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Research paper

Synthesis and antiproliferative activity of [2-(phthaloylamino)alkyl]triphenyl phosphonium derivatives against K562 cell line

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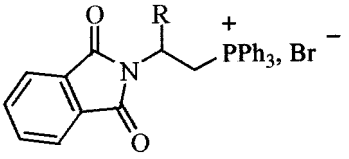
Given the reported cytotoxicity of phthaloylaminoethyltriphenylphosphonium bromide 2a in the P-388 cell line, we have developed new [2-(phthaloylamino)alkyl]phosphonium derivatives 2b–e and evaluated their cytotoxic activity. These compounds have been synthetized from *N,N*-phthaloylaminoalcohols and triphenylphosphonium hydrobromide via a one-pot reaction. 2a was found inactive in the K562 cell line, but 2c–e exhibited a cytotoxic activity with IC_{50} values about 1 μM. [© 2001 Lippincott Williams & Wilkins.]

Key words: [2-(Phthaloylamino)alkyl]phosphonium, cytotoxicity, phtaloylaminoalcohol.

Introduction

Phosphonium salts are lipophilic cationic molecules and some of them display cytotoxic activity against carcinoma cells.[1–3] Among these, compound **2a** (R=H) possesses significant antimitotic activity in lymphocytic leukemia P-388.[1]



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There are relatively few reports of such phosphonium salts and to our knowledge only two are described: **2a** (R=H), which was obtained from *N*-(2-bromoethyl)phthalimide and triphenylphosphonium,[1] and **2f** (R=COOH), obtained from serine via a multistep synthesis.[4] As a part of our research program in the area of peptides and pseudopeptides,[5] we planned to synthesize alkyl derivatives **2** from amino acids in order to examine the antitumoral activities of such compounds. Furthermore, these new products can be useful intermediates for the preparation of ethylenic isoster pseudopeptides.[5]

Our purpose here is thus to report the straightforward synthesis of [2-(phthaloylamino)alkyl]phosphonium bromide derivatives **2** and the evaluation of their cytotoxicity in the human leukemia cell line K562.

Materials and methods

Analytical thin-layer chromatography was carried out using aluminum-backed plates coated with Merck (Nogent-sur Marne, France) Kieselgel 60GF254, visualized under UV light at 254 nm. Flash chromatography was carried out using silicagel (MN Kieselgel; Macherey-Nagel, Hoerdt, France; 0.04–0.063 mm) with a mixture of petroleum ether/ethyl acetate (95/5) for compounds **1** and a mixture of dichloromethane/methanol (95/5) for compounds **2** as elution solvents (v/v). Melting points were recorded using a Kofler (Fisher-Bioblock, Ilkrich, France) apparatus.[1] H-NMR spectra were recorded in $CDCl_3$ using a Brücker (Wissembourg, France) AM 300 (300 MHz) instrument. Mass spectra were recorded using a Finnigan (Bremen, Germany) Mat 95XL.

General procedure for the synthesis of the *N,N*-Phthaloylaminoalcohols **1**

Suitable amino alcohol (13.3 mmol) and phthalic anhydride (16.0 mmol) were fused at 150°C for 1 h. After cooling at room temperature, the residue was dissolved in diethylether, washed successively with an aqueous saturated solution of $NaHCO_3$ (2 × 20 ml) and an aqueous saturated solution of NaCl (2 × 20 ml). The organic layer was dried with Na_2SO_4, filtered and evaporated *in vacuo* to obtain the crude product which was purified by chromatography.

(2S)-2-(Phthaloylamino)propan-1-ol ***(1b)***. Yield 81%. M.p. 88°C. $[\alpha]_D^{25}$=+15.9 (*c*=4.4, $HCCl_3$), lit.[6]+13.7 (*c*=4.2, $HCCl_3$).

