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Synthesis and antimicrobial activity of new adamantane derivatives III

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Abstract

Some novel ester imides synthesised from trimellitic acid anhydride and 1-adamantanol or 2-adamantanol, were tested as antimicrobial compounds. Unfortunately, these agents showed a modest antibacterial activity (MIC > 6 μg/ml). However, a comparison of these *N*-substituted adamantylester imides with the series published previously, indicated that the incorporation of L-alanine and L-phenylalanine into the phthalimide moiety was the best choice regardless of the series and leads to antimicrobial activity against *Staphylococcus aureus* strains. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Adamantane derivatives; Imides; Antimicrobial activity

1. Introduction

Imide derivatives are a valuable group of bioactive compounds showing analgesic activity, antiviral and antibacterial properties [1–5].

Previously, we had found that some 4-[(adamant-1-yl)-methylenoxycarbonyl]phthalimides (**2a**–**d**,**f**,**g**) and 4-[(adamant-1-yl)ethylen-oxycarbonyl]phthalimides (**3a-c**,**f**,**g**) with *N*-substituents derived from amino acids have a strong antibacterial activity [6,7]. The most active compounds were obtained from L-alanine, L-phenylalanine and some α,ω-aminoacids. Minimal inhibitory concentrations (MICs) for these ester imides were comparable with that of clinically used antibiotics (1–0.02 μg/ml). These findings prompted us to further study this class of compounds.

In the present paper, we synthesised two new series of adamantyl derivatives using 1-adamantanol (**1a**–**g**) and 2-adamantanol (**4a**–**g**), respectively, and chose L-alanine, L-phenylalanine, α,ω-aminoacids as *N*-substituents. Since we intended to compare the properties of the four homogeneously substituted series **1**–**4** and to study the influence of their structure on the antibacterial activity, we completed the series **2** and **3** obtained previously [6,7]. Furthermore, we synthesised new compounds **2e** and **3d**,**e** omitted previously, to make the four series complete and thus comparable. The synthesis, analytical and biological data of **2a**–**d**,**f**,**g** and **3a**–**c**,**f**,**g** were published previously [6,7]. Additionally we used some dipeptides as *N*-substituents to synthesise 4-[(adamant-1yl)metylenoxycarbonyl]phthalimides **2h**–**m** [5]. 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 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 KBr pellet method. Analyses indicated by symbols were within ± 0.4% of theoretical values. Adamantane and phthalic acid derivatives were

