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Tumor necrosis factor-alpha production-regulating activity of phthalimide derivatives in genetically modified murine melanoma cells B78H1

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Abstract

The effect of imides, monothioimides, trimellitimides, as well as 5′-deoxy-5′-phthaloylamino-derivatives of azidothymidine on tumor necrosis factor-alpha (TNF-α) production by genetically modified murine B78H1 melanoma cells transduced with the gene for human TNF-α (B78/TNF) was investigated. It was found that *N*-(adamant-1-yl)monothiophthalimide (**1e**) and *N*-(adamant-2-yl)-monothiophthalimide (**1f**) showed over 200% enhancing of TNF-α production while some of imides were inhibitors.

Keywords: Imide; Thioimide; TNF-α

1. Introduction

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine produced by numerous cell types among which monocytes/macrophages play a major role. TNF-α exerts its biological activity by binding to TNF-RI (p55) or TNF-RII (p75) receptors and activating several signaling pathways. TNF-RI mediates mainly cell death (proapoptotic) signals, whereas TNF-RII primary induces cell growth and survival pathways [1]. Investigation of the biological properties of TNF-α, in vitro as well as in vivo models, has revealed that this cytokine has both beneficial and unfavorable effects [2,3]. The beneficial effects include direct antitumor activity because it exhibits cytotoxicity selectively against various tumor cells. On the other hand, the overproduction of TNF-α has been strongly implicated in the pathogenesis of such diseases as septic shock, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis [4]. These pleiotropic effects of TNF-α indicate that TNF-α production enhancers in some cases and production inhibitors in other cases would be useful as biological response modifiers under various circumstances. In this context, it is interesting to search for new drugs working as stimulators or inhibitors of TNF-α production.

Usually, for such biological evaluations human leukaemia cell lines (HL-60, K592, U937) are used [2,3,5]. These cells do not secrete TNF-α without stimulation, but they are beginning to produce the cytokine in response to stimulators including two types of tumor promotors 12-*O*-tetradecanoylphorbol-13-acetate or okadaic acid. It was found that many chemicals exert TNF-α production-enhancing activity and production-inhibiting activity in such systems. There are two main groups of compounds showing TNF-α production-regulating properties including ‘thalidomide like’ phthalimide derivatives and aminoheterocycles [2–8]. Recently, also we have found that some of imide derivatives caring adamantane or nucleoside moieties were biologically active as antibacterial and anticancer agents [9–12]. Now, we would like to check an influence of these and similar compounds on TNF-α production.

Recently, we have presented the studies on cultures of genetically modified mouse melanoma cells B78H1

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secreting TNF-α at a constant rate without stimulators (B78/TNF) and by this reason useful for testing the effects of various chemicals on TNF-α production. We have found that some adamantylaminopyridines enhance TNF-α production several times in B78/TNF melanoma cells [7,8].

In the presented paper we have synthesized a series of imides, thioimides, trimellitimides as well as 5′-deoxy-5′-phthaloylaminoderivatives of azidothymidine (AZT). Then, these compounds were studied as TNF-α production-regulating agents.

2. Experimental

2.1. General methods

Melting points (m.p.) were taken in open capillary tubes on a Gallenkamp 5 m.p. apparatus and were uncorrected. The structures of products were confirmed by elemental analysis, FTIR and ^{1}H NMR spectroscopy. The NMR spectra were measured on a Varian Gemini 200 MHz spectrometer in $CDCl_3$ solutions. FTIR spectra were recorded on a Perkin Elmer 2000 apparatus using KBr pellet method. Analyses indicated by symbols were within ±0.4% of theoretical values.

