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Antibacterial Activity of Ethylpyrrolobenzodiazepines

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ABSTRACT

Pyrrolo [2, 1-c][1, 4] benzodiazepines (PBDs) are naturally occurring compounds isolated from various *Streptomyces* species. The PBDs exert their biological activity through covalent binding. Extensive studies have been carried out on the antitumoral and antibacterial activities of the PBDs. In present study, we have prepared PBDs alkylated and we report results of their antibacterial activity against a range of Gram positive and Gram negative bacteria, which proved successful for the thionated PBD alkylated. The results of the minimal inhibiting concentration (MIC) varied from one bacterium to another. A great affinity of the PBD was found against *Enterococcus* species, *Enterobacter cloacae*, *Listeria monocytogenes*, *Streptococcus agalactiae* and *Salmonella* species.

Key Words: Benzodiazepine; Antibacterial activity; Bacteria; Antitumor activity

INTRODUCTION

In recent years, the PBDs compounds have been designed, synthetized and evaluated for their biological activity, in particular the antitumoral and the antibacterial activity. These compounds are known to exert their cytotoxic effects by covalently binding to the exocyclic C2-NH_2 of guanine residues within the minor groove of DNA (Sagnou *et al.*, 2000; Krčméry & Sefton, 2000; Djipa *et al.*, 2000; Kamal *et al.*, 2001; Kamal *et al.*, 2002a & b; Ritch-Krc *et al.*, 2002; Kamal *et al.*, 2004a & b). The PBD defined as antitumour antibiotics are produced by various *Streptomyces* species and are generally referred to the anthramycin family, which comprise anthramycin, tomaymycin, chicamycin A, neothramycin and DC-81 (Fig. 1).

In present work, novel PBDs derivatives of the benzodiazepine by alkylation of pyrrolo [2, 1-c][1, 4] benzodiazepines are studied for their antibacterial activity. For that we alkylated the PBDs with the ethylbromide. Then, we carried out the thionation of the alkylated PBDs that were tested in the same conditions. An attempt has been made to highlight the antibacterial activity of EthylPBDs by testing their antibacterial action against several types of bacteria.

MATERIALS AND METHODS

Synthesis of ethylpyrrolobenzodiazepines. To develop this study, we prepared two product derivatives of PBDs (Fig. 2). EthylPBD was obtained by alkylation of the PBD by the ethylbromide (Scheme 1).

The second product: The ethylpyrrolo [2, 1-c][1, 4] benzodiazepine-5,11-dithione was obtained by sulfuration of the EthylPBD with the phosphorus pentasulfide in reflux of pyridine according to the following reaction (Scheme 2).

Bacterial strains and culture conditions. Bacterial strains used in this study were obtained from American Type Culture Collection (ATCC), USA; National Institute of Healthy (NIH), Morocco and Institute of Agronomy and Veterinary medicine (IAV), Morocco. All bacteria were stored in trypticase soy (Sanofi Diagnostic Pasteur, France) broth containing 25% (v/v) glycerol (Sigma-Aldrich) at -20°C. Prior to use, the culture were propagated twice in the appropriate media as mentioned above to make them physiologically active. We selected various bacteria showing distinct characteristics (Rehman *et al.*, 2003). Culture conditions for all strains were aerobic at 37°C.

Antibiotic susceptibility testing. Antibiotic as amoxicillin, ticarcillin, piperacillin, amoxicillin, clavulanic acid, ampicillin, ticacillin, cephalotin, cefoxitin, cefamandole, cefotaxim, ceftazidim, kanamycin, gentamicin, tobramycine, penicullin g, oxacillin, erythromycin, clindimycin, pristinamycin, trimethoprim, sulphamethoxazole, cipofloxacin, ofloxacin, chloramphenicol, tetracycline, rifampicin, vancomycin, imipenem, teicoplanin and fusidic acid were studied by using a slightly modified version of the agar diffusion method (Kirby *et al.*, 1966). Strains were grown on the appropriate media, thus a suspension with a density of Mc Farland 0.5 in saline water (8.5%; w/v) was swabbed in three directions on 4 mm thick Mueller Hinton (MH) agar (Oxoid, England) with a cotton swap. Then antibiotics discs

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were placed in the inoculated plates using the oxoid disc dispenser. After 24 h of incubation at 37°C, inhibition zones around the discs were measured.

