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Synthesis and anticancer activity of 5′-phthaloylnucleosides

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A series of novel 5′-phthaloylnucleosides were synthesized via Mitsunobu reaction starting from AZT or 2′-deoxyadenosine and numerous phthalimides and their sulphur analogues-thioimides. Some of them showed significant anticancer activity.

1. Introduction

Phthalimide moieties are present in numerous biologically active compounds. For example, adamantyl ester imides containing amino acids as *N*-substituents exhibit strong antibacterial activity [1–3]. Thalidomides with *N*-phenyl and *N*-benzylphthalimide skeletons, as well as simple halogenated phthalimides have shown bi-directional TNF-α production-regulating activity [4–10]. Also, thioimide derivatives influenced TNF-α production significantly. Naphthalimides such as amonafide and mitonafide, exhibit substantial anticancer activities in various animal tumors [11]. Dendric imides with *N*-polyamine tails have been synthesized and evaluated as antitumor agents [12]. Recently it has been found that similar imides with a 2-chloroethyl group have anticancer properties [11]. 3′-Azido-2′,3′-dideoxythymidine (AZT) and its derivatives are mainly used to treat patients with HIV infections [13–15]. However, AZT shows many limitations in clinical practica and then there are numerous reasons to use AZT prodrugs. Most prodrugs of AZT have been prepared by derivatization at 5′-*O*-position, but there is no available data for 5′-*N*-derivatives. On the other hand, it has been found that phthalimide-derived nucleoside analogs can bind a CG base pair via specific hydrogen bonds [16]. So, the combination of imide and nucleoside structures seems to be interesting from a pharmaceutical point of view. For this reason we have decided to synthesize a series of new 5′-*N*-phthaloylderivatives of some nucleosides and to test them as anticancer agents. Also, for comparison, we have prepared and evaluated numerous nucleoside free ester imides with bioactive *N*-substituents like 2-chloroethyl and 2-(diethylamino)ethyl.

2. Investigations, results and discussion

2.1. Synthesis of 5′-phthaloylnucleosides and imides

The synthetic pathway to 5′-phthaloylnucleosides (Scheme 1) is based upon the Mitsunobu reaction [17, 18].

The starting imides, as well as other reagents were commercial products, but thioimides **1h** and **1j** were synthesized using Lawesson's reagent according to our previous publication [19]. For the synthesis we have chosen those phthalimides which have been used in the previous experiments and showed the strongest biological activity. Particularly, halogenated phthalimides seem to be very promising moieties in such compounds. Adamantyl ester imides **1a**, **b,** due to their hydrophobic, cage-like structure, have been used to enhance lipophilicity of nucleoside derivatives. As nucleoside core we have used AZT (**2**) as well as 2′-deoxyadenosine (**3**) due to their strong cytotoxicity.

The compounds **5a–d** and **6** were synthesized and identified according to the method described previously [1–3, 20].

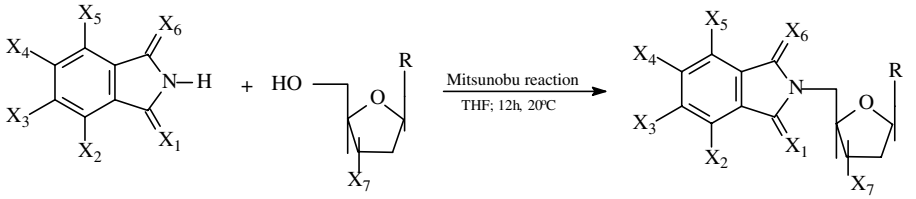
The yields, melting points and ^{1}H NMR data for **4**, **5** and **6** are given in Tables 1 and 2. Analyses indicated by symbols were within ±0.4% of theoretical values.

2.2. Cytotoxicity against normal and malignant human cells

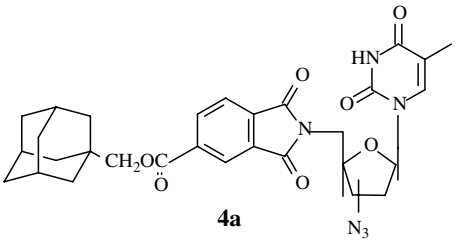
The toxicity of the compounds was tested, in addition to normal activated polyclonal T cells, against three hematopoietic malignancies and two cell lines representing the most common forms of human cancer. A 80% or stronger growth inhibition against at least one cell type was achieved with 5 out of 18 tested compounds i.e. **4e, f, h, m** and **6**.