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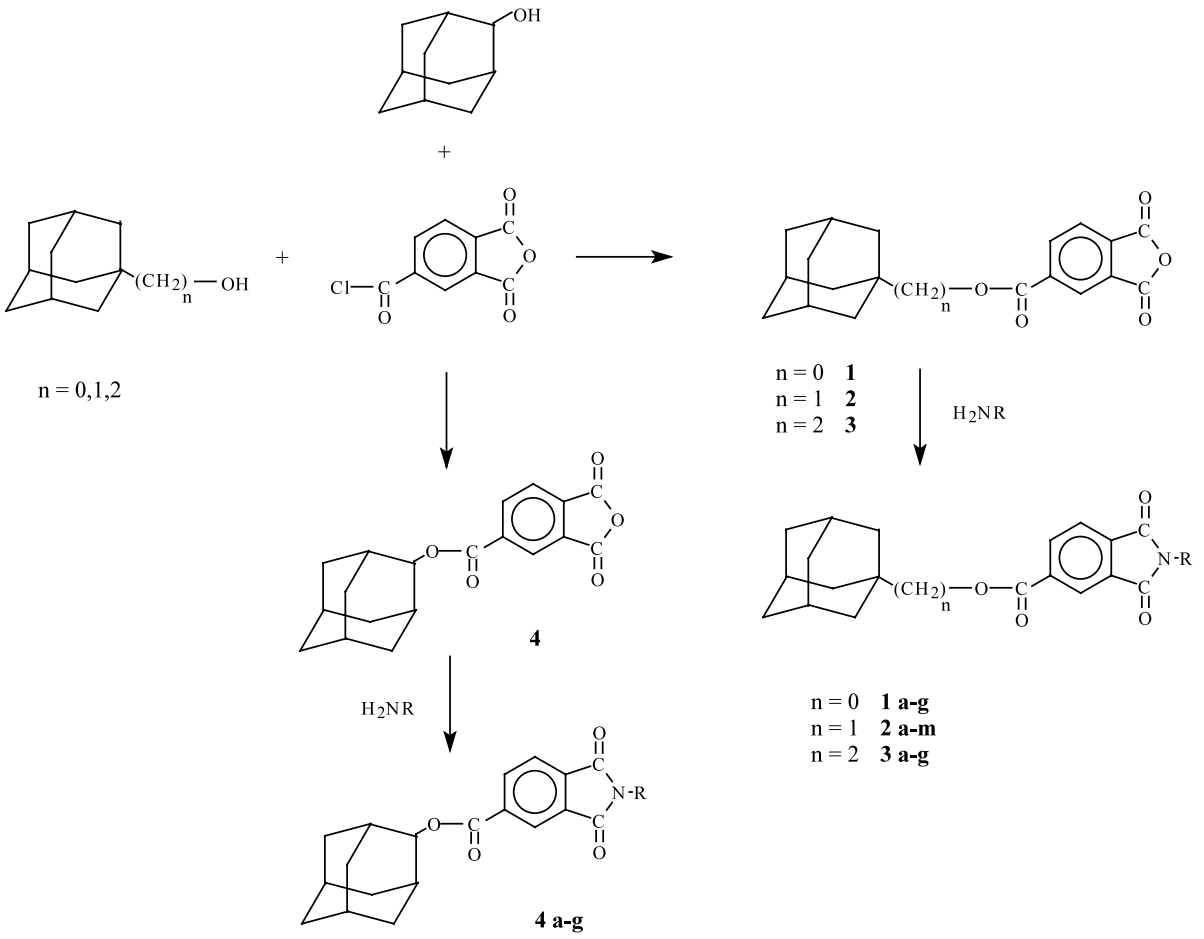
purchased from Aldrich. Preliminary scanning of the antimicrobial activity of the newly synthesised compounds was performed by disc diffusion method using Mueller–Hinton agar medium under standard conditions as described by NCCLS [8,9]. Sterile filter paper discs were soaked in test compounds solutions prepared in EtOH–DMSO mixture (1:1). The results were read following 18 h incubation at 37 °C. 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. *Staphylococcus aureus NCTC* 4163 was purchased from National Institute of Hygiene, Warsaw; the other microorganisms used were from own collection of Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

*2.2. 4-[(Adamant-1-yl)oxycarbonyl]phthalic anhydride $C_{19}H_{18}O_5$ (**1**) and 4-[(adamant-2-yl)oxycarbonyl]-phthalic anhydride $C_{19}H_{18}O_5$ (**4**)*

A total of 2.12 g (10 mmol) trimellitic anhydride chloride and 1.52 g (10 mmol) of 1-adamantanol or 2-adamantanol were dissolved in 20 cm^3 of dry pyridine, respectively. The mixtures were stirred at room



R:

a	CH_2COOH	**h**	Gly-L-Ala
b	$(CH_2)_2COOH$	**i**	L-Ala-Gly
c	$(CH_2)_3COOH$	**j**	L-Ala-L-Ala
d	$(CH_2)_5COOH$	**k**	D-Ala-D-Ala
e	$(CH_2)_7COOH$	**l**	L-Ala-L-Phe
f	L-Ala	**m**	L-Phe-L-Ala
g	L-Phe		

Scheme 1.

Table 1
Synthesis of **1**–**4** series of ester imides studied

Compound	Formula	Yield (%)	M.p. (°C) [a]
1a	$C_{21}H_{21}NO_6$	55	210
1b	$C_{22}H_{23}NO_6$	65	167–168
1c	$C_{23}H_{25}NO_6$	58	125–126
1d	$C_{25}H_{29}NO_6$	42	143
1e	$C_{27}H_{33}NO_6$	35	105–107
1f	$C_{22}H_{23}NO_6$	33	145
1g	$C_{28}H_{27}NO_6$	33	246
2e [b]	$C_{28}H_{35}NO_6$	36	209
2h	$C_{25}H_{28}N_2O_8$	31	171–173
2i	$C_{25}H_{28}N_2O_8$	36	199
2j	$C_{26}H_{30}N_2O_8$	25	181
2k	$C_{26}H_{30}N_2O_8$	25	169
2l	$C_{32}H_{35}N_2O_8$	30	164–165
2m	$C_{32}H_{35}N_2O_8$	28	165
3d [b]	$C_{27}H_{33}NO_6$	40	108
3e	$C_{29}H_{37}NO_6$	32	108
4a	$C_{21}H_{21}NO_6$	40	190
4b	$C_{22}H_{23}NO_6$	44	145–147
4c	$C_{23}H_{25}NO_6$	39	164–166
4d	$C_{25}H_{29}NO_6$	39	116–118
4e	$C_{27}H_{33}NO_6$	45	122
4f	$C_{22}H_{23}NO_6$	49	99
4g	$C_{28}H_{27}NO_6$	31	75

