Synthesis of Some 1-Aryl-2,3-dibromophospholanes as Novel Anti-Cancer Agents

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ABSTRACT

Novel phosphorus heterocyclic compounds, 3-methyl-1-(3-bromophenyl as well as some 3-substituted phenyl)-2-phospholene 1-oxides (**1d** as well as **1b**, **1c**, and **1f**), were synthesized from 1-phenyl-2-phospholene 1-oxide **1a** via 3-methyl-1-(3-nitrophenyl)-2-phospholene 1-oxide (**1b**). 1-(4-Bromophenyl)-2-phospholene **1e** was prepared by Grignard coupling reaction of 1-chloro-3-methyl-2-phospholene 1-oxide with 4-bromophenylmagnesium bromide. 2,3-Dibromo-3-methyl-1-arylphospholane 1-oxides (**2a-2e**) were prepared by the addition reaction of bromine to the C=C double bond of 2-phospholenes **1a-1e**. The substituent effect of the phenyl group of the 1-aryl-phospholanes **2** on the observed anti-proliferative effect against U937 leukemia cell lines evaluated by MTT *in vitro* methods showed that 2,3-dibromo-3-methyl-1-(4-bromophenyl)phospholane (**2e**) was the most active among **2**. These novel dibromophosphorus heterocyclic derivatives exhibit much higher anti-cancer activity than Gleevec® (molecular targeting chemotherapeutic agent) against U937 cells.

Keywords: Phosphorus heterocycles; 1-Aryl-2,3-dibromophospholane; Anti-tumors; Leukemia cells; MTT *in vitro* method

INTRODUCTION

Phospha sugars /1-3/, one new category of the pseudo sugars which have a phosphorus atom in the hemiacetal ring of sugars, are not yet found in nature and the synthesis of them are rather difficult compared with the typical pseudo sugars. Phospha sugars exert important biological activities and have quite efficient anti-cancer activities for leukemia cells /4/. By the further research on phospha sugar anti-tumor agents, it may be more plausible that the medicinal chemistry will lead to find novel phospha sugars as multi-molecular targeting chemotherapeutic drugs for the human cancers, and then researches on the synthesis and evaluation of phospha sugars as a new type of molecular targeting chemotherapeutic

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anti-tumor agents will be developed. In this paper, we will deal with the preparation of derivatives of 1-aryl-2-phospholenes **1** and 1-aryl-2,3-dibromophospholanes **2**, and the evaluation of their anti-cancer activity by MTT *in vitro* methods against leukemia cells.

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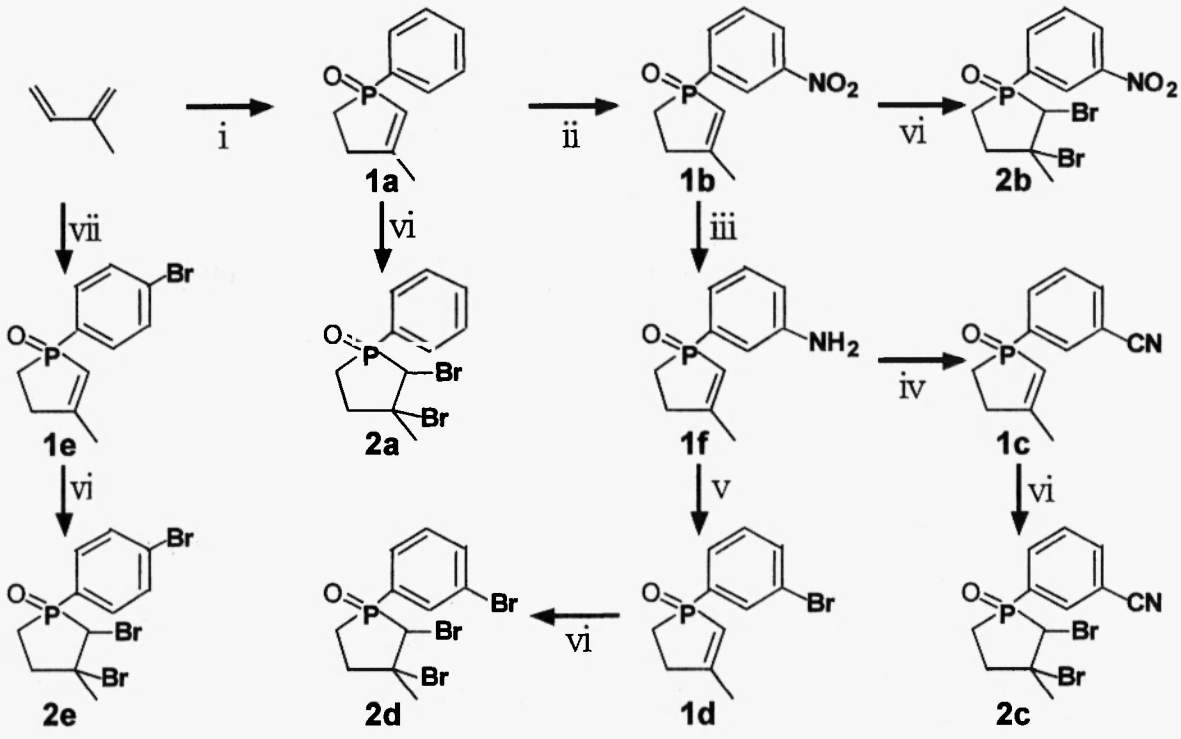
Fig. 1: 1-Aryl-2,3-dibromo-3-methylphospholane 1-oxides **2** (substituents of **2a**: R_1=R_2=H; **2b**: R_1=NO_2, R_2=H; **2c**: R_1=CN, R_2=H; **2d**: R_1= Br, R_2=H, **2e**: R_1= H, R_2=Br).