The results in Tables 3a and 3b demonstrate the sensitivity of individual cell types. No cell-type selectivity could be demonstrated, although normal polyclonal activated T cells tended to be more resistant than cancer cell lines. Owing to a similar response of all test cells, some compounds were screened only with U-937 cells in a concentration of 25 μg/ml. The compounds were **4b, c, i, j, k, l**, **5a, b** and no significant inhibition (>20%) was observed.

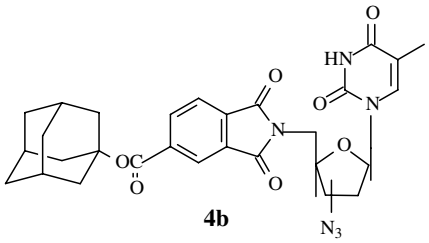
Scheme 1



1a-k **2: Thy 3: Ade** **4a-m**

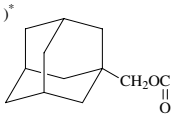


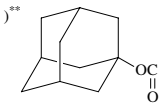
4a



4b

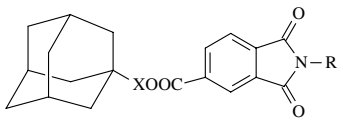
4	X_1	X_2	X_3	X_4	X_5	X_6	X_7	R
a	O	H	)*	H	H	O	N_3	Thy
b	O	H	)**	H	H	O	N_3	Thy
c	O	H	Cl	Cl	H	O	N_3	Thy
d	O	Cl	Cl	Cl	Cl	O	N_3	Thy
e	O	F	F	F	F	O	N_3	Thy
f	O	Br	Br	Br	Br	O	N_3	Thy
g	O	NO_2	H	H	H	O	N_3	Thy
h	S	H	H	H	H	S	N_3	Thy
i	O	H	NO_2	H	H	O	N_3	Thy
j	S	H	H	H	H	O	N_3	Thy
k	O	H	H	H	H	O	N_3	Thy
l	S	H	H	H	H	S	OH	Ade
m	O	H	Cl	Cl	H	O	OH	Ade





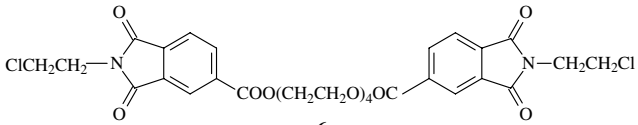
The most biotransformations of nucleoside agents occur by phosphorylation to 5′-*O*-monophosphate. For 5′-esters this process could be easily achieved after hydrolytic removal of ester group. Contrary to ester linkages, N–C bonds in cycling imides are more chemically and biologically stable. It explains why the cytotoxicity of nucleoside derivatives strongly depends on the structure of the 5′-phthaloyl groups (see Tables 3a, b). On the other hand *N*-substituents in phthalimide moieties also play an important role. Compounds **5a, b** are completely inactive while **5c, d** and **6** show moderate anticancer effect.

Scheme 2



5a–d

Compd. 5	X	R
a	–	$CH_2CH_2N(C_2H_5)_2$
b	CH_2	$CH_2CH_2N(C_2H_5)_2$
c	–	CH_2CH_2Cl
d	CH_2	CH_2CH_2Cl



6

3. Experimental

All chemicals used were analytical-grade commercial products (Aldrich) and were used without any further purification. FTIR spectra were recorded on a Perkin Elmer 2000 spectrometer in CH_2Cl_2. ^{1}H NMR spectra were performed on a Gemini Varian 200 in $CDCl_3$. Melting points were taken in open capillary tubes on a Gallenkamp 5 apparatus and were uncorrected.

3.1. Synthesis of 5′-phthaloylnucleosides 4a–m

Typical procedure for the synthesis of 5′-phthaloylnucleosides: a THF solution of mixture of respective nucleoside (10 mmol), imides **1a–k** (10 mmol), triphenyl phosphine (10 mmol) and diethyl azodicarboxylate (10 mmol) was stirred in room temperature for 12 h. Then THF was evaporated and solid crude product was purified by means of flash-column chromatography (silica gel 60; 230–400 mesh) using as an eluent ethyl acetate/hexane (2 : 1) system and crystallized from ethyl acetate. The yields, melting points and ^{1}H NMR data are given in Table 1. The structures were confirmed also using FT-IR spectroscopy. For example, spectroscopic data for **4k**: FT-IR (KBr): 3690 (NH), 2105 (CN), 1769 (C=O), 1703 (C=O), 1606 (C=N) cm^{-1}.