[a] Analysis indicated by the symbols were within $\pm 0.4\%$ of theoretical values.
[b] Compounds **2a**–**d** and **3a**–**c** were obtained and described in our previous publications [6,7].

temperature over 2 h. Then pyridine was evaporated under reduced pressure and the crude products were crystallised from dry toluene. Yields for both anhydrides were about 55%; for **1** m.p. 165–167 °C; FTIR data (in cm^{-1}): 1847 and 1775 C=$O_{anh.}$, 1716 C=O_{ester}; for **4** m.p. 137–138 °C; FTIR data (in cm^{-1}): 1858 and 1758 C=$O_{anh.}$, 1720 C=O_{ester}.

*2.3. 4-[(Adamant-1-yl)methylenoxycarbonyl]phthalic anhydride $C_{20}H_{20}O_5$ (**2**) and 4-[(adamant-1-yl)-ethylenoxycarbonyl]phthalic anhydride $C_{21}H_{22}O_5$ (**3**)*

Both anhydrides were synthesised according to the method described previously [6,7].

*2.4. Trimellitimide series **1**–**4***

Synthesis of the studied compounds performed according to the method described previously [6,7]. The yields and phisico-chemical data for all imides are listed in Table 1. Representative spectroscopical data are reported for compound **4d** only.

^{1}H NMR (in ppm): 1.34–2.24 (m, 20H), 2.36 (t, 2H, $J = 7.3$ Hz), 3.70 (t, 2H, $J = 7.3$ Hz), 5.13 (s, 1H), 7.88–8.53 (m, 3H), 8.78–10.7 (s, 1H).

FTIR (in cm^{-1}): 1776 C=O_{imide}, 1720 C=$O_{imide,ester}$.

3. Results and discussion

We had previously shown that adamantane ester imides derived from L-alanine, L-phenylalanine and some α,ω-aminoacids showed a strong antibacterial activity [6,7]. In this paper we have completed those series (**2e**,**h**–**m** and **3d**,**e**) and we have also obtained similar groups of compounds from 1-adamantanol and 2-adamantanol, respectively. In these cases the proper anhydrides **1** and **4** were synthesised in a different manner than the one described earlier for **2** and **3**, whereas the imidization reactions were the same for all compounds.

The antimicrobial activity of adamantane derivatives was first evaluated by the agar disc-diffusion method against strains belonging to the *Staphylococcus*, *Bacillus* and for *Micrococcus flavus*. The results, expressed as

Table 2
Antibacterial in vitro activity expressed as diameter of growth inhibition area for series **1**

Bacteria strain	Diameter of growth inhibition area (mm)							
	Compound tested [a]							
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	Control
Staphylococcus aureus ATCC 25923	11	13	19	Traces	Traces	11	15	24 [c]
Staphylococcus aureus NCTC 4163	12	19	22	19	12	12 (19 [b])	16	22
Staphylococcus aureus ATCC 6538 P	17 [b]	22	25	19	14	11 (19 [b])	16	22
Micrococcus flavus NCIB 8166	16 [b]	16	18	11 [b]	11 [b]	14 (18 [b])	19	24
Bacillus stearothermophilus ATCC 7953	13 (19 [b])	11	12 (19 [b])	16 (24 [b])	11 (19 [b])	14 (22 [b])	15	16
Bacillus subtilis ATCC 6633	12	13 [b]	22 [b]	13 (25 [b])	Traces	17	18	18
Bacillus cereus ML 98	12	Traces	12 (15 [b])	12	12	16	16	16

[a] 400 μg per 8 mm disc.
[b] Secondary growth.
[c] Nitrofurantoine 300 μg per 8 mm disc.

