and K562 tests, except molecule **7a,** which was inactive toward k562 cells. Also, most of the compounds **5a–7c** showed an enhanced activity compared to cyclophosphamide against MDA-MB-231 cells. Since hydrogen bonds play a key role in biology processes [28,29], these results suggest that an increase in the hydrogen bonds of new derivatives toward cyclophosphamide may be beneficial for drug-target interactions. Also, the present study reveals that HepG2 cells were more sensitive to all the tested compounds than other cell lines.

On the other hand, although the lipophilicity plays an important role during the penetration of compounds into the cells and increases the activity [30], Table 4 demonstrates that there were no direct correlations between the anticancer activity and the lipophilicity of the tested molecules. This may suggest that the antitumor activity depends on other properties, such as the electronic nature of the substituent, a reduction in potential [31], an increase in the number, and the kind of heteroatom in the phosphorinane ring in these molecules.

Finally, to study the degree of selectivity in the cytotoxic activity of the compounds, assays using lymphocyte isolation from whole human blood were carried out on some representative compounds such as **5a, 6b**, and **7a**, which showed relatively high activity in tumor cells. The assay showed that survival values were 98–100% for these compounds.

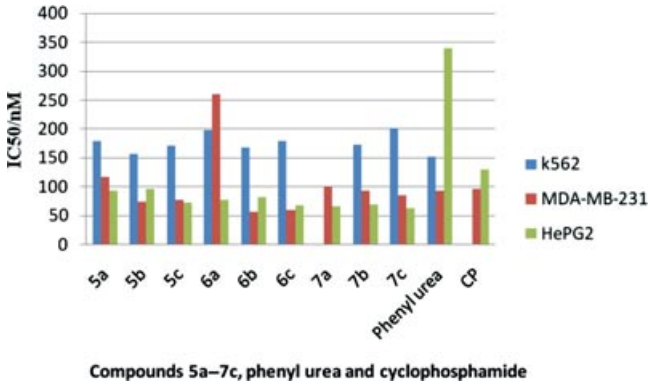


FIGURE 3 IC_{50} values of compounds **5a–7c**, CP and phenyl urea against K562, MDA-MB-231, and HepG2.

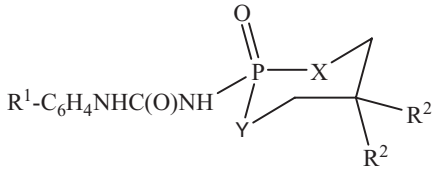
SUPPORTING INFORMATION

Supporting Information is available from the corresponding author (*gholi_kh@modares.ac.ir*.) on request.

SUPPLEMENTARY MATERIAL

CCDC 817643 contains the supplementary crystallographic data for **5b** ($C_{13}H_{21}N_4O_2P_1$). These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

TABLE 4 Cytotoxicity Activity of Compounds in K562, MDA-MB-231 and HepG2 Cell Cultures



					Cell Line, (IC_{50} (nM))[a]			
Compound	R^1	R^2	*X*	*Y*	*K562*	*MDA-MB-231*	*HepG2*	*log P*[b]
5a	H	CH_3	NH	NH	179 ± 2.1	118 ± 7.6	94 ± 5.9	0.77
5b	4-CH_3	CH_3	NH	NH	157 ± 2.0	75 ± 2.5	97 ± 6.5	1.19
5c[c]	4-NO_2	CH_3	NH	NH	171 ± 3.2	78 ± 1.4	73 ± 1.1	0.73
6a	H	H	O	NH	198 ± 5.9	260 ± 18.6	78 ± 3.3	−0.19
6b	4-CH_3	H	O	NH	168 ± 1.2	57 ± 1.9	82 ± 2.4	0.23
6c	4-NO_2	H	O	NH	180 ± 3.1	60 ± 2.9	69 ± 1.3	−0.23
7a	H	CH_3	O	O	NA[d]	100 ± 1.4	67 ± 1.1	2.3
7b	4-CH_3	CH_3	O	O	173 ± 1.5	94 ± 1.5	70 ± 6.7	2.76
7c	4-NO_2	CH_3	O	O	202 ± 7.3	86 ± 5.4	63 ± 1.0	0.82
CP[e]					NA	97 ± 5.5	130 ± 6.2	0.75
Phenyl urea					153 ± 2.8	94 ± 4.6	339 ± 25.7	0.86

[a]Dose required to inhibit cell growth by 50%. Values are the means of at least three independent determinations.
[b]Log *P* values of the synthesized compounds were calculated using ChemDraw Ultra 8.0 calculation software.
[c]For compound**5c**, see [4].
[d]NA: not active ($IC_{50} > 400$ nM).
[e]Cyclophosphamide.

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