RESULTS AND DISCUSSION

The syntheses of various 1-aryl-2,3-dibromo-3-methyl-2-phospholene 1-oxide (**1b-1f**), with one of the substituents of 3-nitro, 3-cyano, 3-bromo, and 3-amino groups, were performed by substitution reaction on the phenyl group of 1-phenyl-2-phospholene **1a** (Scheme 1). Synthesis of 3-methyl-1-phenyl-2-phospholene 1-oxide (**1a**) was carried out by a known [4+2] cycloaddition reaction of 2-methyl-1,3-butadiene with phenylphosphonous dichloride and the successive hydrolysis reaction of the [4+2] cycloadduct (McCormac reaction /5/) in yield of 68% (Scheme 1, step (i)). 3-Methyl-1-phenyl-2-phospholene **1a** was converted into 3-methyl-1-(3-nitrophenyl)-2-phospholene **1b**, in which the nitro group replaced selectively at the 3-position of the phenyl by an action of fuming nitric acid and concentrated sulfuric acid in yield of 97% (Scheme 1, step (ii)). 3-Methyl-1-(3-aminophenyl)-2-phospholene **1f** was prepared from 1-(3-nitrophenyl)-2-phospholene **1b** by the reduction of the nitro group with tin chloride and concentrated hydrochloric acid in yield of 88% (Scheme 1, step (iii)). 3-Methyl-1-(3-cyanophenyl)-2-phospholene **1c** was prepared from 1-(3-aminophenyl)-2-phospholene **1f** by the Sandmeyer reaction of the amino group with sodium nitrite, concentrated sulfuric acid, and copper(I) cyanide in yield of 66% (Scheme 1, step (iv)). Similarity, 3-methyl-1-(3-bromophenyl)-2-phospholene **1d** was prepared from 1-(3-aminophenyl)-2-phospholene **1f** by the Sandmeyer reaction of the amino group with sodium nitrite, concentrated sulfuric acid and copper(II) bromide in yield of 47% (Scheme 1, step (v)).

3-Methyl-1-(4-bromophenyl)-2-phospholene 1-oxide (**1e**) was prepared from 1-chloro-3-methyl-2-phospholene 1-oxide by the Grignard coupling-reaction using 4-bromophenylmagnesium bromide in yield of 64%. 1-Aryl-2,3-dibromo-3-methylphospholane 1-oxides (**2a-2e**), where the phenyl group was substituted by either one of substituents of 3-nitro, 3-cyano, 3-bromo, and 4-bromo groups, were prepared by an addition reaction of bromine to 1-aryl-3-methyl-2-phospholene 1-oxide (**1a-1e**). The addition reaction of bromine to the electron deficient C=C double bond of 2-phospholene **1** was accelerated by a manganese or copper catalyst. The reaction conditions and the results for preparation of **2a-2e** are summarized in Table 1. Evaluation of 1-aryl-2,3-dibromo-3-methylphospholane 1-oxide (**2a-2e**: mixture of diastereomers) as inhibitors on proliferation of leukemia cells was carried out by *in vitro* MTT assay method. The results against U937 cells are shown in Figure 2. U937 cells of leukemia cell lines were incubated with dibromophosphorus derivatives **2a** to **2e** at the indicated concentrations (0-1000 μM) at 37 °C for 48 h. The cell proliferation or inhibition was evaluated as the function of the absorbance at 560 nm visible light by MTT *in vitro* assay.