(2S,3S)-3-Methyl-2-(phthaloylamino)pentan-1-ol ***(1c)***. Yield 79%. M.p. 66–68°C. $[\alpha]_D^{25}$=+14 (*c*=2, EtOH), lit.[7]+12.47 (*c*=2.16, EtOH).

(2S)-4-Methyl-2-(phthaloylamino)pentan-1-ol ***(1d)***. Yield 68%. M.p. 53°C. $[\alpha]_D^{25}$=+16.8 (*c*=1.8, EtOH), lit.[7]+11.37 (*c*=3.16, EtOH).

(2S)-3-Phenyl-2-(phthaloylamino)propan-1-ol ***(1e)*** Yield 70%. M.p. 106–108°C. $[\alpha]_D^{25}$=−147 (*c*=2, EtOH), lit.[7]−136 (*c*=2, EtOH).

General procedure for the synthesis of the [2-(Phthaloylamino)alkyl]triphenylphosphonium bromides **2**

N,N-Phthaloylaminoalcohols **1** (1.0 mmol) and triphenylphosphonium hydrobromide (1.2 mmol) were fused in a sealed tube at 160°C for 20 h. After cooling at room temperature, the residue was dissolved in chloroform. The resulting solution was dried with Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was chromatographied to obtain the title compound as a white powder.

[2-(Phthaloylamino)ethyl]triphenylphosphonium bromide ***(2a)***. Yield 74%. M.p. 236–238°C, lit.[1] 242–243°C.[1] H-NMR ($CDCl_3$, δ p.p.m.): 4.30 (dt, 2H, $C\underline{H}_2P$, ^2J=18 Hz, $\underline{H}$-P, ^3J=6.2 Hz, H-C-C-H); 4.55 (dt, 2H, $C\underline{H}_2N$, ^2J=12 Hz, $\underline{H}$-P, ^3J=6.2 Hz, H-C-C-H); 7.50 (m, 13H, Ar$\underline{H}$); 7.80 (m, 6H, *ortho* P($C_6\underline{H}_5$)$_3$).

(S)-[2-(Phthaloylamino)propyl]triphenylphosphonium bromide ***(2b)***. Yield 72%. M.p. 280°C. $[\alpha]_D^{25}$=+34.9 (*c*=3.4, EtOH). ^{1}H-NMR ($CDCl_3$, δ p.p.m.): 1.9 (dd, 3H, $C\underline{H}_3$, *J*=2.57 Hz, 6.62 Hz); 4.10 (m, 1H, C$\underline{H}$N); 5.05 (m, 1H, C$\underline{H}$P); 5.70 (m, 1H, C$\underline{H}$P); 7.50 (m, 13H, Ar$\underline{H}$); 7.90 (m, 6H, Ar$\underline{H}$). EP MS *m/z* (450.0, $[M\text{-}Br]^+$).

(S,S)-[3-Methyl-2-(phthaloylamino)pentyl]triphenylphosphonium bromide ***(2c)***. Yield 62%. M.p. 128–130°C. $[\alpha]_D^{25}$=+50.9 (*c*=1.1, EtOH). ^{1}H-NMR ($CDCl_3$, δ p.p.m.): 0.85 (t, 3H, $CH_2C\underline{H}_3$, *J*=6 Hz); 1.15 (d, 3H, C$\underline{H}$3CH, *J*=6 Hz); 1.55 (m, 2H, $C\underline{H}_2CH_3$); 4.35 (m, 1H, CHN); 4.57 (m, 1H, C$\underline{H}$P); 4.87 (t, 1H, C$\underline{H}$P, *J*=15 Hz); 7.55 (m, 11H, Ar$\underline{H}$); 7.70 (m, 2H, Ar$\underline{H}$); 7.80 (m, 6H, Ar$\underline{H}$). FAB MS *m/z* (492, M^+).

