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Antibacterial Activity of novel Pyrrolobenzodiazepines

H. BENZEID, M. CHAMMACHE, E.M. ESSASSI, B. IMELOUANE†, F. OHMANI†, R. CHAROF† AND K. KHEDID[1]†
College of Sciences, Laboratory of Organic Heterocyclic Chemistry, Department of Chemistry, University Mohamed V, BP 1014 Avenue Ibn Batouta, Rabat, Morocco
†*National Institute of Health BP 769, Avenue Ibn Batouta, Rabat, Agdal, Morocco*
[1]Corresponding author's e-mail: kkhedid200605@yahoo.fr

ABSTRACT

The pyrrolobenzodiazepines (PBDs) constitute a class of pharmacological products recognized clinically for their effectiveness as much as antitumoral antibiotics. In present study, we report another pharmacological property as antibacterial activity of PBDs. Antibacterial tests were carried out on a range of gram positive and gram negative bacteria, which proved successful for the sulphurate PBD. The results of the minimal inhibiting concentration (MIC) varied from one bacterium to another. A great affinity of the PBD was found against *Streptococcus* and *Salmonella* species.

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

INTRODUCTION

The important progression of the bacterial infections and the recrudescence of resistance to antibiotics used currently encourage the researchers to look for new active molecules. Naturally occuring pyrrolo [2,1-c] [1,4] benzodiazepines (PBDs) have attracted the attention of many scientists because of their anticancer activity exhibited in most of the compounds with this ring system. 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 (Kaneko *et al.*, 1983; Peña & Stille, 1987; Krčméry & Sefton, 2000; Djipa *et al.*, 2000; Sagnou *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, neothramycin and DC-81 (Fig. 1).

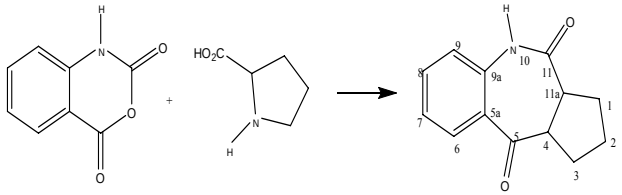
In present work, novel PBDs derivatives of the benzodiazepine are studied for their antibacterial activity. An attempt has been made to highlight the antibacterial activity of PBDs by testing their antibacterial action against several types of bacteria.

MATERIALS AND METHODS

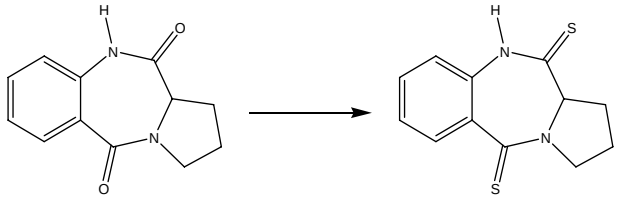
Synthesis of novel pyrrolobenzodiazepines. To develop this study, we prepared two products from the derivatives of PBDs (Fig. 2). PBD was obtained by condensation of isatoic anhydrid with L-proline in the Dimethylformamid (Scheme 1):

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

Scheme 1. Reagents and conditions: DMF, Δ, 5 h



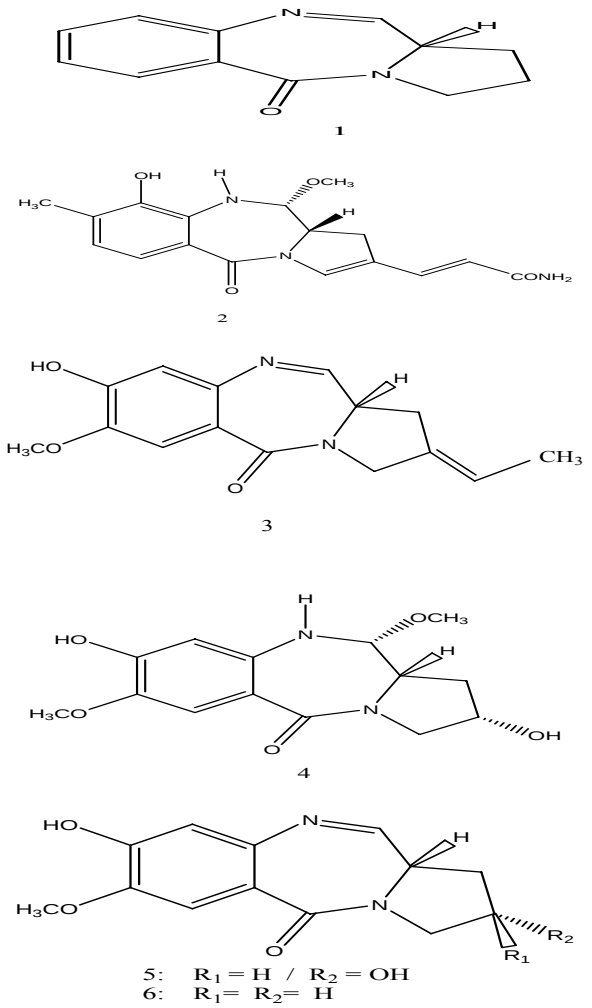
Scheme 2. Reagents and conditions: Pyridin, Δ, 48 h