Antibacterial activity assays. Antibacterial activity of synthetic products was tested against the target of Gram positive and Gram negative bacteria by the well diffusion method as described by (Kim *et al*., 1993). MH agar (1.5%; w/v) plates were overlaid with 5 mL of soft MH agar (0.8%; w/v) containing 100 µL of freshly cultured target microorganisms (approximately 10^6 cfu mL^{-1}). Then agar plates were incubated at 37°C overnight and examined for the presence of clearing zones of growth inhibition.

RESULTS

Structure determination of PBDs. We determined the formula of each product with its ^1H NMR spectra, ^{13}C NMR spectra and spectrum of mass. For the first product, the ethylpyrrolo [2, 1-c][1, 4] benzodiazepine-5,11-dione, ^1H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using tetramethyl silane (TMS) as an internal standard as described below: Chemical shifts are reported in parts per million (ppm) down field from tetramethyl silane. Spin multiplicities are described as: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants are reported in Hertz (Hz).

^1H NMR ($CDCl_3$) (δ ppm, J Hz): 1.23 (t, 3H, CH_3, J=2,9 Hz); 1.84 (m, 3H, CH_2 in 2); 2.51 (m, 1H, CH_2 in 1); 3.26 (m, 2H, CH_2 in 3); 3.91 (d, 1H of CH in 11a 5.4 Hz); 3.61 (q, 2H, CH_2 in 10, J = 2.9 Hz); 7.08-7.33 (m, 4H, CH_{Ar}).

^{13}C NMR ($CDCl_3$) (δ ppm): 14.1 (CH_3); 22.6 (C2); 23.7 (C1); 43.6 (CH_2-10); 46.1 (C3); 47.1 (C-11a); 122.3-132.4 CH_{Ar}; 130.7, 139.3 Cq; 165.0 (C=O); 168.7 (C=O).

IR (KBr): υ(C=O) = 1625 and 1675 cm^{-1}.

Spectrum of mass (I.E.): m/z = 244 (M^+).

Second product was recognized with following specifications.

^1H NMR ($CDCl_3$) (δ ppm, J Hz): 1.22 (t, 3H, CH_3 in 10, J = 3.2 Hz); 2.10 (m, 2H, CH_2 in 2); 2.80 (m, 2H, CH_2 in 1); 3.50 (m, 2H, CH_2 in 3); 4.40 (d, 1H of CH in 11a, J = 7.5 Hz); 4.10-4.90 (CH_2 in 10, J =15 Hz); 7.40 (m, CH_{Ar}).

In the ^{13}C NMR ($CDCl_3$) (δ ppm), we noted: 12.3 (CH_3); 23.0 (C2); 30.0 (C1); 51.6 (C3); 53.5 (C1); 66.2 (C-11a); 122.0-132.5 CH_{Ar};: 138.6, 138.8 Cq; 190.2 (C=S); 197.7 (C=S).

Spectrum of mass (I.E.): m/z = 276 (M^+).

Screening of antibacterial activity. The screening results of antibacterial activity of the pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dione and the pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dithione at: 1 mg mL^{-1} concentration (Table I). For pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dithione, showing activity against Gram positive and Gram negative bacteria, were tested with others concentrations; 0.1 mg mL^{-1} (Table II) and 0.01 mg mL^{-1} (Table III).

Table I. Results of screening antibacterial activity of products 1 and 2 of PBDs

Tested bacteria	Product 1	Product 2
	Diameters (mm)	
Pseudomonas aeruginosa	_	56
Staphylococcus aureus ATCC 25923	_	51
Stap. epidermidis	_	50
Streptococcus agalactiae	_	49
Enterococcus faecalis	_	48
Ent. faecium	_	56
Klebsiella pneumoniae	_	50
K. oxytoca	_	47
Escherichia coli ATCC 25922	_	49
Esch. coli (0157)	_	51
Esch. coli	_	50
Enterobacter cloacae	_	48
Morganella morganii	_	52
Stap. xylosus	_	56
Listeria monocytogenes (1)	_	52
L. monocytogenes (2)	_	50
Neisseria meningitidis	_	50
Streptococcus spp	_	49
Stre. pneumoniae	_	48
Heamophilus influezae b	_	51
Proteus mirabilis	_	49
Serratia marcescens	_	49
Salmonella enteritidis (2)	_	50
Salm. arizonae (2)	_	51