2.2. General procedure for the synthesis of imides *1a–d*

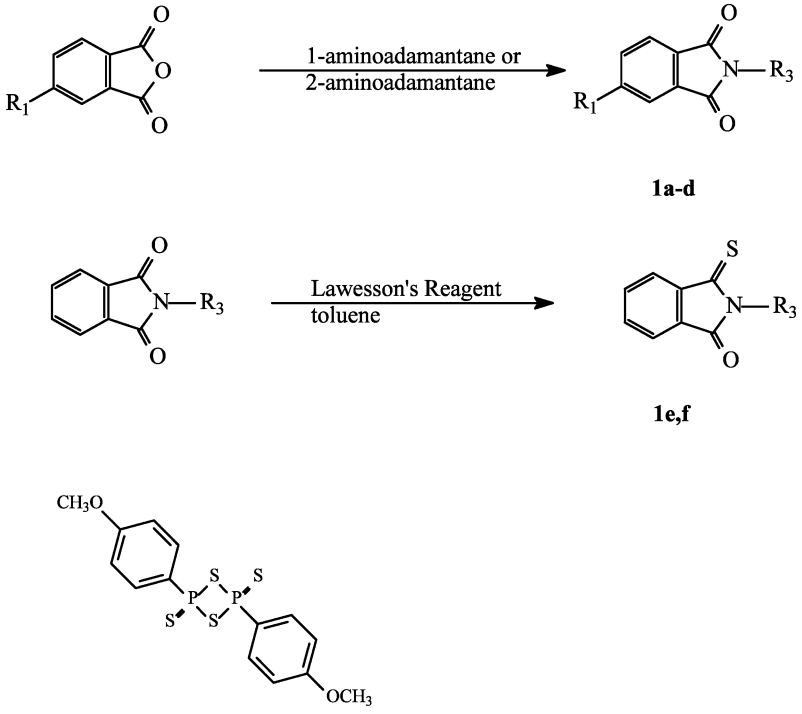
The synthesis of *N*-adamantyl substituted phthalimides and trimellitimides (see Scheme 1) were performed according to the well-known method from proper anhydrides and 1-aminoadamantane or 2-aminoadamantane in boiling DMF, respectively [9]. The crude products were crystallized from ethanol/water (70:30) system.

2.3. General procedure for the synthesis of monothioimides *1e,f*

Both monothioimides were synthesized from proper imides using Lawesson's reagent in boiling toluene according to the procedure described previously [13]. Detailed spectroscopic and crystal data for these compounds were given also in cited publication (Table 1, Scheme 1).

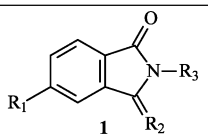
2.4. General procedure for the synthesis of imides *2a–f*

2-Substituted 1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid adamantan-1-ylmethyl esters **2a–f** were the same as described in our previous papers [9–11]. In order to achieve better solubility in water, we have converted these compounds into sodium salts using cation exchange cellulose Cellex CM according to the standard procedure (see Scheme 2).

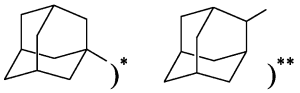


Scheme 1.

Table 1
Synthesis of *N*-substituted imides and thioimides **1a–f** studied



1	R_1	R_2	R_3	M.p. (°C)	Yield (%)
a	H	O	)*	140	45
b	COOH	O	)*	264	53
c	H	O	)**	103	55
d	COOH	O	)**	230	62
e	H	S	)*	151	25
f	H	S	)**	123	22



2.5. The synthesis of imides *2g,h*

2.5.1. *N-(2-chloroethyl)trimellitimide*

A total of 1.92 g (10 mmol) trimellitic anhydride, 1.16 g of 2-chloroethylamine hydrochloride (10 mmol) and 2.02 g (20 mmol) of triethylamine were dissolved in 20 cm^3 of dry DMF and were refluxed over 3 h. Then, mixture was purred into aqueous 5% HCl. Crude product was crystallized from ethanol. M.p. 151 °C; yields 60%.

FTIR (in cm^{-1}): 3300–2500 OH_{acid}, 1780 $C{=}O_{imide}$, 1725 $C{=}O_{imide,acid}$.