Table 3
Antibacterial in vitro activity expressed as diameter of growth inhibition area for compounds **2 h–m**

Bacteria strain	Diameter of growth inhibition area (mm)						
	Compound tested [a]						
	2h [d]	**2i**	**2j**	**2k**	**2l**	**2m**	Control
Staphylococcus aureus ATCC 25923	12	13	13	13	16	15	24 [c]
Staphylococcus aureus NCTC 4163	12	12	14	15	15	15	22
Staphylococcus aureus ATCC 6538 P	14	12	12	15	15	14	22
Micrococcus flavus NCIB 8166	14	14	16		16	19	24
Bacillus stearothermophilus ATCC 7953	14	13	14	13	15	16	16
Bacillus subtilis ATCC 6633	13 [b]	14 [b]	14 [b]	15 [b]	14 [b]	13 [b]	18
Bacillus cereus ML 98	Traces	11	12 [b]	13 [b]	13 [b]	12	16

[a] 400 μg per 8 mm disc.
[b] Secondary growth.
[c] Nitrofurantoine 300 μg per 8 mm disc.
[d] **2e** and **3d**,**e** were practically inactive.

Table 4
Antibacterial in vitro activity expressed as diameter of growth inhibition area for series **4**

Bacteria strain	Diameter of growth inhibition area (mm)							
	Compound tested [a]							
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	Control
Staphylococcus aureus ATCC 25923		13	19	15	Traces	15	16	24 [c]
Staphylococcus aureus NCTC 4163	13	12	20	11 (15 [b])	11	13 (16 [b])	18	22
Staphylococcus aureus ATCC 6538 P	12[b]	23	24	11 (20 [b])	12 [b]	13 (17 [b])	20	22
Micrococcus flavus NCIB 8166	12 [b]	20	18	12 [b]	Traces	17	12 (18 [b])	24
Bacillus stearothermophilus ATCC 7953	12 (19 [b])	16	12	11 (24 [b])	11 (16 [b])	14 (21 [b])	16	16
Bacillus subtilis ATCC 6633	11	13 (20 [b])	12 (17 [b])	13	12[b]	18	20	18
Bacillus cereus ML 98	11	13	14	14	12	17	16	16

[a] 400 μg per 8 mm disc.
[b] Secondary growth.
[c] Nitrofurantoine 300 μg per 8 mm disc.

Table 5
Antibacterial in vitro activity expressed as MIC for series **1**

Bacteria strain	Minimal inhibitory concentration (μg/ml)						
	Compound						
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**
Staphylococcus aureus NCTC 4163	450	75	25	25	25	200–400	25–50
Staphylococcus aureus ATCC 6538 P	450	25	200	100	25	100–200	12.5–25
Micrococcus flavus NCIB 8166	150	100	75	50	50	50–100	12.5
Bacillus stearothermophilus ATCC 7953	350	250	180	25	75	200	12.5–25
Bacillus subtilis ATCC 6633	200	250	180	50	75	200	12.5–25
Bacillus cereus ML 98	400	250	200	12.5	25	200	12.5

the diameter of growth inhibition area in mm, are given in Tables 2–4. Data for **2a**–**d**,**f**,**g** and **3a**–**c**,**f**,**g** were reported previously [6,7]. Compounds **2e**, **3d**,**e** studied at present, are practically inactive. As can be seen from Tables 2–4, the greatest diameters among all compounds tested were found for *S. aureus*. Generally, this is in agreement with our previous findings concerning the series **2** and **3**.

Next, the MICs only for the most active new compounds were determined in liquid Mueller–Hinton

Table 6
Antibacterial in vitro activity expressed as MIC for compounds **2l**,**m**

Bacteria strain	Minimal inhibitory concentration (μg/ml)	
	Compound	
	2l	**2m**
Staphylococcus aureus ATCC 25923	>200	200
Staphylococcus aureus NCTC 4163	>200	50
Staphylococcus aureus ATCC 6538 P	>200	50
Micrococcus flavus NCIB 8166	100–200	12.5–25
Bacillus stearothermophilus ATCC 7953	>200	50

medium. The values of MICs are given in Tables 5–7. As it appears from the present and the former studies, that *N*-alanyl- and *N*-phenylalanyl groups and *N*-(α,ω-aminoacid)s as substituents, cause the greatest antibacterial activity. Unfortunately, the dipeptide derivatives and the whole **3** series showed rather a weak activity.

