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Design and Synthesis of Small and Potent Inhibitors of Urokinase as Antitumor Agents

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Abstract: A set of small nonpeptidic diaryl phosphonate inhibitors was prepared. The phosphonate derivatives were synthesized through Birum-Oleksyszyn reaction followed by reduction of the nitro-group which undergo to Boc-protected guanidine derivatives. After removing of Boc-group by TFAA/DCM afford the guanidine phosphonates.

Key words: Phosphonates · Guanidine · Synthesis · Upa

INTRODUCTION

Organophosphorus compounds have found a wide range of applications in the areas of industrial, agricultural and medicinal chemistry owing to their biological and physical properties as well as their utility as synthetic intermediates [1-4]. Phosphonates-containing molecules are an important class of active compounds and their use and synthesis have received an increasing amount of attention during the last two decades [5]. The utilities of α-amino phosphonates as enzyme inhibitors [6], antibiotics [7], herbicides [8], fungicides and plant growth regulators [9], anti-thrombotic agents [10], as well as peptidases and proteinases [11] are well documented. A number of potent antibiotics, [12] enzyme inhibitors, [13] and pharmacological agents [14] are 1-aminophosphonic acids or peptide analogs thereof. Furthermore, 1-aminoalkyl phosphonates are the key substrates in the synthesis of various phosphonopeptides [15]. The use of 1-aminoalkyl phosphonates as enzyme inhibitors, [16] antibiotics and pharmacological agents, [17] herbicides, [18] and haptens of catalytic antibodies [19] are well documented. On the other hand, 1, 3, 4-Thiadiazoles exhibit broad spectrum of biological activities, possibly due to the presence of toxophoric moiety [20]. They find applications as antibacterial, antitumor, anti-inflammatory agents, pesticides, herbicides, dyes, lubricants and analytical reagents [21-25]. It has been suggested that the anti-HIV activity of nucleosides and their analogs may be critically dependent on their initial intracellular phosphorylation [26, 27]. An approach to bypass the first phosphorylation step more completely is represented by a class of compounds called acyclic nucleoside phosphonates [28-31].

MATERIALS AND METHODS

All solvents were dried by standard methods. Melting points were determined with a Kofler block apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Model 1720 FTIR spectrometer. Specific rotations are for dimethylsulfoxide solutions. ^{1}H NMR spectra were determined with a Bruker AC-250 FT spectrometer. The chemical shifts in ppm are expressed on the δ scale using tetramethylsilane as internal standard. Coupling constants are given in Hz. TLC was performed on Merck silica gel 60-F254 precoated plastic plates. Microanalyses were performed in the unit of microanalysis at Tokyo University (Japan).

(2-Chloro-5-nitrophenyl) (diphenoxyphosphoryl) methylcarbamate derivatives (2a-c)

General Procedure: Cupper II trifilate (0.1 mol equiv) was added to a solution of the aldehyde (**2** mmol) and the carbamate (2.2 mmol) in acetonitrile. The mixture was stirred at reflux for 15 min and then triphenylphosphite (2.2 mmol) was added. After completion of the reaction (6 h), the reaction mixture was neutralized with aq. sat. $NaHCO_3$ followed by brine solution and then extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, concentrated under vacuum and the crude mixture was purified by column chromatography on silica gel (hexane: ethylacetate, 4:1) to afford pure products.

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Methyl (2-chloro-5-nitrophenyl) (Diphenoxyphosphoryl) methylcarbamate 2a: ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 3H, CH3), 4.91 (m, 1H, CH), 7.18–7.64 (m, 13H, Ar-H), 8.03 (s, 1H, NH); MS (ESI) m/z 499 $[M + Na]^+$.

Ethyl (2-chloro-5-nitrophenyl)(diphenoxyphosphoryl) methylcarbamate 2b: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, CH3), 4.12 (q, 2H, CH2), 4.90 (m, 1H, CH), 7.18–7.73 (m, 13H, Ar-H), 8.01 (s, 1H, NH); MS (ESI) m/z 513 $[M + Na]^+$.

