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ORIGINAL ARTICLE

Antimicrobial activity of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione derivatives

Joanna Stefańska · Anna Bielenica · Marta Struga · Stefan Tyski · Jerzy Kossakowski · Paolo La Colla · Elena Tamburini · Roberta Loddo

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Abstract Antibacterial and antifungal activity of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione derivatives were examined by the disk diffusion method (growth inhibition zone diameter in agar medium). The minimal inhibitory concentrations (MICs) for the most active agents were determined. Title compounds were also evaluated in vitro against HIV-1 virus and their cytotoxicity was determined. Aminoalkanol derivatives exhibited activity against the majority of microorganisms studied.

Keywords Antimicrobial activity · 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione derivatives

J. Stefańska (✉) · S. Tyski
Department of Pharmaceutical Microbiology,
Medical University of Warsaw,
3 Oczki Street,
02-007 Warsaw, Poland
emai: joanna.stefanska@wum.edu.pl

A. Bielenica (✉) · M. Struga · J. Kossakowski
Department of Medical Chemistry, Medical University of Warsaw,
3 Oczki Street,
02-007 Warsaw, Poland
email: anna.bielenica@wum.edu.pl

S. Tyski
Department of Antibiotics and Microbiology,
National Medicines Institute,
00-725 Warsaw, Poland

P. La Colla · E. Tamburini · R. Loddo
Department of Biomedical Science and Technology,
University of Cagliari,
Cittadella Universitaria,
09042 Monserrato, Cagliari, Italy

Introduction

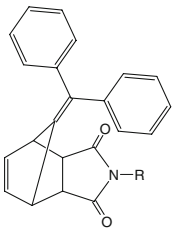
The interest in cyclic imides is due to their biological activity and wide application in the pharmaceutical industry. Some of them are used in polymer production (Cho et al. 2004; Mehdipour-Ataei and Hatami 2005; Hoogboom et al. 2006). Many derivatives possess biological activity. They have antibacterial (Albuquerque et al. 1999; Zentz et al. 2002; Struga et al. 2008a, b), anticonvulsive (Stratford and Curley 1983), hypnotic, sedative (Duarte et al. 2006; Kozioł et al. 2006), anxiolytic (Chilmończyk et al. 1995), and antitumor properties (Di Pardo et al. 2001; Kumar and Rajkumar 2006; Nakamura et al. 2006). It is also known that compounds bearing short-amine fragment possess antibacterial and antifungal activity (Stefańska et al. 2008). The action of these agents could be connected with their influence on microorganisms' cell membrane permeability (Zejc and Gorczyca 2008). The introduction of the aryl group to the amine moiety shifted the activity from antibacterial (Foroumadi et al. 2005) to antiviral, with a specific action against human immunodeficiency virus (HIV) (Pinna et al. 2001; Hadizadeh and Mehrparvar 2004; Richter et al. 2004). It was suggested that the isopropanolamine unit, present in the HIV protease inhibitors (e.g., nelfinavir) and reversed transcriptase inhibitors (e.g., delavirdine), could be responsible for their binding properties (Di Santo et al. 2002). Also, the presence of a polar moiety in the structure of anti-HIV-1 drugs seems to be important for their activity. Most of them contain the piperazine nucleus or arylpiperazine fragment (Hadizadeh and Mehrparvar 2004). For instance, in the structure of indinavir, non-nucleosidic protease inhibitor, both aminoalkanol and arylpiperazine parts can be found (Cheng et al. 2002; Zhang et al. 2003).

In this study, we have combined the structure of polycyclic ring of 10-(diphenylmethylene)-4-azatricyclo [5.2.1.0^{2,6}]dec-8-ene-3,5-dione with the alkyl chains bearing different substituents. Activity of all derivatives against bacteria and fungi, as well as cytotoxicity and HIV-1 activity of selected compounds, were examined.

