10th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-10). 1-30 November 2006. http://www.usc.es/congresos/ecsoc/10/ECSOC10.htm & http://www.mdpi.org/ecsoc-10/

[c014]

Antimicrobial evaluation of benzo[*a*]phenoxazine heterocycles: structure – activity relationships

V. H. J. Frade, M. J. Sousa[a], J. C.V. P. Moura and M. S. T. Gonçalves*

Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[a] *Centro de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

msameiro@quimica.uminho.pt

Abstract – A series of functionalised 5,9-diaminobenzo[*a*]phenoxazinium salts, in the free form or linked to L-glycine and L-valine amino acids were evaluated against *Saccharomyces cerevisiae*, in a microdilution broth assay. The results obtained showed that these compounds exhibited antifungal activity depending on the substituents of the 5,9-diaminobenzo[*a*]phenoxazine nucleous. The best activities were obtained when substituents at the positions 9 and 10 were NHEt, and Me, respectively, and at the position 5, $NH(CH_2)_3Cl$ or $NH(CH_2)_3CO_2Et$.

1. Introduction

Cationic benzo[*a*]phenoxazine heterocycles are long-wavelength dyes, which have been used as fluorescent probes in various applications, such as for monitoring hydrophobic surfaces in proteins, as lipid stains in membranes and also to study the interaction between the probe and DNA and its application in electrochemical recognition.[1-3] 5*H*-Pyridophenoxazin-5-ones were also reported as exhibiting activity against leukemia and solid tumor cell lines at submicromolar concentrations.[4]
Synthesis, photophysical characterisation and application of benzo[*a*]phenoxazines have been a subject of interest in our research work.[5-6] The investigation of their antimicrobial activity started recently[7] and the results obtained were promising. Bearing this in mind, and in view of the continuous interest in new antimicrobial agents, a series of 5,9-diaminobenzo[*a*]phenoxazinium salts, in the free form or linked to L-glycine and L-valine amino acids were studied concerning the effects of their structural modifications on activity against *Saccharomyces cerevisiae* W303-1B.

2. Results and Discussion

5,9-Diaminobenzo[*a*]phenoxazinium salts **1a-o** were prepared by condensation of 5-alkylamino-2-nitrosophenol hydrochlorides with 1-naphthylamine or its *N*-alkylated derivatives, in an acidic

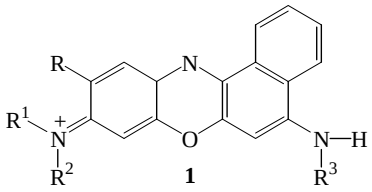
medium.[8] Compounds **1l-o** were obtained by the coupling of the functionalised heterocycles **1c**, **1f**,**g** and **1i** with *N*-*tert*-butyloxycarbonyl-L-glycine, *N*-*tert*-butyloxycarbonyl-L-valine or L-valine methyl ester with the aid of *N*,*N*'-dicyclohexylcarbodiimide (DCC) assisted by 1-hydroxybenzotriazole (HOBt), under standard conditions.[9]

Minimum Inhibitory Concentrations (MIC) were determined for the different compounds using a broth microdilution method for antifungal susceptibility testing of yeasts. The activity of compounds **1a-o** against *Saccharomyces cerevisiae* was dependent on the substituents of the benzo[*a*]phenoxazine moiety (Scheme 1). By comparison of compounds **1a-c**, which have different R, R^1 and R^2 (R^3 = H), it was possible to conclude that dye **1c** (MIC 15 µM) was the most active. When $R^3 = (CH_2)_3OH$, the same combination of R, R^1 and R^2 generated the best activity, although the introduction of this R^3 group decreased the activity in compounds **1e** and **1f**. Maintaining R = Me, R^1 = H and R^2 = Et (**1f-j**), the most active combination, the antimicrobial activity depended on the R^3 substituents. The best activities were obtained when the hydroxyl (**1f**) was substituted by a chloride atom (**1j**) or changed by an ethyl ester (**1h**). The presence of an amine function (**1i**) also increased the activity, whereas the presence of a carboxyl group (**1g**) produced the highest MIC. With the exception of compound **1g**, the activity of compounds was equal or superior to that of Nile Blue A (**1b**, MIC 60 µM).

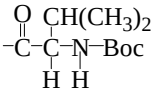
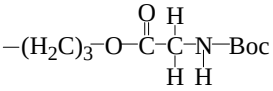
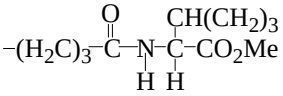
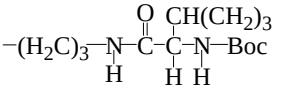
The linkage of the functionalised benzo[*a*]phenoxazine dyes to L-glycine or L-valine amino acids gave compounds **1l-o**, which also showed activity against *Saccharomyces cerevisiae* (MIC 30 µM **1m** and **1o**, or 60 µM **1l** and **1n**). In the case of compounds **1m** (30 µM) and **1n** (60 µM), their activity was superior to that of their precursors **1f** (60 µM) and **1g** (120 µM), respectively.