(-): Antibactirial activity absent

Table II. The effect of product 2 at 0, 1 mg mL^{-1} concentration

Bacteria tested	Diameters (mm)
Pseudomonas aeruginosa	24
Staphylococcus aureus ATCC 25923	15
Stap. epidermidis	19
Streptococcus agalactiae	21
Enterococcus faecalis	14
Ent. faecium	26
Klebsiella pneumoniae	24
K. oxytoca	24
Escherichia coli ATCC 25922	22
Esch. coli (0157)	28
Esch. coli	26
Enterobacter cloacae	24
Morganella morganii	32
Stap. xylosus	25
Listeria monocytogenes (1)	24
L. monocytogenes (2)	24
Neisseria meningitidis	20
Streptococcus spp	21
Stre. pneumoniae	20
Heamophilus influezae b	17
Proteus mirabilis	19
Serratia marcescens	21
Salmonella enteritidis (2)	20
Salm. arizonae (2)	20

DISCUSSION

Naturally occurring pyrrolo [2, 1-c][1, 4] benzodiazepines (PBDs) have attracted the attention of many researchers largely, because of the potent anticancer and antibacterial activities exhibited in most of the

Table III. The effect of product 2 at 0, 01 mg mL^{-1} concentration

Bacteria strains tested	Diameters (mm)
Pseudomonas aeruginosa	8
Staphylococcus aureus ATCC 25923	9
Stap. epidermidis	10
Streptococcus agalactiae	9
Enterococcus faecalis	7
Ent. faecium	9
Klebsiella pneumoniae	8
K. oxytoca	9
Escherichia coli ATCC 25922	10
Esch. coli (0157)	9
Esch. coli	12
Enterobacter cloacae	7
Morganella morganii	8
Stap. xylosus	10
Listeria monocytogenes (1)	7
L. monocytogenes (2)	10
Neisseria meningitidis	8
Streptococcus spp	8
Stre. pneumoniae	9
Heamophilus influezae b	10
Proteus mirabilis	7
Serratia marcescens	10
Salmonella enteritidis (2)	8
Salm. arizonae (2)	8

Table IV. Determination of minimal inhibitory concentration (MIC) of product 2

Bacteria strains tested	MIC (µg mL^{-1})
Pseudomonas aeruginosa	100
Staphylococcus aureus ATCC 25923	100
Stap. epidermidis	0,39
Streptococcus agalactiae	100
Enterococcus faecalis	50
Ent. faecium	50
Klebsiella pneumoniae	100
K. oxytoca	100
Escherichia coli ATCC 25922	100
Esch. coli (0157)	100
Esch. coli	100
Enterobacter cloacae	25
Morganella morganii	100
Stap. xylosus	100
Listeria monocytogenes (1)	12,5
L. monocytogenes (2)	12,5
Neisseria meningitidis	12,5
Streptococcus spp	12,5
Stre. pneumoniae	12,5
Heamophilus influezae b	100
Proteus mirabilis	50
Serratia marcescens	100
Salmonella enteritidis (2)	0,39
Salm. arizonae (2)	0,39

compounds with this ring system (Tsugaya *et al.*, 1986). The PBD compounds exhibited a wide spectrum activity against Gram negative and Gram positive bacteria. Therefore, these products are reported to exert their biological activity by covalently binding to the N2 of guanine in the minor groove of DNA through the imine or imine equivalent functionality at N10-C11 of the PBD ring system and thus interfere with DNA function (Kamal *et al.*, 2000). The carbinolamine containing PBD was first prepared by Leimgruber (Leimgruber *et al.*, 1968).

