ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 619–623

IL FARMACO

Synthesis and antimicrobial activity of new adamantane derivatives II

Andrzej Orzeszko [a,*], Beata Kamińska [a], Grażyna Orzeszko [a], Bohdan J. Starościak [b]

[a] *Agricultural University, Institute of Chemistry, ul. Rakowiecka 26/30, 02-528 Warsaw, Poland*
[b] *Medical University, Department of Pharmaceutical Microbiology, ul. Oczki 3, 02-007 Warsaw, Poland*

Received in revised form 10 August 2000

Abstract

A series of new derivatives of adamantane was synthesised. The new compound 4-(adamant-1-ylethylenoxycarbonyl)phthalanhydride obtained from 1-adamantaneethanol and trimellitic anhydride chloride, as well as 4-(adamant-1-yl-methylenoxycarbonyl)phthalanhydride, appeared very useful for preparation of a number of N-substituted phthalimides. Antimicrobial activity of newly obtained derivatives such as, for example, 4-(adamant-1-ylethylenoxycarbonyl)-*N*-(L-phenylalanyl)phthalimide or 4-(adamant-1-ylmethylenoxycarbonyl)-*N*-(L-leucyl)-phthalimide was tested against *Staphylococcus aureus*, *Bacillus* sp., *Micrococcus flavus* and *Enterococcus faecium*. The minimal inhibitory concentrations for these compounds against *Bacillus cereus* were 15 and 8 μg/ml, respectively.

Keywords: Adamantane derivatives; Imides; Antimicrobial activity

1. Introduction

Many adamantane derivatives have interesting biological properties. The most known of clinical use is the antiviral drug 1-aminoadamantane (amantidine) [1]. Amantidine and many similar compounds with three-dimensional 'box-like' adamantane structure are employed in the treatment of certain neurological disorders [2]. The combination of such aliphatic core with phthalimide ring gives biologically active compounds. For instance, *N*-adamantylphthalimide induces tumour necrosis factor (TNF-α) in human leukaemia HL-60 cells [3], while 1-adamantylmaleinimide and its derivatives, show anticancer activity in mice and inhibition of herpes simplex virus in vitro [4,5]. Moreover, these compounds were tested against some micro-organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and others, however their minimal inhibitory concentration (MIC) were rather high (>100 μg/ml). Adamantaneketoxime ethers and their derivatives were tested as antibacterial and antifungus agents but mostly were found inactive (MIC >200 μg/ml) [6].

In our previous publication we have found that a number of 4-(adamant-1-yl-methylenoxycarbonyl)-*N*-substituted-phthalimides showed a very strong antibacterial activity, especially when as N-substituent, alanine, phenylalanine and some ω-aminoacids were used [7]. Minimal inhibitory concentrations for these compounds against *S. aureus* strain and *Micrococcus flavus* were comparable with that of clinically used antibiotics (1–0.02 μg/ml).

In this paper we would like to develop this line of our early studies and present two series of new phthalimide derivatives of adamantane and their antibacterial properties. The general synthetic pathway is given in Scheme 1.