Dibromo derivatives **2a** to **2e** strongly suppressed the cell proliferation of U937 cells in a dose-dependent manner and the intensity of absorbance at 560 nm decreased at the lower concentration compared with Gleevec®.



Scheme 1

Synthesis of 1-aryl-3-methyl-2-phospholene 1-oxides **1a-1e** and 1-aryl-2,3-dibromo-3-methylphospholane 1-oxides **2a-e** (substituents of the phenyl group in **a** of **1** and **2**: none; **b** of **1** and **2**: 3-NO_2; **c** of **1** and **2**: 3-CN; **d** of **1** and **2**: 3-Br; **e** of **1** and **2**: 4-Br; **f** of **1**: 3-NH_2). Reagents and conditions: (i) ① $PhPCl_2$ (1.0 equiv), 2 weeks, r.t.; ② H_2O, r.t.; (ii) HNO_3 (1.2 equiv)/H_2SO_4, 6 h, 0 °C; (iii) $SnCl_2$ (2.0 equiv)/HCl, 4 h, 0 °C; (iv) ① $NaNO_2$ (1.0 equiv)/H_2SO_4, 5 min, 0 °C, ② CuCN (2.0 equiv), 5 min, 0 °C; (v) ① $NaNO_2$ (1.0 equiv)/H_2SO_4, 5 min, 0 °C; ② $CuBr_2$ (2.0 equiv), 5 min, 0 °C; (vi): Br_2, Catalyst (see Table1), CH_2Cl_2, r.t.; (vii): ① PCl_3 (1.0 equiv), 2 weeks, r.t.; ② CH_3OH, r.t. ③$SOCl_2$, 24 h, r.t., ④Mg (1.3 equiv), 1,4-Dibromobenzene (1.3 equiv), THF, 4 h, r.t.

Evaluation of 1-aryl-2,3-dibromo-3-methylphospholane 1-oxides **2a-2e** (mixture of diastereomers) as inhibitors on proliferation of leukemia cells was carried out by *in vitro* MTT assay method. The results against U937 cells are shown in Figure 2. U937 cells of leukemia cell lines were incubated with dibromophospholane derivatives **2a** to **2e** at the indicated concentrations (0-1000 μM) at 37 °C for 48 h. The cell proliferation or inhibition was evaluated as the function of the absorbance at 560 nm visible light by MTT *in vitro* assay. Dibromo derivatives **2a** to **2e** strongly suppressed the cell proliferation of U937 cells in a dose-dependent manner and the intensity of absorbance at 560 nm decreased at the lower concentration compared with Gleevec®.

Table 1

Preparation of 1-aryl-3-methyl-2-phosoholene 1-oxides **1a-1f** and 1-aryl-2,3-dibromo-3-methyl-2-phospholane 1-oxides **2a-2e**.

Entry	Substituent		2-Phospholene **1**		2,3-Dibromophospholane **2** by step (vi) of Scheme 1		
	R_1(3)	R_2(4)	Reagents and conditions for the preparation of **1** (Step)	Yield (%)	Catalyst (equiv.)	Conditions	Yield (%)
a	H	H	$PhPCl_2$, r.t., 2 weeks (i)	68	$MnBr_2$ (0.5)	CH_2Cl_2, r.t., 1 h	96
b	NO_2	H	HNO_3, H_2SO_4, 0 °C, 6 h (ii)	97	$MnBr_2$ (1.0)	CH_2Cl_2, r.t., 12 h	97
c	CN	H	$NaNO_2$, CuCN, 0 °C, 5 min (iv)	66	MnO_2 (2.0)	CH_2Cl_2, r.t., 16 h	62
d	Br	H	$NaNO_2$, CuBr, 0 °C, 5 min (v)	47	$MnBr_2$ (1.0)	CH_2Cl_2, r.t., 12 h	73
e	H	Br	Mg, 1,4-$Br_2C_6H_4$, THF, r.t., 4 h (vii ④)	64	$MnBr_2$ (1.0)	CH_2Cl_2, r.t., 12 h	88
f	NH_2	H	$TiCl_3$, HCl (iii)	88	-------	--------	-----