The benzo[*a*]phenoxazine derivatives presented significant antifungal activity, arising as good candidates for further studies. From the different group combinations tested (R, R^1 and R^2), the best activity was observed for R = Me, R^1 = H and R^2 = Et, suggesting a possible role for the hydrogen atom of the amino group in the activity of these compounds.

The activities found for compounds with different R^3 substituents apparently did not correlate with the polarity or the size of these groups. In order to elucidate the bases for the differences found, further studies will be carried out.



1

Compound	R	R^1	R^2	R^3	MIC[a]
1a	H	Me	Me	H	> 120
1b [b]	H	Et	Et	H	60
1c	Me	H	Et	H	15
1d	H	Me	Me	$(CH_2)_3OH$	120
1e	H	Et	Et	$(CH_2)_3OH$	120
1f	Me	H	Et	$(CH_2)_3OH$	60
1g	Me	H	Et	$(CH_2)_3CO_2H$	120
1h	Me	H	Et	$(CH_2)_3CO_2Et$	7.5
1i	Me	H	Et	$(CH_2)_3NH_2$	30
1j	Me	H	Et	$(CH_2)_3Cl$	3.75
1l	Me	H	Et		60
1m	Me	H	Et		30
1n	Me	H	Et		60
1o	Me	H	Et		30

Scheme 1. Activity against of *Saccharomyces cerevisiae* W303-1B of 5,9-diaminobenzo[*a*]phenoxazines **1a**-**o**. [a] Minimal Inhibitory Concentration of growth (μM); [b] This compound is Nile Blue A. Boc: *N-tert*-Butyloxycarbonyl.

3. Experimental

Antifungal activity tests. Broth microdilution assays were performed in accordance with the guidelines in CLSI document M27-A2[10] using a RPMI 1640 medium (supplemented with the required amino acids), an inoculum of 0.5 x 10^3 cells per mL, and incubation at 30 °C. MICs were determined visually after 48 hours of incubation, as the lowest concentration of drug that caused no detectable growth. Stock solutions of the compounds were prepared in DMSO and a final dilution was carried out in an RPMI 1640 medium (Sigma, St. Louis, Mo.), buffered to pH 7.0 with 0.165 M morpholenepropanesulfonic acid (MOPS) buffer (Sigma). The yeast strain used was *Saccharomyces cerevisiae* W303-1B (*MATa, ade2, his3, leu2, trp1, ura3*).

Acknowledgments

We thank the Fundação para a Ciência e Tecnologia (Portugal) for their financial support to the Centro de Química and Centro de Biologia (Universidade do Minho).

References

1. Sackett, D. L.; Knutson, J.R.; Wolff, J. *J. Biol. Chem.* **1990**, *265*, 14899-14906.
2. Gao, F.; Mei, E.; Lim, M.; Hochstrasser, R. M. *J. Am. Chem. Soc.* **2006**, *128*, 4814-4822.
3. Ju, H.; Ye, Y.; Zhu, Y. *Electrochimica Acta* **2005**, *50*, 1361-1367.
4. Bolognese, A.; Correale, G. Manfra, M; Lavecchia, A.; Mazzoni, O.; Novellino, E.; Barone, V. Colla, P.; Loddo, R. *J. Med. Chem.* **2002**, *45*, 5217-5223.
5. Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Tetrahedron Lett.* **2005**, *46*, 4949-4952.
6. Frade, V. H. J.; Gonçalves, M. S. T.; Coutinho, P. J. G.; Moura, J. C. V. P. *J. Photochem. Photobiol.*, **2006**, doi:10.1016/j.jphotochem.2006.06.013.
7. Frade, V. H. J.; Gonçalves, M. S. T.; Sousa, M. J.; Moura, J. C. V. P., poster communication (P1), *Medicinal Chemistry of 21st Century*, Lisbon, **2006**.
8. Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Tetrahedron Lett.* **2006**, doi: 10.1016/j.tetlet.2006.09.133.
9. a) V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Proceedings of ECSOC-9, 9th International Electronic Conference on Synthetic Organic Chemistry*, http://www.mdpi.net/ecsoc/, A034, **2006**.

b) Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. poster communication (P64), *9th International Conference on the Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes*, Lisbon, **2005**.

10. *National Committee for Clinical Laboratory Standards*. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.