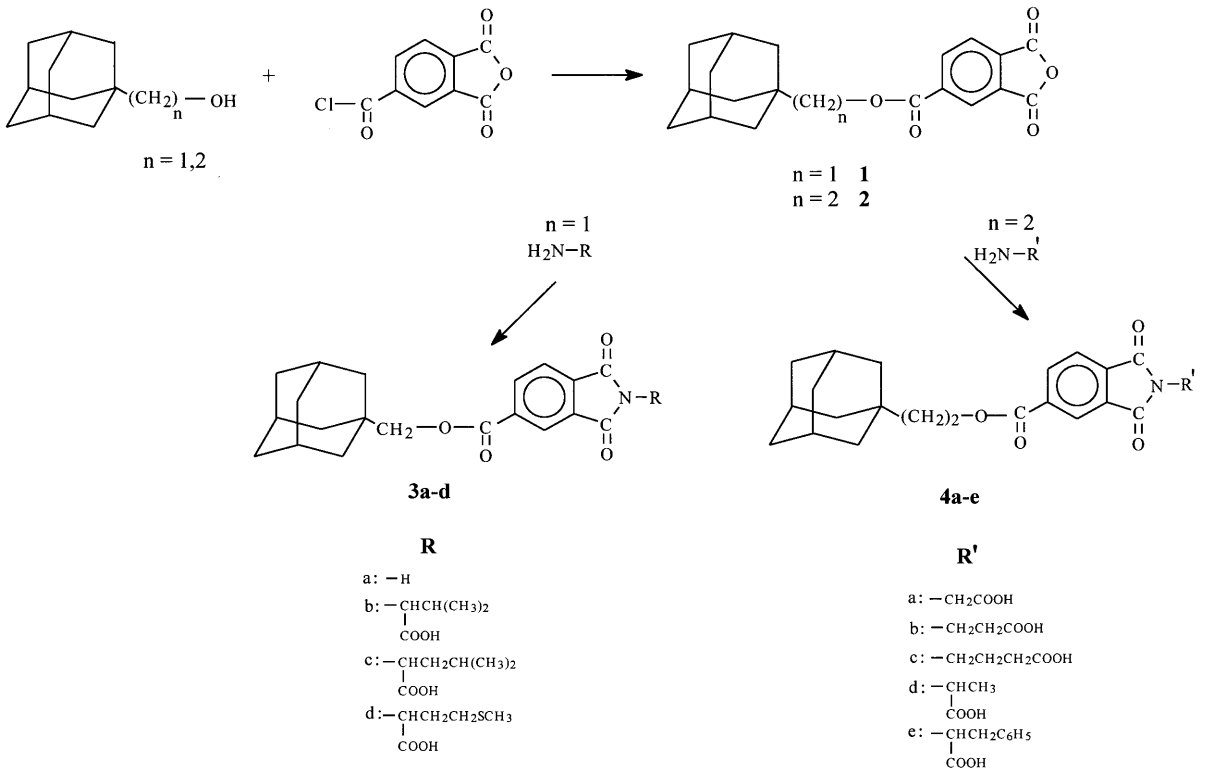
2. Experimental

2.1. General methods

Melting points were taken in open capillary tubes on a Gallenkamp 5 melting point 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

* Corresponding author.

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00075-6



Scheme 1.

Varian Gemini 200 MHz spectrometer in $CDCl_3$ solutions. Column flash chromatography and TLC were performed on silica gel 60 (Merck) using chloroform/methanol/acetic acid (20:1:trace) mixture as eluent. FTIR spectra were recorded on a Perkin–Elmer 2000 apparatus using the KBr pellet method. Analyses indicated by symbols were within $\pm 0.4\%$ of theoretical values. Adamantane and phthalic acid derivatives were purchased from Aldrich. Preliminary testing of the antimicrobial activity of newly synthesised compounds was performed by disc diffusion method using Mueller–Hinton agar medium under standard conditions as described by NCCLS [8]. Sterile filter paper discs were soaked in test compounds solutions prepared in EtOH–DMSO mixture (1:1). The results were read following 18 h incubation at 37°C (for *M. flavus* at 30°C). Compounds showing distinct antimicrobial activity in the above test were next examined for MIC in liquid Mueller–Hinton medium according to the appropriate NCCLS protocol, using original stock solutions [9]. *S. aureus* NCTC 4163 and *Enterococcus faecium* ATCC 6057 were purchased from the National Institute of Hygiene, Warsaw; the other microorganisms used were from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

*2.2. 4-(Adamant-1-ylmethylenoxycarbonyl)-phthalanhydride $C_{20}H_{20}O_5$ (**1**)*

The synthesis of **1** was performed from 1-adamantanemethanol and trimellitic anhydride chloride according to the method described previously [10,11]. Crude product was crystallised from benzene. Yield 42%; m.p. 158°C; FTIR (cm^{-1}): $C{=}O_{ester}$ 1731, $C{=}O_{anh}$ 1785 and 1857.

*2.3. 4-(Adamant-1-ylethylenoxycarbonyl)-phthalanhydride $C_{21}H_{22}O_5$ (**2**)*

The synthesis of **2** was performed from 1-adamantaneethanol and trimellitic anhydride chloride according to method described previously [10,11]. Crude product was crystallised from a toluene–hexane mixture. Yield 49%; m.p. 133–135°C; FTIR (cm^{-1}): $C{=}O_{ester}$ 1726, $C = O_{anh}$ 1785 and 1855.