Materials and methods

All chemicals were of analytical grade (Aldrich) and were used without further purification. The list of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione derivatives that were studied is presented in Table 1. Compounds used in this work were previously synthesized. The starting compound was obtained in Diels-Alder reaction of 1,1′-(cyclopenta-2,4-dien-1-ylidenemethylene)dibenzene with 1*H*-pyrrole-2,5-dione (Kossakowski and Hejchman 2000) and later subjected to the reaction with 2-(chloromethyl)oxirane in anhydrous medium. Subsequently, the resultant oxirane reacted with appropriate amines giving aminoalkanol derivatives **4, 5, 6, 7** (Kossakowski and Wojciechowska 2006). Alkylation of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione with appropriate amines gave compounds **2** and **3** (Kossakowski et al. 2006). By reaction of the title compound with 1-bromo-3-chloropropane or 1,4-dibromobutane, substituted derivatives **8** and **9**, respectively, were obtained. Next, these compounds were condensed with selected arylpiperazines to form derivatives **10–15**. Imide **1** is a product of the reaction of

Table 1 Chemical structures of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione derivatives



	R		R
1	-OH	**9**	—CH_2-CH_2-CH_2-CH_2—Br
2	—CH_2-CH_2—N	**10**	—CH_2-CH_2-CH_2-CH_2—N, N, N
3	—CH_2-CH_2-CH_2—N(CH_3)(CH_3)	**11**	—CH_2—CH_2—CH_2—N, N, F
4	—CH_2—CH(OH)—CH_2—N(CH_2-CH_3)(CH_2-CH_3)	**12**	—CH_2-CH_2-CH_2—N, N, H_3CO
5	—CH_2—CH(OH)—CH_2—NH—C(CH_3)(CH_3)(CH_3)	**13**	—CH_2—CH_2—CH_2—N, N, F
6	—CH_2—CH(OH)—CH_2—N, N, H_3CO	**14**	—CH_2—CH_2—CH_2—N, N—CH_2
7	—CH_2—CH(OH)—CH_2—N, N, F	**15**	—CH_2-CH_2-CH_2-CH_2—N, N, H_3CO
8	—CH_2-CH_2-CH_2-Cl		

Table 2 Antibacterial and antifungal activities of 10-(diphenylmethylene)-4-azatricyclo- [5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione and its derivatives: diameter of the growth inhibition zone [mm] and minimal inhibitory concentration (MICs) values (in parentheses) (μg/ml)

Strain	Compound[a]			
	1	3	4	5
Staphylococcus aureus ATCC 4163	17 (200)	13 (200)	19 (200)	24 (100)
Staphylococcus aureus ATCC 25923	17 (200)	13 (200)	17 (200)	20 (100)
Staphylococcus aureus ATCC 6538	18 (200)	12 (200)	20 (200)	24 (100)
Staphylococcus aureus ATCC 29213	14 (200)	12 (200)	20 (200)	25 (100)
Staphylococcus epidermidis ATCC 12228	13 (200)	12 (200)	23 (200)	25 (100)
Bacillus subtilis ATCC 6633	14 (200)	16 (200)	25 (100)	30 (100)
Bacillus cereus ATCC 11778	13 (200)	14 (100)	20 (100)	22 (100)
Enterococcus hirae ATCC 10541	na (> 400)	11 (400)	22 (200)	24 (200)
Micrococcus luteus ATCC 9341	14 (200)	18 (100)	27 (100)	29 (50)
Micrococcus luteus ATCC 10240	16 (200)	19 (100)	27 (100)	30 (50)
Candida albicans ATCC 10231	na	na	20 (200)	24 (200)
Candida albicans ATCC 90028	na	na	20 (200)	22 (200)
Candida parapsilosis ATCC 220191	na	na	20 (200)	23 (200)

na No activity

[a] Compounds not listed above were completely inactive in concentration up to 400 μg per disk

1,1′-(cyclopenta-2,4-dien-1-ylidenemethylene)dibenzene with 1-hydroxy-1*H*-pyrrole-2,5-dione (Kossakowski and Prędka 2003). For biological studies, free bases were converted into corresponding hydrochloride salts.

Biological assays

The antibacterial activity of compounds was tested against series of collection strains of Gram-positive bacteria: *Staphylococcus aureus* ATCC 4163, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Enterococcus hirae* ATCC 10541, *Micrococcus luteus* ATCC 9341, and *Micrococcus luteus* ATCC 10240, and Gram-negative rods: *Escherichia coli* ATCC 10538, *Escherichia coli* ATCC 25922, *Escherichia coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* NCTC 6749, *Pseudomonas aeruginosa* ATCC 27853, and *Bordetella bronchiseptica* ATCC 4617. Antifungal activity was tested against yeasts: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, and *Candida parapsilosis* ATCC 220191. Microorganisms used in this study were obtained from the appropriate collection and were stored in the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