2.5.2. *2-(2-chloroethyl)-1,3-dioxo-2,3-dihydro-1H-isoindole-5-carboxylic acid adamantan-1-ylmethyl ester (**2g**) and 2-(2-chloroethyl)-1,3-dioxo-2,3-dihydro-1H-isoindole-5-carboxylic acid adamantan-1-yl ester (**2h**)*

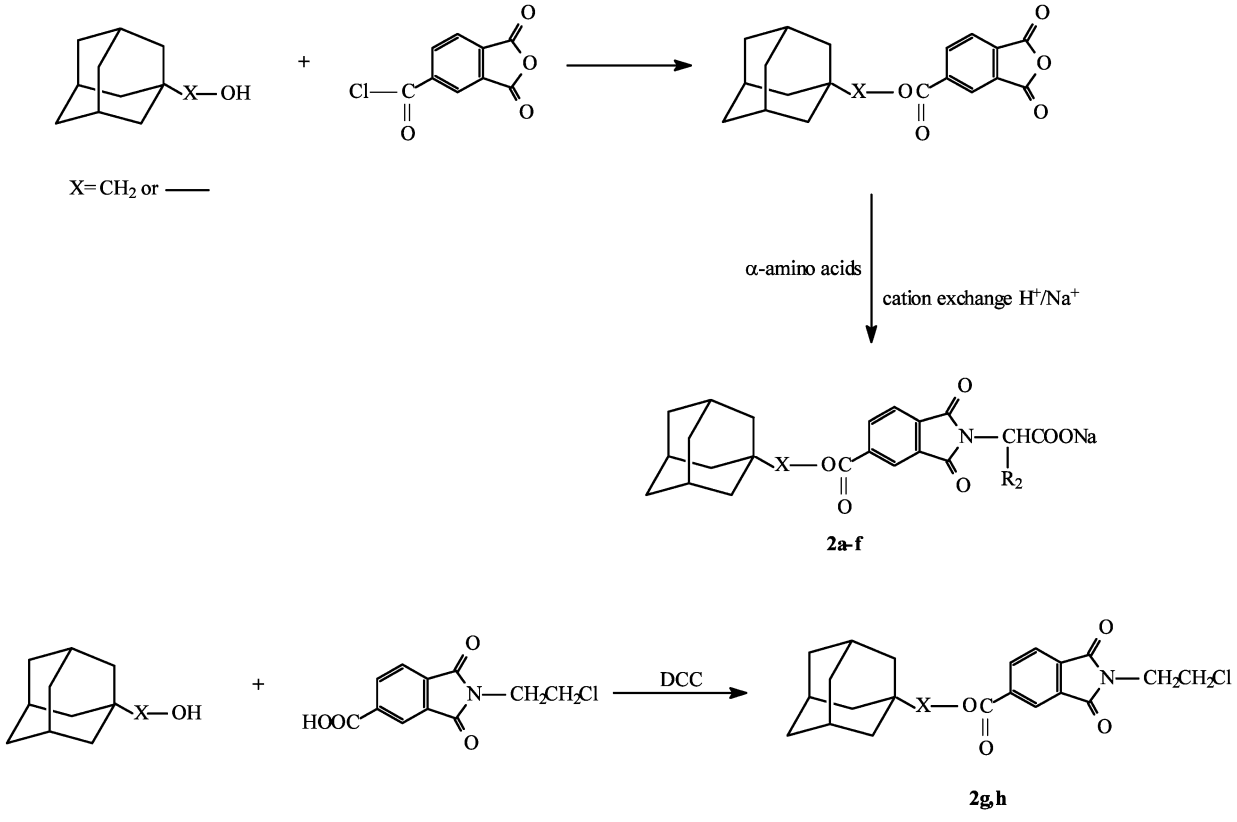
A total of 2.54 g (10 mmol) of *N*-(2-chloroethyl)trimellitimide and 10 mmol of 1-adamantanemethanol or 1-adamantanol, respectively, were dissolved in dry methylene chloride. Next, 2.06 g (10 mmol) of DCC was added. The mixture was stirred over 6 h. Then, after filtration, the solvent was evaporated and crude products were crystallized from ethanol/H_2O system. Representative spectroscopic data are reported for **2h**.

^1H NMR (in ppm): 1.22–2.50 (m, $15H_{adam.}$), 3.86 (m, 2H), 3.93 (m, 2H), 8.00–8.31 (m, 3H).

FTIR (in cm^{-1}): 2918, 2856 ($CH_{adam.}$), 1779 ($C{=}O_{imide}$), 1725 ($C{=}O_{imide,ester}$) (Table 2, Scheme 2).