*2.4. 4-(Adamant-1-ylmethylenoxycarbonyl)-phthalimide $C_{20}H_{21}NO_4$ (**3a**)*

A total of 1.36 g (5 mmol) of **1** and 0.15 g (2.5 mmol) of urea were melted and the reaction was run over 10 min at 250°C. Crude product was crystallised from a

benzene–methanol mixture. Yield 78%; m.p. 214°C. $R_f = 0.21$; FTIR (cm^{-1}): N–H 3275, C=O_{imide} 1779 and 1753, C = O_{ester} 1726; ^1H NMR δ (ppm): 1.63–2.03 (m, $H_{adamantane}$), 3.99 (s, CH_2O), 7.94–8.50 (m, H_{arom}), 7.98 (s, N–H).

2.5. 4-(Adamant-1-ylmethylenoxycarbonyl)-N-substituted-phthalimides (3b–d)

A total of 1.36 g (5 mmol) of **1** and the proper amino acid were refluxed in dry DMF for 3 h. The mixtures were poured into diluted HCl (1%). Crude products were filtered off and purified by column chromatography (silica gel) using chloroform/methanol/acetic acid (v/v, 25:1:traces) as eluent.

2.6. 4-(Adamant-1-ylmethylenoxycarbonyl)-N-(L-valinyl)phthalimide $C_{25}H_{29}NO_6$ (3b)

M.p. 140–142°C, 1.33 g, 65%); $R_f = 0.33$; FTIR (cm^{-1}): C=O_{imide} 1780 and 1725; ^1H NMR ($CDCl_3$) δ (ppm): 0.91–1.18 (d, CH_3), 1.63–2.02 (m, $H_{adamantane}$), 3.98 (s, CH_2O), 4.65 (d, N–CH), 7.93–8.48 (m, H_{arom}).

2.7. 4-(Adamant-1-ylmethylenoxycarbonyl)-N-(L-leucyl)phthalimide $C_{26}H_{31}NO_6$ (3c)

M.p. 99°C, 0.99 g, 57%); $R_f = 0.35$; FTIR (cm^{-1}): C=O_{imide} 1779 and 1724; ^1H NMR ($CDCl_3$) δ (ppm): 0.87–0.94 (m, $CH_{leucine}$), 1.62–2.02 (m, $H_{adamantane}$), 2.80 (t, $CH_2C{=}O$), 3.98 (s,CH_2O), 4.92 (m, N–CH), 7.90–8.45 (m, H_{arom}).

2.8. 4-(Adamant-1-ylmethylenoxycarbonyl)-N-(L-methionyl)phthalimide $C_{25}H_{29}NO_6S$ (3d)

M.p. 56–58°C, 0.90 g, 56%); $R_f = 0.38$; FTIR (cm^{-1}): C=O_{imide} 1780 and 1724; ^1H NMR ($CDCl_3$) δ (ppm): 1.63–1.73 (m, $H_{adamantane}$), 2.04 (s, CH_3S), 2.48 (m, $-CH_2-$), 3.97 (s, CH_2CO), 5.03 (m, N–CH); 7.89–8.45 (m, H_{arom}).

2.9. 4-(Adamant-1-ylethylenoxycarbonyl)-N-substituted phthalimides (4a–e)

A total of 1.36 g (5 mmol) of **2** and the proper amino acid were refluxed in dry DMF for 3 h. The mixtures were poured into diluted HCl (1%). Crude products **4a**–**c** were filtered off and crystallised from ethanol; crude products **4d** and **4e** were purified by column chromatography (silica gel) using chloroform/methanol/acetic acid (v/v, 25:1:traces) as eluent.

2.10. 4-(Adamant-1-ylethylenoxycarbonyl)-N-glycylphthalimide $C_{23}H_{25}NO_6$ (4a)

M.p. 103°C, 1.23 g, 72%; $R_f = 0.37$; FTIR (cm^{-1}): C=O_{imide} 1778 and 1720; ^1H NMR ($CDCl_3$) δ (ppm): 1.56–2.02 (m, $H_{adamantane}$), 4.40 (t, CH_2O), 4.51 (s, N–CH_2), 7.94–8.51 (m, H_{arom}).

2.11. 4-(Adamant-1-ylmethylenoxycarbonyl)-N-(2-carboxydimethylene)phthalimide $C_{24}H_{27}NO_6$ (4b)

M.p. 181°C, 1.44 g, 76%; $R_f = 0.37$; FTIR (cm^{-1}): C=O_{imide} 1778 and 1720; ^1H NMR ($CDCl_3$) δ (ppm): 1.56–2.02 (m, $H_{adamantane}$), 2.82 (t, CH_2COO), 4.03 (t, CH_2O), 4.44 (t, N–CH_2), 7.90–8.48 (m, H_{arom}).