Benzyl (2-chloro-5-nitrophenyl)(diphenoxyphosphoryl) methylcarbamate 2c: ^{1}H NMR ($CDCl_3$) δ 4.90 (m, 1H, CH), 5.11 (s, 2H, CH2), 7.18–7.84 (m, 18H, Ar-H), 8.01 (s, 1H, NH); MS (ESI) m/z 575 $[M + Na]^+$.

(5-Amino-2-chlorophenyl)(diphenoxyphosphoryl) methylcarbamate derivatives 3a-c: To a solution of carbamate derivative (**2**) in ethyl acetate, H_2O (2ml) and catalytic amount of $SnCl_2$ were added. The mixture was stirred at 70°C for 3 h. After completion, the reaction mixture was neutralized with aq. sat. $NaHCO_3$ followed by brine solution and then extracted with ethyl acetate, dried over anhydrous Na_2SO_4, concentrated under vacuum and washed by dry ether to afford pure products.

Methyl (5-amino-2-chlorophenyl) (diphenoxyphosphoryl) methylcarbamate 3a: ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 3H, CH_3), 4.90 (m, 1H, CH), 6.22 (s, 2H, NH_2), 6.88–7.62 (m, 13H, Ar-H), 8.03 (s, 1H, NH); MS (ESI) m/z 469 $[M + Na]^+$.

Ethyl (5-amino-2-chlorophenyl) (diphenoxyphosphoryl) methylcarbamate 3b: ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, CH3), 4.13 (q, 2H, CH2), 4.89 (m, 1H, CH), 6.30 (s, 2H, NH2), 6.68–7.58 (m, 13H, Ar-H), 8.00 (s, 1H, NH); MS (ESI) m/z 483 $[M + Na]^+$.

Benzyl (5-amino-2-chlorophenyl) (diphenoxyphosphoryl) methylcarbamate 3c: ^{1}H NMR ($CDCl^3$) δ 4.92 (m, 1H, CH), 5.08 (s, 2H, CH2), 6.71–7.64 (m, 18H, Ar-H), 8.03 (s, 1H, NH); MS (ESI) m/z 545 $[M + Na]^+$.

(5-(*tert*-butyloxycarbonylamino)-2-chlorophenyl)(diphenoxyphosphoryl)-methylcarbamatederivatives4a-c: Amixture of the phosphonate **3** (1 equiv), *N, N*'- bis (*tert*-butyloxycarbonyl-1-guanyl-pyrazole (1 equiv) and triethylamine (3 equiv) in chloroform (20 ml) was stirred at room temperature for 3 days. The solvent was evaporated and the residue was dissolved in ethylacetate and washed with 1 N HCl, saturated solution of $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by chromatography (0–80% AcOEt in hexane) to obtain the protected guanidine. Yield 62%.

Methyl(5-(*tert*-butyloxycarbonylamino)-2-chlorophenyl) (diphenoxy-phosphoryl) methylcarbamate 4a: ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 6CH3, 18H), 3.45 (s, 3H, CH3), 4.76 (m, 1H, CH), 7.21–7.66 (m, 13H, Ar-H); MS (ESI) m/z 711 $[M + Na]^+$.

Ethyl(5-(*tert*-butyloxycarbonylamino)-2-chlorophenyl) (diphenoxy-phosphoryl)methylcarbamate 4b: ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, CH3), 1.47 (d, 6CH_3, 18H), 4.15 (q, 2H, CH2), 4.76 (m, 1H, CH), 7.15–7.61 (m, 13H, Ar-H); MS (ESI) m/z 725 $[M + Na]^+$.

Benzyl(5-(*tert*-butyloxycarbonylamino)-2-chlorophenyl)(diphenoxy-phosphoryl)methylcarbamate4c: ^{1}H NMR ($CDCl_3$) δ 1.51 (d, 6CH_3, 18H), 4.15 (s, 2H, CH_2), 4.76 (m, 1H, CH), 7.18–7.61 (m, 18H, Ar-H); MS (ESI) m/z 787 $[M + Na]^+$.