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 (IAVM), 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 having various 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,

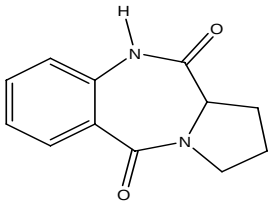
Fig. 1. Some examples of PBDs defined as antitumour antibiotics



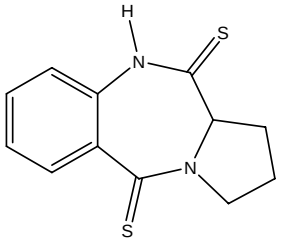
ampicillin, ticacillin, cephalotin, cefoxitin, cefamandole, cefotaxim, ceftazidim, kanamycin, gentamicin, tobramycine, penicullin g, oxacillin, erythromycin, clindimycin, pristinamycin, trimethoprim, sulphamethoxazole, cipofloxacin, ofloxacin, chloramphenicol, tetracycline, rifampicin, vancomycin, imipenem, teicoplanin and fusidic acid were studied by using a slighthly 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 McFarland 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 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

Fig. 2. Representative of PBDs tested



(1):The pyrrolo [2, 1-c] [1, 4] benzodiazepine - 5, 11- dione



(2):The pyrrolo [2, 1-c] [1, 4] benzodiazepine - 5, 11- dithione

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 106 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 ^{1}H NMR spectra, ^{13}C NMR spectra and spectrum of mass. For the first product, the pyrrolo [2, 1-c] [1, 4] benzodiazepine-5, 11-dione, ^{1}H 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) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants are reported in Hertz (Hz).

IR (R): υ(C=O) =1625 et 1675 cm^{-1}; υ(NH) =3200-3400 cm^{-1}; spectrum of mass (I.E.): m/z = 216 ($M^{+\cdot}$); ^{1}H NMR (DMSO-d_6) (δ ppm, J Hz): 1,89 (m, 4H, CH_2 in 1 & 2); 3,55 (m, 2H,CH_2 in 3); 4,09 (d, 1H, J=2,28 Hz, CH in 11a); 7,10 - 7,80 (m, 4H, CH_{Ar}); 10,49(s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ ppm): 25,7 (C1); 22,9 (C2); 46,8 (C3); 56,1 (C11a); CH_{Ar}: 122,2; 123,4; 130,8 et 132,0; Cq: 126,5 (Benz); 136,2 (Benz); 164,4 (C=O); 170,6 (C=O).

Second product was recognized with following specifications. In the ^{1}H NMR spectra, we used m: 2.21 ppm ; C1 and C2. m: 3,9 ppm; C3. d: 4.9 ppm; H in 11a. In the ^{13}C NMR spectra, we noted three signals at 23.2 ppm; 29.9 ppm and 55.3 ppm; CH_2 in positions 1, 2 and 3, a signal towards 65.5 ppm corresponding at proton in position 11a and a signal at 190.5 ppm and 200.3 ppm; presence of two thiolactames groups. Spectrum of mass (I.E.): m/z = 248 ($M^{+\cdot}$).

Result screening of antibacterial activity. The screening results of antibacterial activity of the pyrrolo [2,1-c] [1,4]

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

Tested bacteria	Product 1	Product 2
	Diameters (mm)	
Pseudomonas aeruginosa	–	47
Staphylococcus aureus ATCC 25923	–	48
Staphylococcus epidermidis	–	47
Streptococcus agalactiae	–	48
Enterococcus faecalis	–	44
Enterococcus faecium	–	52
Klebsiella pneumoniae	–	44
Klebsiella oxytoca	–	48
Escherichia coli ATCC 25922	–	48
Escherichia coli (0157)	–	51
Escherichia coli	–	51
Enterobacter cloacae	–	44
Morganella morganii	–	52
Staphylococcus xylosus	–	47
Listeria monocytogenes (1)	–	55
Listeria monocytogenes (2)	–	45
Neisseria meningitidis	–	48
Streptococcus spp	–	50
Streptococcus pneumoniae	–	51
Heamophilus influezae b	–	49
Proteus mirabilis	–	47
Serratia marcescens	–	50
Salmonella enteritidis (2)	–	49
Salmonella arizonae (2)	–	50

