Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2435–2437

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Activity of *p*-Methoxybenzylisothiocyanate Against *Neisseria gonorrhoeae*, *Haemophilus ducreyi*, and Other Microorganisms

Henk Swart,* Sandra Van Dyk and Sarel F. Malan

Pharmaceutical Chemistry, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, Private Bag X6001, Potchefstroom 2520, South Africa

Received 28 January 2002; accepted 13 May 2002

Abstract—*p*-Methoxybenzylisothiocyanate was isolated from *Lepidium bonariense* and found to be responsible for the plants antimicrobial and STD activity. MIC determinations were conducted for *p*-methoxybenzylisothiocyanate on *Haemophilus ducreyi, Neisseria gonorrheae, Candida albicans*, *Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Enterobacter* sp., *Escherichia coli, Klebsiella pneumoniae,* and *Psuedomanas aeruginosa*. An in vitro cellular toxicity assay showed that at 100 μM (17,9 μg/mL) *p*-methoxybenzylisothiocyanate is not toxic to living cells.

It has only been within the last 20 years that an interest has developed with regard to the true potential of traditional medicines.[1] The interest in the use of traditional medicines is fuelled by the development of resistance to certain 'western' medicines by the pathogens responsible for disease. It is hoped that research into the traditional medicinal plants could lead to the discovery of new drugs or that the use of indigenous medicines will be promoted as a method of enhancing orthodox treatments of disease.[1]

The global problem pertaining to sexually transmitted diseases (STDs) is well documented[2,3] as is the development of resistance by some of these organisms against traditional treatment regimens, particularly in third world countries. During the advent of antibiotics, *Neisseria gonorrhoeae* and *Haemophilus ducreyi* were highly sensitive to them. The development of resistance has caused penicillin and tetracyclines to be virtually ineffective in the treatment of *N. gonorrhoeae*. As resistance against the antibiotics developed, the resistant strains could initially be treated by increased dosage, but in 1985 the emergence of total therapeutic failure by antibiotics and tetracyclines was described.[4] A possible solution to the problem is the discovery of new compounds, from plants, which are effective against the resistant STD strains.

A thorough literature study was conducted on plants commonly used by South African traditional healers in the management of the curable STD's. After initial screening, *Lepidium bonariense*[1] was selected for further antibacterial and STD screening.

L. bonariense is a dicotyledon and member of the Cruciferae family.[5,6] It is native to South America, but is widespread throughout South Africa as a weed and is commonly known as 'umathoyisa', birdseed, pepper cress, pepper weed, and 'peperbossie'.[6]

Mustard oil glycosides are known to be present in plants belonging to the Cruciferae family.[7] When plant cells are disrupted, the mustard oil glycosides are enzymatically converted to isothiocyanic acid esters (mustard oils) that have been found to possess antimicrobial activity against certain moulds and bacteria.[8] These compounds and the above plant species have never been tested against STDs and antibiotic resistant bacteria.

H. ducreyi, N. gonorrheae, and *Candida albicans* were chosen as STD test pathogens. The following antibiotic and penicillin sensitive organisms (henceforth collectively known as the antibiotic sensitive organisms) were also included for screening: *Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Enterobacter*

*Corresponding author. Fax: +27-18-299-4243; e-mail: fchhs@puknet.puk.ac.za

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00437-7

sp., *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*.

L. bonariense possessed antibacterial activity against all the organisms screened. The roots presented with the greatest degree of antibacterial activity and were selected for extraction purposes (Fig. 1).

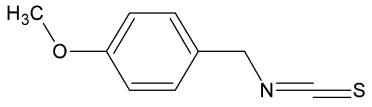

Figure 1. *p*-Methoxybenzylisothiocyanate.

The data obtained revealed that the compound responsible for the antibacterial and STD activity within *L. bonariense* is *p*-methoxybenzylisothio cyanate.[16] The physicochemical data corresponds to that described by Migirab et al.[9] The compound was tested for verification against the bacteria and STDs. It possessed antibacterial activity against all the organisms tested.

The MIC values obtained for the organisms screened are presented in Table 1. Ampicillin, Doxycycline, and Ciprofloxacin were used as positive controls for the bacteria, and Amphoteracin B and Fluconazole were used as positive controls for the fungus *C. albicans*. Antibiotic resistant strains of *P. aeruginosa*, *S. aureus,* and Penicillinase producing *N. gonorrhoeae* (PPNG), were also included in the MIC evaluations.