3.2. Synthesis of imide derivatives 5a–d and 6

The synthesis of imide derivatives were performed according to the method described previously [1–3]. As starting reagents trimellitic anhydride and proper amines (2-chloroethyl amine or 2-(diethylamino)ethyl amine) were used. Then, imides obtained were condensed with adamantoles (for **5a–d**) or tetraethylene glycol (for **6**) using DCC solution in CH_2Cl_2. The crude products were crystallized from an ethanol/H_2O system.

3.3. Cytotoxicity tests

Toxicity of the compounds was determined by their effects on protein synthesis (^{14}C-L-leucine incorporation). The cell lines IM-9, MOLT-3 and U-937 were obtained from the American Type Culture Collection. MCF-7 and PC-3 lines were a generous gift from Dr. Jorma Isola (Department of Cancer Biology, University of Tampere). Mononuclear cells for phytohemagglutinin stimulation were obtained from healthy donors. These cells

Table 1: Analytical data of 5′-phthaloylnucleosides (4)

Compd. 4	^{1}H NMR data (DMSO d_6), δ	Yield (%)	M.p. (°C)
a	1.25–2.55 (m, 15 $H_{adam.}$), 1.72 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4,22 (m, 1 H, CHO), 5.99 (t, CH), 6.63 (s, 1 H, CH), 8.05–8.70 (m, 3 $H_{ar.}$), 11.30 (s, NH)	65	135
b	1.25–2.65 (m, 15 $H_{adam.}$), 1.66 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.97 (m, 2 H, CH_2N), 4,22 (m, 1 H, CHO), 6.05 (t, CH), 7.46 (s, 1 H, CH), 8.07–8.44 (m, 3 $H_{ar.}$), 11.31 (s, NH)	44	162
c	1.57 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4,46 (m, 1 H, CHO), 6.05 (t, CH), 7.45 (s, 1 H, CH), 8.05–8.70 (m, 3 $H_{ar.}$), 11.25 (s, NH)	45	165
d	1.70 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 26 (m, 1 H, CHO), 6.02 (t, CH), 7.45 (s, 1 H, CH), 11.29 (s, NH)	34	185
e	1.25 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 22 (m, 1 H, CHO), 6.05 (t, CH), 7.43 (s, 1 H, CH), 11.29 (s, NH)	25	245
f	1.68 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.05 (t, CH), 7.45 (s, 1 H, CH), 11.29 (s, NH)	32	240
g	1.57 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.05 (t, CH), 7.45 (s, 1 H, CH), 8.05–8.55 (m, 3 $H_{ar.}$), 11.25 (s, NH)	33	226
h	1.60 (s, 3 H, CH_3), 2.44 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 89 (m, 1 H, CHO), 6.02 (t, CH), 7.45 (s, 1 H, CH), 7.64–8.35 (m, 4$H_{ar.}$), 11.29 (s, NH)	35	160
i	1.57 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.05 (t, CH), 7.45 (s, 1 H, CH), 8.05–8.70 (m, 3 $H_{ar.}$), 11.25 (s, NH)	41	112
j	1.57 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 04 (m, 1 H, CHO), 6.03 (t, CH), 7.45 (s, 1 H, CH), 7.50–8.98 (m, 4$H_{ar.}$), 11.31 (s, NH)	22	205
k	1.66 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.05 (t, 1 H, CH), 7.45 (s, 1 H, CH), 7.56–7.92 (m, 4$H_{ar.}$), 11.29 (s, NH)	45	240
l	1.59 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.08 (t, CH), 7.45 (s, 1 H, CH), 8.05–8.88 (m, 4$H_{ar.}$)	23	220
m	1.57 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 2.58 (m, 1 H, CH–CN), 3.98 (m, 2 H, CH_2N), 4, 46 (m, 1 H, CHO), 6.05 (t, CH), 7.45 (s, 1 H, CH), 8.05–8.70 (m, 2 $H_{ar.}$)	21	200