An interesting tendency was observed when comparing the MICs of *N*-(α,ω-amino acid) substituted compounds. The collected data from our previuos papers (Table 8), and from the present study, have been expressed together as diagrams (Fig. 1) showing the relation of MIC value versus the number of methylene groups in amino acid chains.

As can be seen from the plots the minima of MICs for the majority of homologous compounds correspond mostly to five methylene groups, i.e. for 6-aminocapronic acid derivatives. It should be noticed that the dependence of the length of aliphatic linker on MIC value was observed also for organic thiosulfates, so-called Bunte salts [10].

4. Conclusion

The comparison of antimicrobial properties of four adamantylester imide series shows that derivatives of tertiary alcohol 1-adamantanol and secondary 2-adamantanol exhibit rather weak activity, whereas trimellitimides synthesised from primary alcohols, i.e. 1-adamantanmethanol and 1-adamantaneethanol, are effective antibacterial agents [6,7].

The results shown in this paper and our former findings, point to a significant influence of *N*-substituents such as *N*-alanyl, *N*-phenylalanyl, as well as some *N*-(α,ω-aminoacid)s groups in the appropriate adamantane derivatives on the antibacterial activity. Therefore it was surprising for us to find out that dipeptide *N*-substituents carrying such moieties, make ester imides inactive.

The most sensitive microorganisms in our studies were Gram-positive bacteria *S. aureus*, *M. flavus* and some strains of *Bacillus*.

Table 7
Antibacterial in vitro activity expressed as MIC for series **4**

Bacteria strain	Minimal inhibitory concentration (μg/ml)						
	Compound						
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**
Staphylococcus aureus NCTC 4163	150	150	100	6.25	25	25–50	25
Staphylococcus aureus ATCC 6538 P	100	150	50	12.5	25	>200	12.5–25
Micrococcus flavus NCIB 8166	100	25	75	25	40	50–100	12.5
Bacillus stearothermophilus ATCC 7953	200	50	100	50	40	50	12.5–25
Bacillus subtilis ATCC 6633	100	12.5	75	50	40	50	12.5–25
Bacillus cereus ML 98	250	12.5	100	50	25	25–50	12.5

Table 8
Antibacterial in vitro activity expressed as MIC for *N*-(α,ω-aminoacid) substituted ester imides

Bacteria strain	Minimal inhibitory concentration (μg/ml)									
	Compound									
	2a [a]	**2b** [a]	**2c** [a]	**2d** [a]	**2e**	**3a** [b]	**3b** [b]	**3c** [b]	**3d**	**3e**
Staphylococcus aureus NCTC 4163	7.5	5	5	0.08	>250	>100	100	20	>100	>100
Micrococcus flavus NCIB 8166	10	2.5	2.5	0.8	>250	>100	>100	>100	>100	>100

[a] See Ref. [6].
[b] See Ref. [7].