Table 1. MIC values obtained for the bacteria and STDs screened

Organisms	**1** (μg/mL)	**2** (μg/mL)	**3** (μg/mL)	**4** (μg/mL)
B. subtilus	>32	8	2	32
M. luteus	2	8	2	8
S. aureus	2	8	2	2
Enterobacter sp.	16	8	2	8
E. coli	8	2	1	32
P. aeruginosa	>32	8	2	16
N. gonorrhoeae	0.25	0.5	0.25	4
PPNG	>32	>32	0.25	4
H. ducreyi	2	4	1	64
Resistant *S. aureus*	>32	>32	>32	8
Resistant *P. aeruginosa*	>32	>32	>32	16

1 = Ampicillin; **2** = Doxycycline; **3** = Ciprofloxacin; **4** = *p*-methoxybenzylisothiocyanate

The MIC value of *p*-methoxybenzylisothiocyanate for *C. albicans* of 2 μg/mL also compared reasonably well with the control MIC values of 2 μg/mL for Amphoteracin B and 0.25 μg/mL for Fluconazole.

Analysis of the Graham cell line graph (Fig. 2), shows a Log [*p*-methoxybenzylisothiocyanate] value of 2 at approximately 100% cellular viability. The concentration of *p*-methoxybenzylisothiocyanate at 100% cellular viability is thus 100 μM (17.9 μg/mL).

The screening results showed that *L. bonariense* possesses antibacterial activity against the STDs, *N. gonorrhoea*,

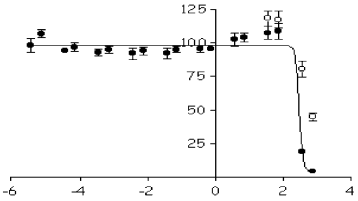

Figure 2. Graham cell line viability in the presence of *p*-methoxybenzylisothiocyanate.

H. ducreyi, and *C. albicans*, as well as the antibiotic sensitive and resistant organisms. The compound present in *L. bonariense*, responsible for its antibacterial properties was found to be *p*-methoxybenzylisothiocyanate.

An evaluation of the MIC results showed that *p*-methoxybenzylisothiocyanate presents with reasonably good activity against the selected range of antibiotic sensitive organisms when compared to the control antibiotics. The MIC values of 4 μg/mL against *N. gonorrhoeae* and 64 μg/mL against *H. ducreyi* are higher that the control antibiotics MICs, but it is none the less significant that *p*-methoxy-benzylisothiocyanate does possess antibacterial activity against these two STDs. It is also important to note that the MIC values of 4 μg/mL against PPNG, 8 μg/mL against antibiotic resistant *S. aureus*, and 16 μg/mL against antibiotic resistant *P. aeruginosa* are significantly lower than the MIC values of the control antibiotics. The antibacterial potency of *p*-methoxybenzylisothiocyanate is thus not decreased by antibiotic resistant *S. aureus*, *P. aeruginosa*, and PPNG strains.

The results of the in vitro cellular toxicity assay show that at a concentration of 100 μM (17.9 μg/mL), which is higher than the MIC values of *N. gonorrhoeae,* PPNG, antibiotic resistant *S. aureus,* and *P. aeruginosa*, *p*-methoxybenzylisothio-cyanate is not toxic to Graham cell lines. The use of *p*-methoxybenzylisothiocyanate for the treatment of *H. ducreyi* (MIC = 64 μg/mL), would warrant further cellular toxicity assays to determine the toxicity at this high concentration.

The method employed for the initial bacterial screening was the direct plate test as performed by Van der Vijver and Lötter.[10]

Extraction of *L. bonariense* roots were done on a Soxhlet extractor with petroleum ether, chloroform, ether and ethanol as solvents. The finely chopped roots were extracted with each solvent for 24 h at 60 °C. Screening of the extracts revealed that the active compound was present within the ether extract.

Isolation of the active compound from the ether extract was done by column chromatography with a mobile phase of 10:1:1 of petroleum ether–dichloromethane–ethyl acetate. Once isolated, structure elucidation of the active compound was conducted using NMR, IR, and MS.