2.12. 4-(Adamant-1-ylethylenoxycarbonyl)-N-(3-carboxytrimethylene)phthalimide $C_{25}H_{29}NO_6$ (4c)

M.p. 163°C, 1.33 g, 75%; $R_f = 0.41$; FTIR (cm^{-1}): C=O_{imide} 1776 and 1720; ^1H NMR ($CDCl_3$) δ (ppm): 1.56–2.02 (m, $H_{adamantane}$ and CH_2), 2.43 (t, CH_2COO), 3.79 (s, CH_2O), 4.33 (t, N–CH), 7.93–8.46 (m, H_{arom}).

2.13. 4-(Adamant-1-ylethylenoxycarbonyl)-N-(L-alanyl)phthalimide $C_{24}H_{27}NO_6$ (4d)

M.p. 84°C, 1.00 g, 55%; $R_f = 0.37$; FTIR (cm^{-1}): C=O_{imide} 1779 and 1724; ^1H NMR ($CDCl_3$) δ (ppm): 1.55–1.97 (m, $H_{adamantane}$ and CH), 4.43 (t, CH_2O), 4.98 (q, N–CH), 7.87–8.44 (m, H_{arom}).

2.14. 4-(Adamant-1-ylethylenoxycarbonyl)-N-(L-phenylalanyl)phthalimide $C_{30}H_{31}NO_6$ (4e)

M.p. 72–74°C, 1.11 g, 57%; $R_f = 0.35$; FTIR (cm^{-1}): C=O_{imide} 1780 and 1727; ^1H NMR ($CDCl_3$) δ (ppm): 1.57–1.97 (m, $H_{adamantane}$), 3.61 (m, CH_2–phenyl), 4.41 (t, CH_2O), 5.22 (m, N–CH), 7.17 (s, $H_{phenylala}$), 7.37–8.35 (m, H_{arom}).

3. Results and discussion

The trimellitimides obtained from **1** and glycine, 4-aminobutyric acid, β-alanine, L-alanine and L-phenylalanine described previously, exhibited strong antimicrobial activity [7]. These findings encouraged us to develop this series of compounds. Unsubstituted imide **3a**, as well as its derivatives synthesised from L-valine, L-leucine and L-methionine **3b**–**d**, respectively, were first testing by the agar disc-diffusion method using the Gram-positive strains of *S. aureus*, *M. flavus*, and certain strains of *Bacillus* (Table 1). The Gram-negative bacteria: *Bordetella bronchiseptica*, *Pseudomonas aerug-*

Table 1
Antibacterial in vitro activity expressed as diameter of growth inhibitory area

Bacteria strain	Diameter of growth inhibitory area (mm)								
	Compound [a]								
	3b [b]	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	Control
Staphylococcus aureus ATCC 25923	20	20	22	11		12	11	15	24 [c]
Staphylococcus aureus NTCC 4163	24	22	23			11	18	28	22
Staphylococcus aureus ATCC 6538 P	24	25	25	10		20	12	16	22
Micrococcus flavus NCIB 8166	21	19	21	11	13	14	13	16	24
Bacillus stearothermophillus	26	19	20		11	12	12	16	16
Bacillus subtilis ATCC 6633	27	24	20		11	12	13	15	18
Bacillus cereus	24	27	18		13	14		20	16

[a] 800 μg per 8 mm disc.
[b] Compound **3a** was completely inactive.
[c] Nitrofurantoine 300 μg per 8 mm disc.

Table 2
Antibacterial in vitro activity expressed as MIC

Bacteria strain	Minimal inhibitory concentration (μg/ml)							
	Compound							
	3b	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**
Staphylococcus aureus ATCC 25923	100	20	100	>100	>100	100	100	30
Staphylococcus aureus NTCC 4163	100	15	25	>100	100	20	30	20
Staphylococcus aureus ATCC 6538 P	50	15	25	>100	100	30	50	20
Micrococcus flavus NCIB 8166	80	80	100	>100	>100	>100	>100	20
Bacillus stearothermophillus	50	25	100	>100	>100	100	75	30
Bacillus subtilis ATCC 6633	50	50	100	>100	>100	80	75	30
Bacillus cereus	50	8	80	>100	>100	80	75	15

inosa and strains belonging to the family *Enterobacteriaceae* were resistant to all testing compounds.