(-): Antibactirial activity absent

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

Bacteria tested	Diameters (mm)
Pseudomonas aeruginosa	21
Staphylococcus aureus ATCC 25923	20
Staphylococcus epidermidis	20
Streptococcus agalactiae	18
Enterococcus faecalis	14
Enterococcus faecium	22
Klebsiella pneumoniae	12
Klebsiella oxytoca	22
Escherichia coli ATCC 25922	15
Escherichia coli (0157)	20
Escherichia coli	16
Enterobacter cloacae	18
Morganella morganii	14
Staphylococcus xylosus	16
Listeria monocytogenes (1)	17
Listeria monocytogenes (2)	16
Neisseria meningitidis	18
Streptococcus spp	20
Streptococcus pneumoniae	17
Heamophilus influezae b	19
Proteus mirabilis	18
Serratia marcescens	20
Salmonella enteritidis (2)	19
Salmonella arizonae (2)	18

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, which showed activity against Gram positive and Gram negative bacteria, were been tested with others concentrations: 0.1 mg mL^{-1} (Table II) and 0.01 mg mL^{-1} (Table III).

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

Bacteria strains tested	Diameters (mm)
Pseudomonas aeruginosa	7
Staphylococcus aureus ATCC 25923	8
Staphylococcus epidermidis	10
Streptococcus agalactiae	7
Enterococcus faecalis	8
Enterococcus faecium	7
Klebsiella pneumoniae	6
Klebsiella oxytoca	10
Escherichia coli ATCC 25922	7
Escherichia coli (0157)	9
Escherichia coli	9
Enterobacter cloacae	8
Morganella morganii	8
Staphylococcus xylosus	7
Listeria monocytogenes (1)	6
Listeria monocytogenes (2)	7
Neisseria meningitidis	9
Streptococcus spp	7
Streptococcus pneumoniae	10
Heamophilus influezae b	6
Proteus mirabilis	8
Serratia marcescens	6
Salmonella enteritidis (2)	10
Salmonella arizonae (2)	9

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

Bacteria strains tested	MIC (µg mL^{-1})
Pseudomonas aeruginosa	100
Staphylococcus aureus ATCC 25923	100
Staphylococcus epidermidis	1,56
Streptococcus agalactiae	100
Enterococcus faecalis	25
Enterococcus faecium	100
Klebsiella pneumoniae	100
Klebsiella oxytoca	100
Escherichia coli ATCC 25922	100
Escherichia coli (0157)	100
Escherichia coli	100
Enterobacter cloacae	100
Morganella morganii	100
Staphylococcus xylosus	100
Listeria monocytogenes (1)	100
Listeria monocytogenes (2)	100
Neisseria meningitidis	100
Streptococcus spp	12,5
Streptococcus pneumoniae	10
Heamophilus influezae b	100
Proteus mirabilis	50
Serratia marcescens	100
Salmonella enteritidis (2)	1,56
Salmonella arizonae (2)	1,56

DISCUSSION

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 PBDs have shown to interfere with the interaction of endonuclease enzymes of DNA and block the transcription by inhibiting RNA polymerase in a sequence specific manner, which are thought to account for the biological activity of PBDs. The PBDs have also been used as a scaffold to attach different type of moieties leading to novel sequence selective DNA cleaving and crosslinking 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 is currently in Phase I clinical development 19. This compound 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 two derivatives of PBDs, the PBD1 and the PBD2 against various Gram-positive and of Gram-negative bacteria. However, the PBD1 did not show antibacterial activity, while PBD2 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 is forms a part of precise sites on the bacterial genome.

The studies showed that PBDs cross the bacterial cytoplasmic membrane effectively (Vassilva *et al.*, 2005). The effectiveness of the PBD2 is probably attributed to the presence of two atoms of sulphur on the product. The sulphur is an atom, which is recognized for its toxicity, instead of the oxygen atom fixed on the PBD1. The results obtained for the determination of the MIC showed a great effectiveness of the PBD2 as much as antibacterial agent for all the species of bacteria (Table IV). The MIC of *Salmonella* and *Streptococcus* spp. was higher than all tested bacteria.

In conclusion, the potency of this new class of agents against bacteria is high and there appears to be an opportunity for the design of sequence-selective PBDs that have a high affinity for motifs within the DNA of key Gram-positive and Gram negative pathogens. This is the reason why we tested other derivatives of the PBDs against various bacteria.

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