Table 2: Analytical data of imides 5a–d and 6

Comp.	^{1}H NMR data (DMSO d_6), δ	Yield (%)	M.p. (°C)
5a	0.96 (t, 6 H, CH_3), 1.58–2.69 (m, 15 $H_{adam.}$), 2.52 (q, 4 H, CH_2), 2.70 (t, 2 H, CH_2), 3.78 (t, 2 H, NCH_2), 7.85–7.98 (m, 3 $H_{arom.}$)	39	132
5b	0.96 (t, 6 H, CH_3), 1.58–2.69 (m, 15 $H_{adam.}$), 2.52 (q, 4 H, CH_2), 2.70 (t, 2 H, CH_2), 3.55 (s, 2 H, CH_2O) 3.78 (t, 2 H, NCH_2), 7.85–7.98 (m, 3 $H_{arom.}$)	55	145
5c	1.67–2.51 (m, 15 $H_{adam.}$), 3.85–3.94 (m, 4 H, CH_2CH_2), 8.00–8.30 (m, 3 $H_{arom.}$)	42	138
5d	1.61–2.58 (m, 15 $H_{adam.}$), 3.31 (s, 2 H, CH_2), 3.85–3.95 (m, 4 H, CH_2CH_2), 8.00–8.30 (m, 3 $H_{arom.}$)	41	149
6	1.89–2.88 (m, 8 H, CH_2O), 3.87–3.99 (m, 8 H, CH_2CH_2Cl), 8.03–8.37 (m, 6 $H_{arom.}$)	35	60

Table 3a: The best results of inhibition (ID_{80})[a] of cell growth by compounds tested

Compd.	ID_{80}[a] (μg/mL)					
	Cell types investigated					
	Phytohemagglutinin stimulated human lymphocytes	IM-9 Multiple myeloma	MOLT-3 T-cell acute lymphoblastic leukemia	U-937 Monocytic leukemia	MCF-7 Breast carcinoma	PC-3 Prostate carcinoma
4a	>25[b]	>25[c]	>25[b]	>25[c]	>25[b]	>25[c]
4d	>25[c]	>25[c]	>25[b]	>25[c]	>25[c]	>25[c]
4e	20	18	18	>25[c]	>25[b]	>25
4f	>25[c]	19	18	18	14	15
4g	>25[c]	>25[c]	>25[c]	>25[c]	>25[c]	>25[b]
4h	>25	18	16	18	17	16
4m	>25[b]	>25[c]	>25[b]	>25[b]	>25[b]	21
5c	>25[c]	>25[c]	>25[b]	>25[b]	>25[b]	>25[c]
5d	>25[c]	>25[c]	>25[c]	>25[b]	>25[b]	>25[c]
6	>25[c]	>25[b]	20	>25[b]	>25[b]	>25[b]

[a] ID_{80} is the concentration (μg/ml) of compound which caused a 80% decrease in [^{14}C]-leucine incorporation by the cells in the culture. Values were calculated from dose-response curves done in duplicate for each compound.
[b] The ID_{80} was not achieved by 25 μg/mL but a significant inhibition was observed with this concentration – see Table 3b.
[c] no significant inhibition (>20%) was observed.

Table 3b: The moderate results of inhibition (percent of inhibition at a concentration of 25 μg/mL) of cell growth by compounds tested

Compd.	Percent of inhibition at a concentration of 25 μg/mL of compound tested					
	Cell types investigated					
	Phytohemagglutinin stimulated human lymphocytes	IM-9 Multiple myeloma	MOLT-3 T-cell acute lymphoblastic leukemia	U-937 Monocytic leukemia	MCF-7 Breast carcinoma	PC-3 Prostate carcinoma
4a	26	–[a]	30	–	38	–
4d	–	–	74	58	–	–
4e	–	–	–	–	35	–
4f	–	–	–	–	–	–
4g	–	–	–	–	–	32
4h	–	–	–	–	–	–
4m	52	–	26	66	79	–
5c	–	–	25	21	50	–
5d	–	–	–	22	33	–
6	–	32	–	55	76	77