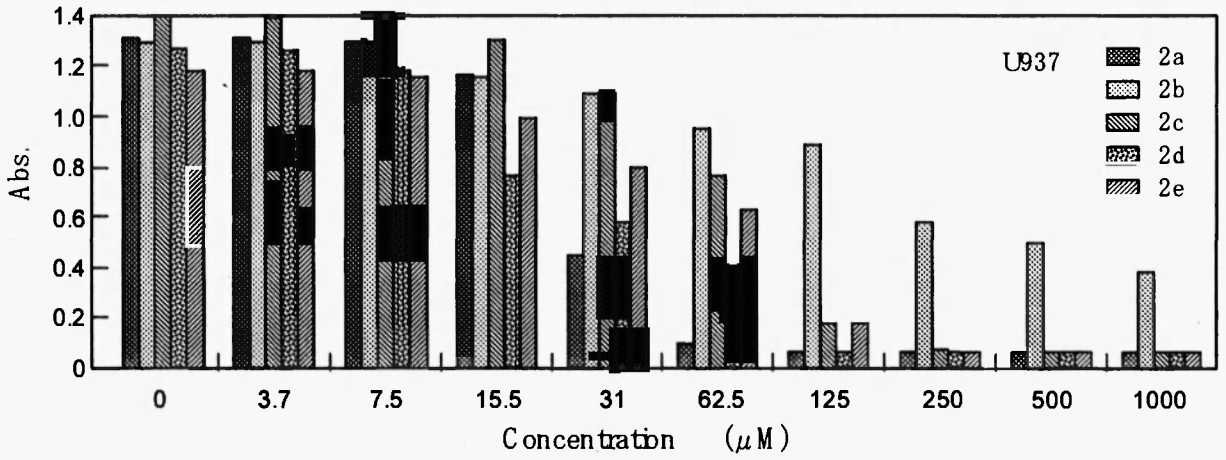


Fig. 2: Inhibition from cell proliferation against U937 cells by treatment with 1-aryl-2,3-dibromo-3-methylphospholane 1-oxides **2a-2e** at 37 °C for 48 h.

The decrease of the absorbance means the death of the cells and clearly indicates that dibromo derivatives **2a-2e** possess the strong growth inhibitory effect on U937 cells and that half of the absorbance intensities (IC_{50}) observed were 28, 250, 70, 15, and 58 μM for **2a-2e**, respectively (Table 2). The observed anti-proliferative effect of dibromo derivatives **2a-2e** on U937 cells observed was much more efficient than that of Gleevec® (IC_{50} = 500 μM) /6-9/. The effect of increase in the number of bromo substituents in the phospha sugars or phopholane derivatives **2** suggests the decrease of the IC_{50} and a good anti-proliferative effect. Substituent effects of bromo groups in heterocyclic compounds and/or aromatic compounds on improvement of bioactivities of meridianins and isatin derivatives, which activates protein kinase inhibitory effects and induces apoptosis, are reported/10,11/. These protein kinase inhibitory effects are important for drugs for targeting oncology/12/. The substituent effect of the phenyl group on IC_{50} seems to be influenced either by electronic and steric factors of the substituents.

Table 2

Half of the absorbance intensity (IC_{50}) by phosphorus heterocyclic compounds **1a** and **2a-2e**.

Compound	Substituted and/or unsubstitited phenyl derivative of **1a** and **2**		Number of Br	IC_{50} [a]
	$R_1(3)$	$R_2(4)$		(μM)
1a	H	H	0	>1000
2a	H	H	2	28
2b	NO_2	H	2	250
2c	CN	H	2	70
2d	Br	H	3	15
2e	H	Br	3	58

[a] The concentration of compounds **1a** and **2** which caused half absorbance intensity by MTT evaluation against U937 cells.

In conclusion, we have synthesized novel dibromophospholane derivatives, 1-aryl-2,3-dibromo-3-methyl-2-phospholane 1-oxides **2b-2e** substituted by either one substituents of 3-nitro, 3-cyano, 3-bromo, and 4-bromo groups on the phenyl group, in good yields by addition reaction of bromine in the presence of catalyst. MTT *in vitro* bio-assay method revealed that the prepared phosphorus heterocyclic compounds **2a-2e**, especially 2,3-dibromo-3-methyl-1-(3-bromophenyl)phospholane 1-oxide (**2d**), have quite efficient anti-cancer activities for leukemia cells in manners of (i) wide spectra, (ii) high activities, and (iii) high specificities and selectivities.