Antimicrobial activity was examined by the disk diffusion and determination of minimal inhibitory concentrations (MICs) methods under standard conditions, using Mueller-Hinton II agar medium (Becton Dickinson) for bacteria and RPMI agar with 2% glucose (Sigma) for yeasts, according to CLSI guidelines (CLSI 2006a). Solutions containing the tested agents were prepared in methanol or DMSO. For the disk diffusion method, sterile paper disks (9 mm diameter, Whatman No. 3 chromatography filter paper) were dipped with the tested compound solutions to obtain 400 μg of substance per disk. Dry disks were placed on the surface of an appropriate agar medium. The results (diameter of the growth inhibition zone) were read after 18 h of incubation at 35°C. MICs were examined by the twofold serial agar dilution technique (CLSI 2006b). Concentrations of the tested compounds in solid medium ranged from 3.125 to 400 μg/mL. The final inoculum of studied organisms was 10^4 CFU/mL (colony forming units per ml), except the final inoculum for *E. hirae* ATCC 10541, which was 10^5 CFU/mL. Minimal inhibitory concentrations were examined after 18 h (for bacteria) and 24 h (for yeasts) of incubation at 35°C.

Table 3 Cytotoxicity of 10-(diphenylmethylene)-4-azatricyclo-[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione and its derivatives

Compound	MT-4 CC_{50} [μM][a]
4	44
5	15±5
6	47
7	47
8	> 100
9	> 100
10	47
11	> 100
12	> 100
13	> 100
14	47
15	> 100
EFV[b]	37

[a] CC_{50} Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.

[b] *EFV* Efavirenz; positive control

Cytotoxicity assays

Compounds Compounds for cytotoxicity assays were dissolved in DMSO at concentration of 100 mM and then diluted in culture medium.

Cells Cell lines were purchased from the American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4).

Cytotoxicity evaluation Exponentially growing cells derived from human haematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1×10^5 cells/mL in 96-well plates in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 100 units/mL penicillin G, and 100 μg/ml streptomycin. Cell cultures were then incubated at 37°C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37°C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (auwels et al. 1988).

Anti HIV-1 activity Activity of compounds (**4–15**) against human immunodeficiency virus type-1 (HIV-1) was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infections (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing 2×10^4 MT-4 were added to each well of flat-bottom microtiter trays containing 50 μL of RPMI, without or with serial dilutions of test compounds. Then, 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method (Pauwels et al. 1988).

Results and discussion

In the present study, 10-(diphenylmethylene)-4-azatricyclo-[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione derivatives were tested in vitro against bacteria, yeasts, and HIV-1 virus. Preliminary antimicrobial test by disk diffusion method showed activity of compounds **1, 3, 4, 5** against Gram-positive bacteria and yeasts. Gram-negative rods were resistant to all tested agents. The activity giving compounds in this assay were tested in order to determine their minimal inhibitory concentration values. The cytotoxicity and anti HIV-1 activity (data not shown, the results are available from authors) of derivatives **4–15** were determined. Aminoalkanol derivatives of 10-(diphenylmethylene)-4-azatricyclo [5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione presented the lowest cytotoxicity. None of title compounds, however, turned out to be active against HIV-1. The results are summarized in Table 2 and Table 3.

The obtained data may serve as a good subject for discussion of the influence of chain length and subjected amine type of a compound on its antimicrobial activity. It seems that antibacterial activity is determined by kind of a substituent at the imide's nitrogen atom. Derivatives containing (hydroxy)propyl linker combined with short-amine moiety possess significant activity, whereas these with cyclic or arylpiperazine units appear inactive. The shortening or lengthening of a hydrocarbon chain resulted in a decreasing of the compound's activity. The best results were found for compounds **4** and **5**, in which diethyl and *tert*-butyl branches are present. They were active mainly against various *Staphylococcus aureus* and *Micrococcus luteus* strains, as well as *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 11778. Compounds **1** and **3** (with hydroxyl and 3-dimethylaminopropyl group attached to the imide ring) were also characterized by broad antibacterial activity. The mentioned derivatives were active against all tested Gram-positive bacteria and show antifungal activity against selected *C. albicans* and *C. parapsilosis* strains. The MICs values were in the range between 50 and 200 μg/mL. The presence of butyl spacer and/or heteroarylpiperazine ring, and therefore in consequence high lipophilicity, could be the reason of lack of activity of compounds **2** and **6–15**.

The cytotoxicity values are in the range between 15 and above 100 μM. For the compound **5**, the observed value is lower than for the standard efavirenz (EFV). The compounds with lowest cytotoxicity possess hydroxypropyl linker connected to diethylamine (**4**) and *tert*-butyl (**5**) units.

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