In recent years, many synthetic PBDs have been

Scheme 1: Reagents and conditions: DMF, K_2CO_3, BTBA, 24 h at room temperature

Scheme 2: Reagents and conditions: Pyridin, Δ, 48 h

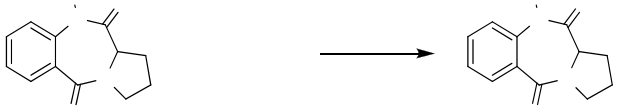
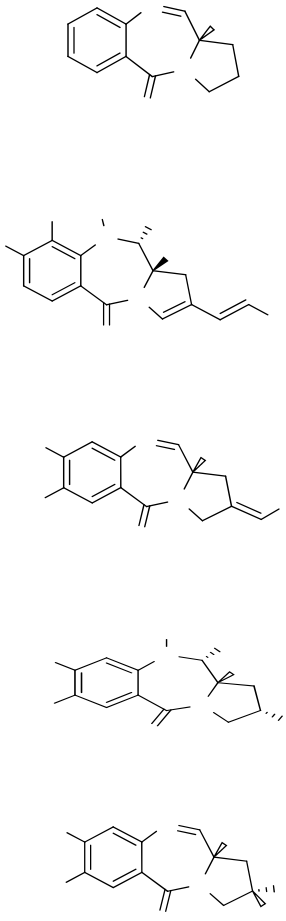


Fig. 1. Some examples of PBDs defined as antitumour antibiotics



prepared to study their recognizing and bonding capabilities. Indeed, the PBDs have been shown to interfere with the interaction of endonuclease enzymes of DNA and block the transcription by inhibiting RNA polymerase in a sequence specific manner. The PBDs have also been used as a scaffold to attach different type of moieties leading to novel sequence selective DNA cleaving and cross-linking agents. This improvement in the biological profile has been explained on the basis of certain factors like DNA cross-linking and doubling of DNA binding sites (Kamal *et al.*, 2002a & b).

A novel sequence selective PBD dimer has been developed, which produces inter-strand cross-links at embedded Pu-GATC-Py target sites within duplex DNA, these PBDs dimer are currently in Phase I clinical development. One of these compounds comprised two PBD units tethered through a three-carbon diether linkage, possesses potent bactericidal activity against five species of Gram positive bacteria, whereas the PBD is included in the adenine dinucleotide (AND) of the bacterium modifying its genome and thus interfering in the process of replication of the ADN and inducing the death of the cell (Vassilva *et al.*, 2005).

We confirmed the antibacterial activity of PBDs, by testing the simple PBD against various Gram positive and Gram negative bacteria. However, the pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dithione exerted a very clear effect against various bacteria. Thus this product can be recognized as an effective antibacterial drug against several species of bacteria. Indeed, the PBD2 forms a part of precise sites on the bacterial genome (Benzeid *et al.,* 2008) and to increase the antibacterial effectiveness of the PBD, we subjected the simple pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dione to an alkylation by the ethylbromide, the product EthylPBD was ineffective, but we had recourse to a reaction of thionation of the EthylPBD. The Ethylpyrrolo [2, 1-c][1, 4] benzodiazepine-5,11-dithone was greatly effective against all the types of bacteria as the results of the MIC (Table IV). This is because the sulphur is an atom, which is recognized for its toxicity, instead of the oxygen atom fixed on the EthylPBD (Benzeid *et al.,* 2008).

Let use note that, by the alkylation of the pyrrolo [2, 1-c] [1, 4] benzodiazepine-5,11-dione, we succeeded in increasing its antibacterial activity, indeed the MIC had dropped at a lot of types of bacteria by report with the results of the antibacterial activity of the simple PBD (Benzeid *et al*., 2008), by order *Staphylococcus epidermidis* and *Salmonella* species have the most important MIC (0,39 $\mu g.mL^{-1}$), follow up by *Listeria* species, *Neisseria meningitidis* and *Streptococcus* species (12,5 $\mu g\ mL^{-1}$), *Enterobacter cloacae* (25 $\mu g\ mL^{-1}$) and *Enterococcus* species and *Proteus mirabilis* (50 $\mu g\ mL^{-1}$). All other bacteria have 100 $\mu g\ mL^{-1}$ as MIC.

In conclusion, the antibacterial activity of the PBD rose after alkylation then thionation, which led us to think of the application of new agents alkylants to highlight the antibacterial activity of the PBD to constitute a new class of antibacterial agents against Gram positive and Gram negative pathogens. This is the reason why we tested other derivatives of the PBDs alkylated against various bacteria, which we will report shortly.

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