(2-Chloro-5-guanidinophenyl)(diphenoxyphosphoryl) methyl-carbamate trifluoroacetate derivatives 5a-c: The Boc-protected intermediate (1) was dissolved in 50% trifluoroacetic acid in DCM (2–5 ml). After stirring for 3 h at room temperature, the solvent was evaporated. The crude oil was washed with cold ether and a precipitate was isolated.

Methyl(2-chloro-5-guanidinophenyl) (diphenoxyphosphoryl) methyl-carbamate trifluoroacetate 5a: ^{1}H NMR ($CDCl_3$) δ 3.49 (s, 3H, CH_3), 4.71 (m, 1H, CH), 7.19–7.64 (m, 13H, Ar-H); MS (ESI) m/z 511 $[M + Na]^+$.

Ethyl(2-chloro-5-guanidinophenyl) (diphenoxyphosphoryl)methyl-carbamate trifluoroacetate 5b: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, CH_3), 4.25 (q, 2H, CH_2), 4.76 (m, 1H, CH), 7.25–7.68 (m, 13H, Ar-H); MS (ESI) m/z 525 $[M + Na]^+$.

Benzyl(2-chloro-5-guanidinophenyl) (diphenoxyphosphoryl) methyl-carbamate trifluoroacetate 5c: ^{1}H NMR ($CDCl_3$) δ 4.18 (s, 2H, CH_2), 4.78 (m, 1H, CH), 7.18–7.59 (m, 18H, Ar-H); MS (ESI) m/z 587 $[M + Na]^+$.

***Tert*-butyl(3-(amino(diphenoxyphosphoryl)methyl)-4-chlorophenylamino)-(tertbutoxycarbonylamino) methylenecarbamate 6:** The Z-protected phosphonate (1 equiv) **4c** was dissolved in 20 ml MeOH. Pd/C (10%) was added. Under N_2 gas, H_2 was bubbled through the solution. The solution was stirred for 8 h at room temperature. The solution as filtered over Celite and the filtrate was evaporated. Compound **6** was used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 6CH_3, 18H), 4.76 (m, 1H, CH), 6.51 (s, 2H, NH_2), 7.11–7.56 (m, 13H, Ar-H); MS (ESI) m/z 654 [M + Na]$^+$.

Diphenyl amino(2-chloro-5-guanidinophenyl) methylphosphonate trifluoro-acetate 7: The phosphonate **6** was dissolved in 50% trifluoroacetic acid in CH_2Cl_2 (2-5ml). After stirring for 3 h at room temperature, the solvent was evaporated. The crude oil was washed with cold ether and a precipitate was isolated. Yield 83% ^{1}H NMR ($CDCl_3$) δ 4.82 (m, 1H, CH), 6.51 (s, 2H, NH_2), 7.15–7.66 (m, 13H, Ar-H), 8.58 (brs, 2H, NH_2); MS (ESI) m/z 453 [M + Na]$^+$.

***Tert*-butyl(3-(amino(diphenoxyphosphoryl)methyl)-4-chlorophenylamino)-(*tert*-butoxycarbonylamino) methylenecarbamate derivatives 8a-c**

General Procedure: To a stirred solution of phosphonate **6** (1 equiv) in a mixture of DCM, Et_3N and DMAP, alkyl chloride (1.1 equiv) was added dropwise. The reaction mixture was allowed to stir overnight. Saturated solution of $NaHCO_3$ was added, after extraction, the organic layer, dried over anhydrous Na_2SO_4 and evaporated. The product was purified by washing 3 times with cold ether.

***Tert*-butyl(*tert*-butoxycarbonylamino)(4-chloro-3-((diphenoxyphosphoryl)-(methylamino)methyl) phenylamino) methylenecarbamate 8a:** ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 6CH_3, 18H), 3.25 (s, 3H, CH_3), 4.76 (m, 1H, CH), 7.26–7.70 (m, 13H, Ar-H); MS (ESI) m/z 667 [M + Na]$^+$.