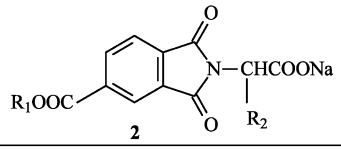
2.6. Synthesis of AZT derivatives *3a–c*

The synthesis of 2-[3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-tetrahydrofuran-2-yl-

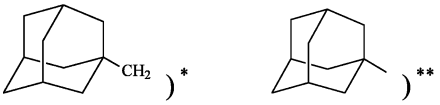


Scheme 2.

Table 2
Synthesis of *N*-substituted trimellitimides **2a–h** studied [9–11]



2	R_1	R_2	M.p.(°C)	Yield (%)
a	)*	H	decomp.	34
b	)*	CH_3	decomp.	44
c	)*	$CH_2C_6H_5$	decomp.	32
d	)*	$CH_2(CH_3)_2$	decomp.	45
e	)*	$(CH_2)_5$	decomp.	43
f	)*	$CH_2CH_2SCH_3$	decomp.	23
g	)*	CH_2CH_2Cl	149	67
h	)**	CH_2CH_2Cl	138	66

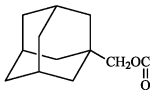


methyl]-1,3-dioxo-1*H*-isoindole derivatives **3a,b** and 2-[3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-tetrahydrofuran-2-yl-methyl]-3-thioxo-2,3-dihydroisoindol-1-one **3c** were perform according to the general synthetic pathway via Mitsunobu reaction given in Scheme 3, [14]. A THF solution of mixture of 3′-AZT (10 mmol), proper imides (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 products were purified by means of flash-column chromatography (SiO_2) using ethyl acetate/hexane (2:1) as an eluent system. The structures were confirmed also using ^1H NMR and FTIR spectroscopy. For example, spectroscopic data for **3b**:

Table 3
Synthesis of AZT derivatives [12]

3	X_1	X_2	X_3	X_4	X_5	X_6	M.p. (°C)	Yield (%)
a	O	H	[a]	H	H	O	135	65
b	O	Br	Br	Br	Br	O	240	32
c	S	H	H	H	H	S	160	35

[a]



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^1H NMR (in ppm): 1.68 (s, 3H, CH_3), 2.37 (m, 2H, H-2′), 2.58 (m, 1H, H-3′), 3.98 (m, 2H, H-5′), 4,46 (m, 1H, H-4′), 6.05 (t, 1H, H-1′), 7.45 (s, 1H, 6-H), 11.29 (s, 1H, NH).

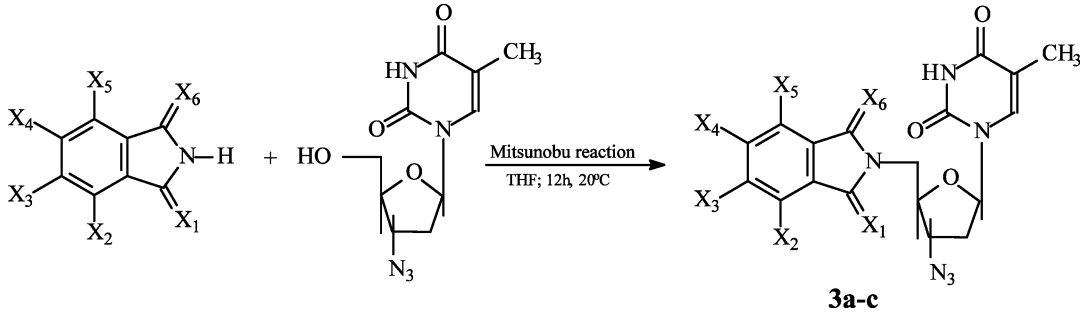
FTIR (in cm^{-1}): 3690 (NH), 2105 (CN), 1769 (C=O), 1703(C=O), 1606 (C=C) (Table 3, Scheme 3).