EXPERIMENTAL SECTION

General Procedures and Methods:

TLC (Silica gel: Wako Chromato Sheet and/or Merk Kieselgel 60; Eluent : $CHCl_3$: MeOH = 20 : 1, in R*f* value); Melting point apparus (Gallenkamp, in °C); MS (MALDI-TOF-MS: GL Science (Voyager-DE Porimerix); Matrix: α-Cyano-4-hydroxycinnamic acid, in *m/z*); IR (JASCO FT/IR 410 (KBr), in cm^{-1}); ^{1}H-NMR (JEOL JNM-AL300 (300 MHz) and Hitach R90H (90 MHz); Solvent: $CDCl_3$, in δ (ppm)) were used for analyzing the products.

Synthesis of Phosphorus Heterocyclic Compounds:

*Synthesis of 3-methyl-1-phenyl-2-phospholene 1-oxide (**1a**):*

Isoprene 70 ml (0.398 mol) and phenylphosphonous dichloride 68 ml (0.663 mol) were mixed and reacted at room temperature for 2 weeks. The formed solid, 3-methyl-1-phenyl-2-phospholenium dichloride, was dissolved in chloroform and hydrolized at 0 °C. The reaction mixture was neutralized with sodium hydrogencarbonate and then filtered and extracted with chloroform (3 × 15 ml). The chloroform extarct was washed with saturated sodium hydrogencarbonate solution (50 ml) and saturated sodium chloride solution (50 ml). Drying over the chloroform extarct with anhydrous sodium sulfate followed by filtration, evapolation of the solvent *in vacuo*, and distillation under the reduced pressure afforded 3-methyl-1-phenyl-2-phospholene 1-oxide (**1a**: Registry number: 707-61-9; 64 g) in 68% yield.

B.p.: 156-161 °C /0.10 mmHg; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 2.08 (s, 3H, CH_3), 2.21-2.29 (m, 2H, H-5), 2.64-2.81 (m, 2H, H-4), 5.95 (ddt, 1H, H-2), and 7.47-7.71 (m, 5H, Ph); MS (*m/z*): 193.5 (M^+ + H).

*Synthesis of 3-methyl-1-(3-nitrophenyl)-2-phospholene 1-oxide (**1b**):*

To a sulfuric acid solution (1 ml) of 2-phospholene **1a** (192 mg, 1.00 mmol) was added fuming nitric acid (0.089

ml), and then the mixture was kept at room temperature for 30 min with stirring. To the reaction miture was added ice/water and extracted with chloroform (10 ml x 3). The chloroform extract was washed with water (10 ml x 1) and saturated sodium chloride solution (10 ml x 1), and then dried over with anhydrous sodium sulfate. Filtration of the chloroform extarct and evaporation of the filtrate gave the residue, which was column chromatographed (silica gel; eluent: chloroform: methanol = 20 : 1) to give 3-methyl-1-(3-nitrophenyl)-2-phospholene 1-oxide (**1b**: Registry number 91207-34-0; 162 mg) in 68% yield.

Rf: 0.64; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 2.15 (s, 3H, CH_3), 2.26 (t, 2H, H-5), 273-2.89 (dd, 2H, H-4), 5.95 (d, 1H, H-2), and 7.68-8.42 (m, 4H, Ph); MS (*m/z*): 238.6 (M^+ + H).

*Synthesis of 3-methyl-1-(3-aminophenyl)-2-phospholene 1-oxide (**1f**):*

To chloroform solution (10 ml) of nitric acid (0.089 ml) of 2-phospholene **1b** (4.50 mg, 19.9 mmol) was added concentrated hydrochloric acid solution (5 ml) of tin chloride (4.5 g, 20 mmol) at 0 °C, and then the mixture was kept at 0 °C for 4 h and room temperature for 4 days with stirring. The reaction miture was poured into ice/water and the diluted reaction mixture was neutralized with sodium hydrogencarbonate, and the mixture was filtrated and evaporated *in vavuo*. The residue was column chromatographed (alumina; eluent: chloroform : methanol = 10 : 1) to afford 3-methyl-1-(3-aminophenyl)-2-phospholene 1-oxide (**1f**, 0.91 g) in 88% yield.