***Tert*-butyl(*tert*-butoxycarbonylamino)(4-chloro-3-((diphenoxyphosphoryl)-(ethylamino)methyl) phenylamino) methylenecarbamate 8b:** ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H, CH3), 1.39 (d, 6CH_3, 18H), 2.60 (q, 2H, CH_2), 4.70 (m, 1H, CH), 7.25–7.69 (m, 13H, Ar-H); MS (ESI) m/z 681 [M + Na]$^+$.

***tert*-butyl (3-((benzylamino) (diphenoxyphosphoryl) methyl)-4-chloro-phenylamino) (*tert*-butoxycarbonylamino) methylenecarbamate 8c:** ^{1}H NMR ($CDCl_3$) δ 1.48 (d, 6CH_3, 18H), 3.84 (s, 2H, CH_2), 4.73 (m, 1H, CH), 7.19–7.58 (m, 18H, Ar-H); MS (ESI) m/z 743 [M + Na]$^+$.

Diphenyl amino (2-chloro-5-guanidinophenyl) methylphosphonate trifluoro-acetate derivatives 9a-c

General Procedure: The phosphonate **8** was dissolved in 50% trifluoroacetic acid in CH_2Cl_2 (2-5ml). After stirring for 3 h at room temperature, the solvent was evaporated. The crude oil was washed with cold ether and a precipitate was isolated, yield 80%.

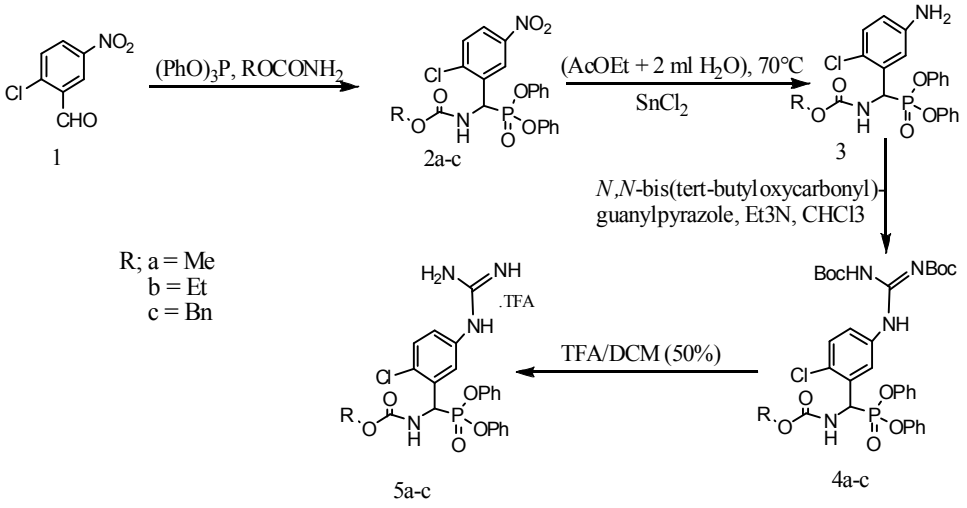
Diphenyl (2-chloro-5-guanidinophenyl) (methylamino) methylphosphonate trifluoroacetate 9a: ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H, CH_3), 4.72 (m, 1H, CH), 7.21–7.60 (m, 13H, Ar-H), 8.57 (s, 2H, NH_2); MS (ESI) m/z 667 [M + Na]$^+$.

Diphenyl (2-chloro-5-guanidinophenyl) (ethylamino) methylphosphonate 9b: ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3H, CH3), 2.62 (q, 2H, CH_2), 4.74 (m, 1H, CH), 7.25–7.69 (m, 13H, Ar-H), 8.61 (s, 2H, NH_2); MS (ESI) m/z 681 [M + Na]$^+$.