(S)-[4-Methyl-2-(phthaloylamino)pentyl]triphenylphosphonium bromide ***(2d)***. Yield 61%. M.p. 130–132°C. $[\alpha]_D^{25}$=+13.5 (*c*=2.6, EtOH). ^{1}H-NMR ($CDCl_3$, δ p.p.m.): 1.10 (d, 3H, $C\underline{H}_3$, *J*=6 Hz). 1.20 (d, 3H, $C\underline{H}_3$, *J*=6 Hz); 1.65 (m, 1H, $C\underline{H}(CH_3)_2$); 2.6 (m, 2H, $C\underline{H}_2CH(CH_3)_2$). 4.5 (m, 1H, C$\underline{H}$N); 5.25 (m, 1H, CHP); 5.75 (t, 1H, C$\underline{H}$P, *J*=15 Hz). 7.90 (m, 13H, Ar$\underline{H}$); 8.10 (m, 6H, Ar$\underline{H}$). FAB MS *m/z* (492, M^+).

(S)-[3-Phenyl-2-(phthaloylamino)propyl]triphenylphosphonium bromide ***(2e)***. Yield 56%. M.p. 144–146°C. $[\alpha]_D^{25}$=26.5 (*c*=3.5, EtOH). ^{1}H-NMR ($CDCl_3$, δ p.p.m.): 3.5 (dd, 1H, $C\underline{H}C_6H_5$, *J*=9 Hz, 15 Hz). 4.0 (dd, 1H, $C\underline{H}C_6H_5$, *J*=6 Hz, 15 Hz); 4.10 (m, 1H, C$\underline{H}$N); 5.00 (m, 1H, C$\underline{H}$P); 6.70 (t, 1H, C$\underline{H}$P, *J*=14.7 Hz); 7.00 (m, 5H, $CH_2C_6\underline{H}_5$); 7.50 (m, 13H, Ar$\underline{H}$); 7.80 (m, 6H, Ar$\underline{H}$). FAB MS *m/z* (526, M^+).

Cytotoxicity essays

Cell line and drugs. The human chronic myeloid leukemia (CML) K562 cell line was used to evaluate the cytotoxicity of compounds **2**. The cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Life Technology, Cergy Pontoise, France), 2 mM L-glutamine, 100 IU/ml penicillin G and 100 ml/ml streptomycin in 5% humidified CO_2 in air at 37°C. A 10 mM stock solution of each compound was prepared in water or in ethanol/water mixture. Drug solutions were obtained by appropriate dilution with culture medium. The final concentration in ethanol never exceeded 0.1%. Exponentially growing cell cultures were exposed to the medium

containing the tested compounds in the concentration range of 10 μM to 1 nM for 3 days. Cell growth and cell viability were assessed by phase contrast microscopy using a hemocytometer and the Trypan blue exclusion methodology respectively.

Cell proliferation and erythroid differentiation studies Some compounds (antitumor drugs, chemical or physiological molecules) are able to induce the erythroid differentiation program of the K562 cell line and to activate hemoglobin synthesis. The hemoglobin cell content was quantified by a benzidine staining method as previously described.[8] Each experiment was repeated at least twice and the data represents the average.

Results and discussion

Compounds **1b–e** were obtained in satisfactory yields (68–81%) from fusion of phthalic anhydride with alcohols (Scheme 1). *N*-(2-hydroxyethyl)phthalimid **1a** is commercially available (Sigma-Aldrich, St Quentin Fallavier, France).

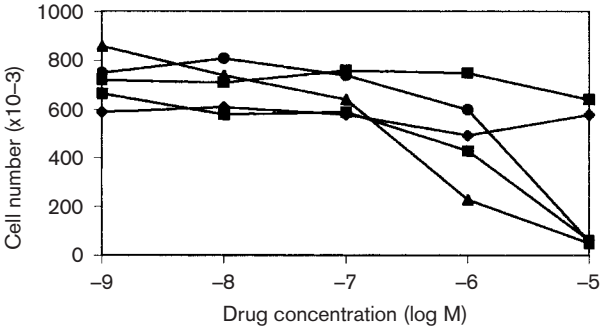


Figure 1. Cytotoxicity versus concentration of **2a** (◆), **b** (■), **c** (▲), **d** (■) and **e** (●).