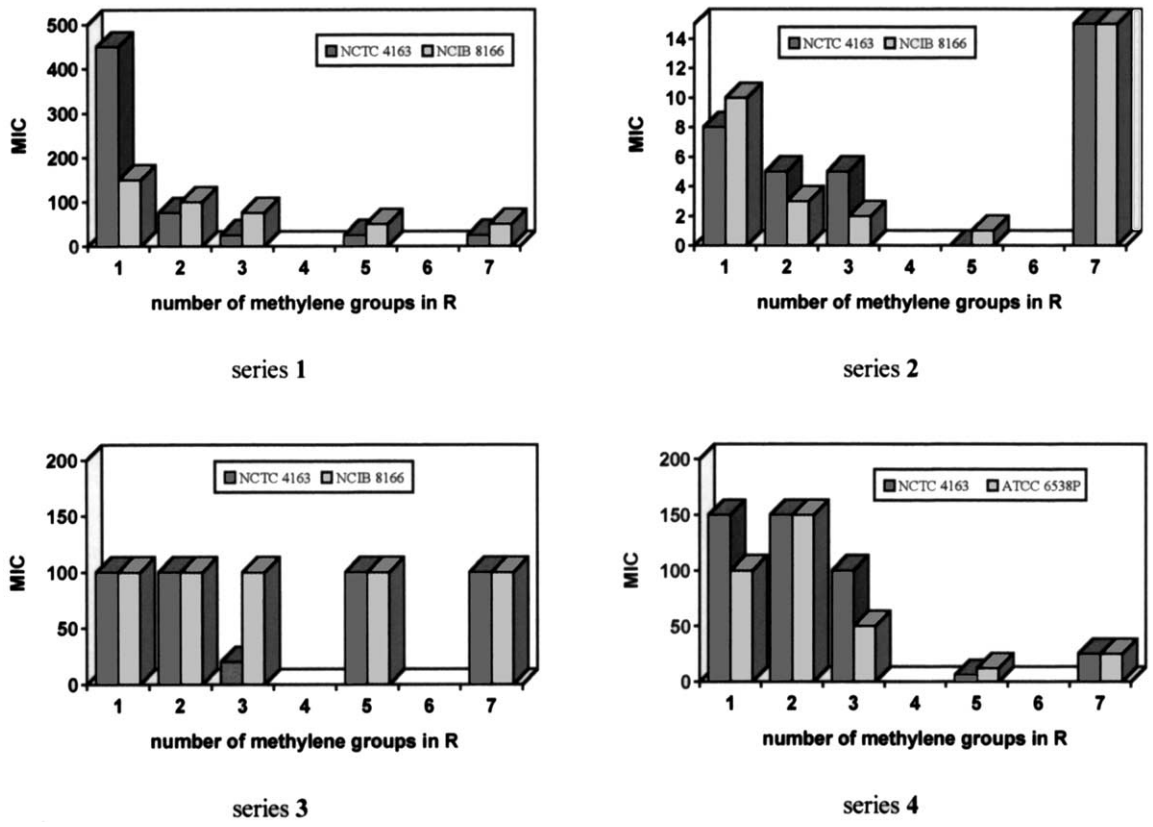


Fig. 1. The dependence of the length of aliphatic linker on MIC (μg/ml) value for *S. aureus* (NCTC 4163, ATCC 6538P) and *M. flavus* (NCIB 8166).

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References

[1] R. Antunes, H. Batista, R.M. Srivastava, G. Thomas, C.C. Araujo, New phthalimide derivatives with potent analgesic activity, Bioorg. Med. Chem. Lett. 8 (1998) 3071–3076.
[2] Y. Hasimoto, Novel biological response modifiers derived from thalido-mide, Curr. Med. Chem. 5 (1998) 163–178.
[3] A. da Settimo, A.M. Marini, G. Primafiore, F. da Settimo, Sythesis and evaluation of aminoadamantane derivatives for in vitro anti-HIV and antitumor activities, Farmaco 50 (1995) 321–326.
[4] N. Kolocouris, A. Kolocouris, G.B. Foscolos, G. Fytas, J. Neyts, E. Padalko, J. Balzarini, R. Snoeck, G. Andrei, E. De Clercq, Synthesis and antiviral activity evaluation of some new aminoadamantane derivatives 2, J. Med. Chem. 39 (1996) 3307–3318.
[5] C.T. Miller, R. Weragoda, E. Izbicka, B.L. Iverson, The synthesis and screening of 1,4,5,8-naphthalenetetrecarcoxylic diimide-peptide conjugates with antibacterial activity, Bioorg. Med. Chem. 9 (2001) 2024–2025.
[6] A. Orzeszko, R. Gralewska, B.J. Starościak, Z. Kazimierczuk, Synthesis and antimicrobial activity of new adamantane derivatives I, Acta Biochim. Pol. 47 (2000) 87–94.
[7] A. Orzeszko, B. Kamińska, G. Orzeszko, B.J. Starościak, Synthesis and antimicrobial activity of new adamantane derivatives II, Farmaco 55 (2000) 619–623.
[8] National Committee for Clinical Laboratory Standards NCCLS Approved standard document M7-A, Villanova, PA, 1985.
[9] National Committee for Clinical Laboratory Standards, 5th edn. Approved standard NCCLS Document M2-A5, Villanova, PA, 1993.
[10] J.Z. Stefańska, B.J. Starościak, A. Orzeszko, Z. Kazimierczuk, Antimicrobial activity of organic thiosulfates (Bunte salts), Pharmazie 53 (1998) 190–192.