As one can see from Table 1, for Gram-positive bacteria, the diameters of growth inhibition area of compounds studied were in the range 11–27 mm. Noteworthy, for compounds tested previously by us, the greatest diameters were 45–47 mm (for the *Bacillus* strain).

Next, the MIC of the most active compounds was determined in liquid Mueller–Hinton medium. As can be seen from Table 2, **3c** is the most effective agent and shows a particularly strong antimicrobial activity against *Bacillus cereus* and *Staphylococcus* strains.

The trimellitimides obtained from **2** make a new series of compounds (**4a**–**e**). For their synthesis we have chosen, among others, glycine, L-alanine and L-phenylalanine. These amino acids, in the former case, gave the imides with the strongest antibacterial activity. For newly obtained esterimides **4a**–**e** the diameters of growth inhibition area and MICs measured are shown in Tables 1 and 2, respectively.

It should be noted that the core structures of **3** and **4** are similar. The first series is obtained from 1-adamantanemethanol while the second is synthesised from 1-adamantaneethanol. Antibacterial studies of series **4**, as well as our earlier findings, show that adamantaneethanol derivatives are less active against microorganisms than compounds **3a**–**d**. We believe that the reason is the alongation of the spacer between the adamantane core and phthalimide ring in these molecules (**4a**–**e**).

The results shown in this paper and our former findings, point to a significant influence of amino acids used in the synthesis, on trimellitimides' biological properties. On the other hand, L- and D-enantiomers of amino acid derivatives showed almost the same antiseptic efficacity [7]. Therefore *N*-alanyl and *N*-phenylalanyl substituents in appropriate adamantane derivatives, caused greatest antibacterial activity, while, the unsubstituted imide **3a**, as well as **4a**,**b** were almost inactive.

References

[1] W.L. Davies, R.R. Grunert, R.F. Haff, J.W. McGahen, E.M. Neumayer, M. Paulshock, J.C. Watts, T.R. Wood, E.C. Hermann, C.E. Hoffmann, Antiviral activity of 1-adamantanamine (amantadine), Science 144 (1964) 862–863.

[2] R.S. Schwab, A.C. England, Jr., D.C. Poskanzer, R.R. Young, Amantadine in the treatment of Parkinson's diasease, J. Am. Med. Assoc. 208 (1969) 1168–1170.

[3] Y. Sabata, M. Shichita, K. Sasaki, Y. Hashimoto, S. Iwasaki, N-Alkylphthalimides: Structural requirement of thalidomidal action on 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor necrosis factor (production by human leukemia HL-60 cells, Chem. Pharm. Bull. 43 (1995) 177–179.

[4] J.-J. Wang, S.-S. Wang, C.h.-F. Lee, M.-A. Chung, Y.-T. Chern, In vitro antitumor and antimicrobial activities of N-substituted of maleimide by adamantane and diadamantane, Chemotherapy 43 (1997) 182–189.

[5] Y. Igarashi, K. Yagami, R. Imai, S. Watanabe, Antimicrobial activities of some N-alkylmaleimides, J. Ind. Microbiol. 6 (1990) 223–225.

[6] A. Papadaki-Valiraki, S. Papakonstantinou-Garoufalias, P. Makaros, A. Chytyroglou-Lada, M. Hosoya, J. Balzarini, E. De Clercq, Synthesis, antifungal, antibacterial and antiviral effects of some adamantaneketoxime ethers, Farmaco 48 (1993) 1091–1102.

[7] A. Orzeszko, R. Gralewska, B.J. Starościak, Z. Kazimierczuk, Synthesis and antimicrobial activity of new adamantane derivatives I, Acta Biochim. Pol. 50 (2000) 87–94.

[8] National Committee for Clinical LaboratoryStandards, NCCLS Approved Standard Document M7-A, Villanova, PA, USA, 1985.

[9] National Committee for Clinical Laboratory Standards, Approved Standard NCCLS Document M2-A5, fifth ed., Villanova, PA, USA, 1993.

[10] I. Śledziñska, E. Białecka-Florjañczyk, A. Orzeszko, Synthesis and liquid crystalline properties of cholesteryl bisester imides with poly(ethylene oxide)s as central spacer, Eur. Polym. J. 32 (1996) 1345–1350.

[11] (a) Polish Patent 175659, 1999. (b) Chem. Abstr. 130 (1999) 330860.