2.7. Biological evaluation

The ability of the imide derivatives to stimulate TNF-α production described above was studied in cultures of B78H1 murine melanoma cells that had been transduced with the gene for human TNF-α (clone 9, hereafter named B78/TNF) [14]. The compounds were incubated with melanoma cells (2×10^5 cells/cm^3) for 24 h as described elsewhere [7]. The concentration of TNF-α in culture supernatant was measured using an enzyme-linked immunosorbent assay. The concentration of compounds tested was 100 μM. TNF-α stimulatory activity of imide compounds were expressed as percent of TNF-α level measured in control cultures (= 100%) [7,8].

3. Results and discussion

The ability of the compounds studied to stimulate TNF-α production has been expressed as diagrams (Fig. 1). It should be noticed that *N*-(adamant-1-yl)phthalimide (**1a**) was examined by Hashimoto as enhancer of



3a-c

Scheme 3.

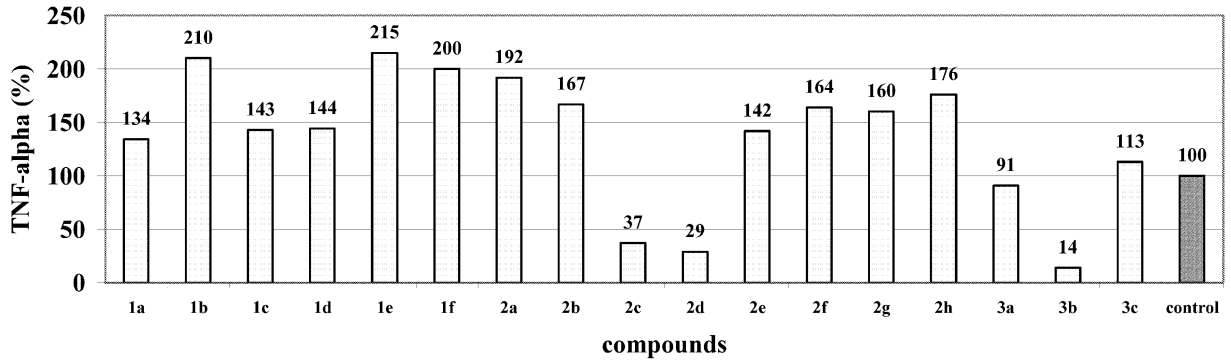


Fig. 1. TNF-a production-regulating activity of phthalimide derivatives expressed as percent of TNF-a level in control cultures (incubated without tested compounds (= 100%).

TNF-α production on human leukaemia cell line HL-60 in presence of 12-*O*-tetradecanoylphorbol-13-acetate and showed 500% overproduction of the cytokine [15]. It is interesting that in our evaluations this compound showed rather weak activity. Among imides studied only **1b**, **1e** and **1f** showed significant activity as enhancers of TNF-α production (at least 200% of the control). It is worth to notice that also in other papers sulfur-containing agents showed strong activity [2,8].

Imides **2c**, **2d**, as well as AZT derivative **3b** exhibited strong inhibition of cytokine production that resulted from the direct toxic effects of these agents on B78/TNF cells (data not shown). Previously we had found that such compounds (**2c**, **2d**) with *N*-substituents derived from L-phenylalanine and L-leucine have strong antibacterial properties [9–11]. Also, tetrabromophthalimide derivative (**3b**) had showed interesting antitumor activity [12]. Remaining compounds are almost inactive under our examination or show rather modest TNF-α production-enhancing activity.

As the recapitulation it should be noticed that many compounds caring phthalimide and adamantane moieties can stimulate TNF-α production in genetically modified mice melanoma cells. Also, a replacement of carbonyl group in the imide ring for thioimides one, enhances TNF-α production. These results are in agreement with Hashimoto's findings concerning HL-60 cells secreting cytokine in the presence of 12-*O*-tetradecanoylphorbol-13-acetate [2–6]. On the other hand, for the present, it is difficult to correlate satisfactorily a structure of compounds studied with TNF-α production-regulating activity. For example, in the group of trimellitimides, **2c** and **2d** were found to be toxic for B78/TNF cells and strongly inhibited TNF-α production while the rest of similar agents showed rather small enhancement of the cytokine secretion. Both groups could have potential application in the antitumor therapy, either as cytostatics or as agents augmenting effectiveness of tumor cell-based vaccines [16].

Acknowledgements

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