Rf: 0.41; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 1.73-1.81 (m, 2H, H-5), 2.06 (s, 3H, CH_3), 260-2.80 (m, 2H, H-4), 3.82 (bs 2H, NH_2), 5.92 (dt, 1H, H-2), and 6.78-7.27 (m, 4H, Ph); MS (*m/z*): 208.5 (M^+ + H).

*Synthesis of 3-methyl-1-(3-bromophenyl)-2-phospholene 1-oxide (**1d**):*

To a diluted sulfuric acid solution (water (2 ml) and sulfuric acid (1 ml)) of 2-phospholene **1f** (0.36 g, 1.7 mmol) was added concentrated sulfuric acid (2 ml), and then sodium nitrite (0.12 g, 1.0 eq) solution was added dropwise for 5 min at 0 °C with stirring to prepare the diazonium salt. To the hydrobromic acid solution (1 ml) of copper(I) bromide (0.49 g, 2.0 eq) was added dropwisely the diazonium salt solution at 0 °C with stirring. After the reaction (Sandmayer reaction) for 5 min, the reaction mixture was extracted with chloroform (10 ml x 3), and then the extract was washed with sodium hydrogencarbonate (10 ml x 2) and saturated sodium chloride solution. Drying over the chloroform extract with anhydrous sodium sulfate followed by filtration and evaporation of the solvent *in vacuo* gave the residual product, whose column chromatography on silica gel (eluent: chloroform : methanol = 30 : 1) afforded 3-methyl-1-(3-bromophenyl)-2-phospholene 1-oxide (**1d**, 0.22 g) in 47% yield.

Rf: 0.58; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 1.73-1.86 (m, 2H, H-5), 2.11 (s, 3H, CH_3), 266-2.84 (m, 2H, H-4), 5.90-6.03 (m, 1H, H-2), and 7.26-8.38 (m, 4H, Ph); MS (*m/z*): 271.4 (M^+ + H).

*Synthesis of 3-methyl-1-(3-cyanophenyl)-2-phospholene 1-oxide (**1c**):*

The similar Sandmayer reaction of **1f** with copper(I) cyanide afforded 3-methyl-1-(3-cyanophenyl)-2-phospholene 1-oxide (**1c**) in 66% yield.

Rf: 0.42; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 1.84 (s, 3H, CH_3), 2.09-2.38 (m, 2H, H-5), 270-2.85 (m, 2H, H-4), 5.94 (dd, 1H, H-2), and 7.47-8.02 (m, 4H, Ph); MS (*m/z*): 218.7 (M^+ + H).

*Synthesis of 3-methyl-1-(4-bromophenyl)-2-phospholene 1-oxide (**1e**):*

To a dry THF solution (15 ml) of 1-chloro-3-methyl-2-phospholene 1-oxide (4.70 g, 31.2 mmol: prepared from 1-methoxy-3-methyl-2-phospholene 1-oxide) was added 4-bromophenylmagnesium bromide (prepared from 4-dibromobenzene (9.58 g, 40.6 mmol) and magnesium (0.99 g, 40.6 mmol) in dry THF (20 ml)) under the argon atmosphere and reacted at room temperature for 4 h with stirring. The reaction mixture was treated with 10% hydrochloric acid and neutralized, and then THF of the reaction medium was evaporated. The residual solution was extracted with ethyl acetate (30 ml x 3) and the extract was washed with saturated sodium hydrogencarbonate solution

and saturated sodium chloride solution. Drying the ethyl acetate extract over anhydrous sodium sulfate followed by filtration and evapolation *in vacuo* gave residual product, which was column chromatographed (silica gel; eluent: chloroform : methanol = 10 : 1) to afford 3-methyl-1-(4-bromophenyl)-2-phospholene 1-oxide (**1e**, 5.46 g) in 64% yield.

R*f*: 0.66; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 2.08 (s, 3H, CH_3), 2.12-2.28 (m, 2H, H-5), 2.60-2.87 (m, 2H, H-4), 5.89 (d, 1H, H-2), and 7.46-7.72 (m, 4H, Ph); MS (*m/z*): 271.7 (M^+ + H).