MIC values were determined for *p*-methoxybenzyl-isothiocyanate using the quick microplate method.[11]

Bacterial cultures were standardised at a concentration of approximately 10^7 CFU/mL with the use of a spectrophotometer at 500 nm.[12]

p-Methoxybenzylisothiocyanate and control antibiotic concentrations of 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 μg/mL were obtained per well. The final bacterial concentration within each well was approximately 5×10^5 to 5×10^6 CFU/mL.

Brown's dilute agar method was used for the MIC determinations of both *N. gonorrhoeae*[13] and *H. ducreyi*.[14] Final concentrations of 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 μg/mL *p*-methoxybenzyl-isothiocyanate were used. For *N. gonorrhoeae*, the media used was GC agarbase, without vancomycin, but 1% Isovitalex and foetal calf serum.[13] For *H. ducreyi*, the medium used was Charcoal Colombia agar base with foetal calf serum and 1% Isovitalex.[14] Bacterial concentrations of 10^8 CFU/mL *N. gonorrhoeae* and *H. ducreyi* were used.

Cellular viability in the presence of *p*-methoxy benzyl-isothiocyanate was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide) microculture tetrazolium assay.[15] The toxicity evaluation was performed on Graham 293 cells. The Graham cells were cultured in Ham's F10 media containing 5% foetal calf serum (FCS) and 0.1% gentamicin.

Taking into regard the problems pertaining to resistance development by the STD pathogens and bacteria to current treatment regimens, the isothiocyanate compounds might become a viable option for anti-STD and antibacterial research against these resistant strains.

References and Notes

1. Hutchings, A.; Scott, A. H.; Lewis, G.; Cunningham, A. *Zulu Medicinal Plants*; University of Natal Press: Pietermaritzburg, 1996; p 450.
2. Sciarra, J. J. *Int. J. Gynecol. Obstet.* **1997**, *58*, 107.
3. World Health Organization. *An Overview of Selected Curable STDs*; http://www.who.int/asd/akagmer.html, 1995 (date of use: 22/02/99 4:13 PM).
4. Ison, C. A.; Dillon, J. R.; Tapsall, J. W. *Lancett* **1998**, *351*, 8.
5. Plowden, C. C. *A Manual of Plant Names*; Allen & Unwin: London, 1968; p 260.
6. Wells, M. J. *A Catalogue of Problem Plants in South Africa, Incorporating the National Weed List of South Africa*; Botanical Research Institute: Pretoria, 1986; p 658.
7. Trease, G. E.; Evans, W. C. *Pharmacognosy*, 12th ed.; Baillière Tindal: Eastbourne, 1983; p 812.
8. Vitanen, A. I. *Phytochemistry* **1965**, *4*, 207.
9. Migirab, S. E.; Berger, Y.; Jadot, J. *Phytochemistry* **1977**, *16*, 1719.
10. Van der Vijver, L. M.; Lötter, A. P. *Planta Med.* **1971**, *20*, 9.
11. Eloff, J. N. *Planta Med.* **1998**, *64*, 711.
12. Tilton, R. C. *Appl. Microbiol.* **1973**, *26*, 658.
13. Ison, C. A. *Genitourin. Med.* **1996**, *72*, 253.
14. Lagergård, T.; Frisk, A.; Trollfors, B. *J. Antimicrob. Chemother.* **1996**, *38*, 849.
15. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.
16. Light-green oil; R_f 0.48 (petroleum ether–dichloromethane–ethyl acetate 10:1:1); C_9H_9NOS; HRMS; M^+ 179.0401; *m/z* (EI,%) 179 (8), 121 (100), 78 (10), 69 (17), 57 (10); ν_{max} (KBr, cm^{-1}) 1046, 1183, 1250, 1304, 1357, 1432, 1463, 1511, 1621, 2086, 2168, 2827, 2925; δ_H (300.075 MHz, $CDCl_3$) 3.8 (s 3H CH_3O), 4.6 (s 2H CH_2), 6.9 (d 2H, $J=8.8$ C-2 C-6), 7.2 (d 2H $J=8.8$ C-3 C-5); δ_C (75.462 MHz, $CDCl_3$) 48.28 (CH_2), 55.29 (CH_3O), 114.42 (C-2; C-6), 126.18 (C-4 or NCS), 128.45 (C-3; C-5), 159.82 (C-1).