[a] see Table 3a

represent mainly activated normal human polyclonal T lymphocytes. Test compounds were added to duplicate suspension cultures in 96-well microplates containing 2×10^4 cells (IM-9, MOLT-3, U-937) or 10^5 peripheral blood mononuclear cells (for phytohemagglutinin stimulation) per a 200 μl-well. These cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, in humidified atmosphere containing 5% CO_2 at 37 °C. The adherent cell cultures (MCF-7 and PC-3) were initiated by splitting 1/5 of the corresponding confluent master culture. The cells were grown in microplates in the presence of the test compounds. MCF-7 cells were grown in Eagle's MEM medium and PC-3 cells in Ham's F-12 medium. After 3 days of culture, ^{14}C-L-leucine (0.5 μCi/ml, specific activity 1.3 μCi/mmol) was added and the cells were incubated for another 24 h. After incubation, the proteins were precipitated with 0.2 N $HClO_4$, and collected on glass fibre filters using a multiple cell harvester (Wallac, Turku, Finland). The radioactivity incorporated into proteins was measured in a scintillation counter (LKB-Wallac 1410, Turku, Finland). The growth inhibition was determined from dose-response curves representing 5 different concentrations of the test compounds. Some compounds were first screened with a single dose of 25 μg/ml using only U-937 cells. If an 80% growth inhibition was not achieved, other cell types were not tested. We have shown in a number of experiments that there is a good correlation between the leucine incorporation and the number of living cells in 4-day cultures like this [21].

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References

1 Orzeszko, A.; Gralewska, R.; Starościak, B. J.; Kazimierczuk, Z.: Acta Biochim. Pol. **47**, 87 (2000)
2 Orzeszko, A.; Kamińska, B.; Orzeszko, G.; Starościak, B. J.: Farmaco **55**, 619 (2000)
3 Orzeszko, A.; Kamińska, B.; Starościak, B. J.: Farmaco **57**, 619 (2002)
4 Hashimoto, Y.: Curr. Med. Chem. **5**, 163 (1998)
5 Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y.: J. Med. Chem. **40**, 2858 (1997)
6 Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S.: Biochem. Biophisical Res. Commm. **205**, 1992 (1994)
7 Miyachi, H.; Ogasawara, A.; Azuma, A.; Hashimoto, Y.: Bioorg. Med. Chem. **5**, 2095 (1997)
8 Shibata, Y.; Shichita, M.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S.: Chem. Pharm. Bull. **43**, 177 (1995)
9 Collin, X.; Robert, J.-M.; Wielgosz, G.; Le Baut, G.; Bobin-Dubigeon, C.; Grimaud, N.; Petit, J-Y.: Eur. J. Med. Chem. **36**, 639 (2001)
10 Gütshow, M.; Hecker, T.; Thiele, A.; Hauschildt, S.; Eger, K.: Bioorg. Med. Chem. **9**, 1059 (2001)
11 Samanta, S.; Pain, A.; Dutta, S.; Sanyal, U.: Acta Polon. Pharm.-Drug Res. **58**, 351 (2001)
12 Braña, M. F.; Dominguez, G.; Sáez, B.; Romerdahl, C.; Robinson, S.; Barlozzari, T.: Bioorg. Med. Chem. Lett. **11**, 3027 (2001)
13 Parang, K.; Wiebe, L. I.; Knaus, E. E.: Curr. Med. Chem. **7**, 995 (2000)
14 De Clercq, E. J.: Antimocrob. Chemother. **23**, Suppl. A., 35 (1989)
15 Hardewijn, P.; Pauwels, R.; Baba, M.; Balzarini, J.; De Clercq, E.: J. Med. Chem. **30**, 2131 (1987)
16 Lengeler, D.; Weisz, K.: Tetrahedron Lett. **42**, 1479 (2001)
17 Mitsunobu, O.: Synthesis 1 (1981)
18 Mitsunobu, O.; Takizawa, S.; Morimato, H.: J. Am. Chem. Soc. **98**, 7858 (1976)
19 Orzeszko, A.; Maurin, J. K.; Melon-Ksyta, D. Z.: Naturforsch. **56b**, 1035 (2001)
20 Śledzińska, I.; Białecka-Florjańczyk, E.; Orzeszko, A.: Eur. Polym. J. **32**, 1345 (1996)
21 Kievekäs, I.; Vilpo, L.; Vilpo, J.: Leukemia Res. in press