Synthesis of 2,3-dibromo-3-methyl-1-(3-nitrophenyl)-phospholane 1-oxide (2b):

To a dichloromethane solution (5 ml) of 2-phospholene **1b** (0.20 g, 1.00 mmol) was added manganese(II) bromide (0.19 g, 1.0 mmol; 1.0 eq), and then to the mixture was added dichloromethane solution (10 ml) of bromine (0.5 ml, excess) dropwisely for 20 min with stirring at room temperature. After the reaction, saturated sodium hydrogensulfite solution was added to reduce the excees amount of bromine and was extracted with chloroform. Neutralization of the chloroform extract with saturated sodium hydrogencarbonate solution followed by washing the chloroform layer with saturated sodium chloride, drying over with anhydrous sodium sulfate, filtration, and evapolation of the solvent gave the residual product. The column chromatography on silica gel (eluent: chloroform : methanol = 30 : 1) afforded colorless crystaline 2,3-dibromo-3-methyl-1-(3-nitrophenyl)phospholane 1-oxide (**2b**, 0.40 g) in 96% yield.

R*f*: 0.49; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 1.83-2.17 (m, 2H, H-5), 2.18-2.19 (m, 3H, CH_3), 2.20-2.84 (m, 2H, H-4), 3.33 (d, 1H, H-2), and 7.72-8.55 (m, 4H, Ph); MS (*m/z*): 398.5 (M^+ + H).

Similarly, 1-aryl-2,3-dibromo-3-methylphospholane 1-oxides (**2a**, and **2c-2e**) were prepared.

2,3-Dibromo-3-methyl-1-phenylphospholane 1-oxide (2a): M.p. 189.20 °C; b.p. 280.24 °C; R*f*=0.52; IR :1126 cm^{-1} (P=O), 748 cm^{-1}, 1396 cm^{-1} (C-Br); ^{1}H-NMR ($CDCl_3$, 300 MHz), δ (ppm); 1.67 (s, 3H, CH_3), 2.36-2.46 (m, 2H, H-4), 2.97-3.02 (m, 2H, H-5) 4.28-4.31 (m, 1H, C-2), 7.51-7.70 (m, 5H, Ph); MS (m/z), 353.24 (M + H^+).

2,3-Dibromo-3-methyl-1-(3-cyanophenyl)phospholane 1-oxides (2c): Yield: 62% (catalyst: manganese(IV) oxide); R*f*: 0.47; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 1.95 (s, 3H, CH_3), 2.09-2.86 (m, 4H, H-4 and H-5), 4.68 (d, 1H, H-2), and 7.65-8.08 (m, 4H, Ph); MS (*m/z*): 379.7 (M^+ + H).

2,3-Dibromo-3-methyl-1-(3-bromophenyl)phospholane 1-oxides (2d): Yield: 73% (catalyst: manganese(IV) oxide); R*f*: 0.54; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 2.10 (s, 3H, CH_3), 2.20-2.56 (m, 2H, H-5), 2.62-2.85 (m, 2H, H-4), 4.65 (d, 1H, H-2), and 7.27-7.67 (m, 4H, Ph); MS (*m/z*): 427.5 (M^+ + H).

2,3-Dibromo-3-methyl-1-(4-bromophenyl)phospholane 1-oxides (2e): Yield: 86% (catalyst: manganese(IV) oxide); R*f*: 0.65; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ: 2.10 (s, 3H, CH_3), 2.32-2.56 (m, 2H, H-5), 2,62-2.85 (m, 2H, H-4), 4.65 (d, 1H, H-2), and 7.27-7.67 (m, 4H, Ph); MS (*m/z*): 427.5 (M^+ + H).

MTT *in vitro* Evaluation:

Reagent and solvent for the in vitro MTT evaluation:

1-Aryl-2,3-dibromo-3-methylphospholane 1-oxides **2a-2e**, the reagents being evaluated by the *in vitro* MT*T method*, were dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Company, St. Louis, MO, USA) as the solvent, and the solutions were diluted into appropriate concentration with DMSO in culture medium immediately before use. The final concentrations of **2** in DMSO in all experiments were less than 0.010%, and all the treatment conditions were compared with vehicle controls. The control experiments for the evaluation were carried out by using DMSO, and the absorption change by DMSO in the MTT method was not observed for U937 cells as well as K562 cells at 37 °C for 48 h.

Human tumor cell lines and culture:

Chronic promyeloid leukemia (U937) cells as well as myeloid leukemia (K562) cells were purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA). The cells were cultured in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal calf serum (FCS) 292 mg/l (or 2.0 mM) L-glutamine, 100 μg/ml streptomycin, and 200 U/ml penicillin (GIBCO-BRL, Gaithersburg, MD, USA). All cells were maintained in a humidified 5 % CO_2 atmosphere at 37 °C.

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