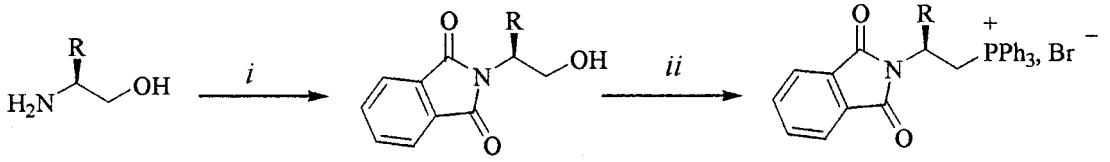
Treatment of halides derived from alcohols with triphenylphosphine is the most common method for the preparation of phosphonium salts. However, all attempts for the synthesis of **2** by this method failed. Consequently, we planned to use triphenylphosphonium hydrobromide PPh_3. HBr in order to obtain phosphonium salts directly from alcohols as described for alkyl alcohols.[9] Thus, the phosphonium salts **2a–e** were successfully prepared in 56–74% yield by fusion of triphenylphosphonium hydrobromide with *N,N*-phthaloylaminoalcohols **1** at 160°C for 20 h in a sealed tube. The use of a sealed tube is essential. Under air atmosphere, lower yields were obtained.

The biological activity of compounds **2** was evaluated using human erythroleukemic K562 cells. Given the IC_{50} value (about 20 nM) of doxorubicin, a well-known cytotoxic compound, we chose to test them at increasing concentrations from 10^{-5} to 10^{-9} M for 3 days. The results are illustrated in Figure 1. We noted that compounds **2c–e** exhibited similar cytotoxic profiles. Cell growth decreased from 0.1 μM onwards and this inhibition reached 100% at the concentration of 10 μM. IC_{50} values were respectively of 0.5 (**2c**), 1.5 (**2d**) and 3.2 (**2e**) μM. No cell growth inhibition was observed for compounds **2a** and **2b**, even at the concentration of 10 μM.

We then evaluated the hemoglobinization content of the K562 cells after treatment with compounds **2c–e** at different inhibiting concentrations. The percentage of hemoglobinized cells never exceeded 9 versus 50% for doxorubicin at 40 nM.[10]

It was reported that compound **2a** possesses substantial antileukemic activity in the P-388 cell line.[1] This compound was found inactive in the K562 cell line, but it is often difficult to establish relationships between the cytotoxic potencies towards two different cell lines.[11]

An increase in growth inhibitory activity against P-388 cells with the size of the substituent or



1a R = H
1b R = CH_3
1c R = -$CH(CH_3)C_2H_5$
1d R = -$CH_2CH(CH_3)_2$
1e R = -$CH_2C_6H_5$

2a-e

Scheme 1. *i*: phthalic anhydride, 1.2 eq, 150°C, 1 h, 68–81% yield. *ii*: PPh_3.HBr, 1.2 eq, 160°C, 20 h, 56–74% yield.

lipophilicity was described for phosphonium salts.[12] We noted a similar effect about compounds **2**. **2c–e** exhibited a higher activity than the unsubstituted parent **2a** and than the methyl substituted compound **2b**. Furthermore, it seems that the bulkier substituant R, the greater the cytotoxic activity (**2c–d** versus **2e**).

Conclusion

Among the tested compounds, three of them exhibited cytotoxic activity in the K562 cell line, with IC_{50} values of about 1 μM. There are less active than the reference compound, doxorubicin, a drug commonly used in clinical anticancer protocols, but these preliminary results indicate the potential of substituted phthalimidoalkyltriphenylphosphonium derivatives as cytotoxic agents.

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