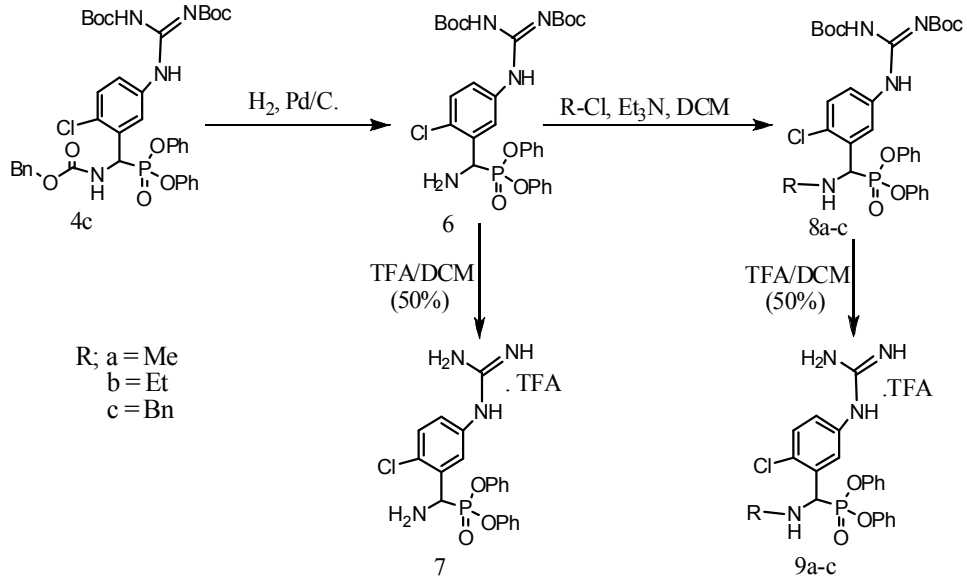
Diphenyl (benzylamino) (2-chloro-5-guanidinophenyl) methylphosphonate 9c: ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 2H, CH_2), 4.72 (m, 1H, CH), 7.23–7.55 (m, 18H, Ar-H), 8.63 (s, 2H, NH_2); MS (ESI) m/z 743 [M + Na]$^+$.

RESULTS AND DISCUSSION

(2-Chloro-5-nitrophenyl) (diphenoxyphosphoryl) methylcarbamate derivatives **2a-c** were synthesized by the stirring of a mixture of three components, triphenyl phosphite, aldehyde **1** and carbamate as one-pot reaction in presence of 5% mol of cupper trifilate. Mass spectra of **2a-c** showed that the molecular weight (M + Na) at 499, 513 and 575 respectively. The *nirto*-group of the derivatives **2a-c** was reduced to *amino*-group by stirring at 70°C in ethyl acetate in presence of 2ml H_2O and catalytic amount of $SnCl_2$ to give **3a-c**. Mass spectra of **3a-c** showed that the molecular weight (M + Na) at 469, 483 and 545 respectively. The guanidine derivatives **5a-c** were prepared by the reaction of the derivatives amino-derivatives **3**a-c with *N,N'*- bis(*tert*-butyloxycarbonyl)-1-guanyl-pyrazole in presence of triethyl amine and DMAP to afford the boc-protected guanidine derivatives **4a-c** followed by deprotection to remove the boc-groups. ^{1}HNMR showed the existence of peaks of methyl groups



Scheme 1



Scheme 2

in the derivatives **4a-c** and disappear of the peak of methyl-groups in the derivatives **5a-c**, (scheme 1).

When the Z-protected phosphonate **4c** was reacted with Hydrogen in presence of 10% mol of Pd/C afforded the free aminophosphonate derivative **6**. Mass spectra of **6** showed that the molecular weight (M + Na) at 654. Removing of Boc-groups from the phosphonate derivative **6** was carried out by the reaction with trifluoroacetic acid in dichloromethane (50%) to afford derivative **7**. ^{1}HNMR showed the absence of the peaks of methyl-groups. Also, the aminophosphnates derivative **6** was reacted with alkyl chloride to give the phosphonate derivatives **8a-c** which were undergo to react with trifluoroacetic acid in dichloromethane (50%) afforded the guanidineo-phosphnate derivatives **9a-c**. The ^{1}HNMR and Mass spectra proves the derivatives **8a-c** and **9a-c**.

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