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The effect of methanesulfonylhydrazine and its hydrazones on yeast *Saccharomyces cerevisiae* viability

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Three cytotoxic Schiff bases, previously synthesized by us: salicylaldehyde methanesulfonylhydrazone, 2-hydroxyacetophenone methanesulfonylhydrazone and 2-hydroxy-1-naphthaldehyde methanesulfonylhydrazone, along with acetone methanesulfonylhydrazone and the parent compound, methanesulfonylhydrazine, have been tested for their effect on *Saccharomyces cerevisiae*. All five compounds exhibited moderate antiyeast activity. The effect of the compounds on cell cycle and DNA integrity have been studied by FACS analysis and Comet Assay. The compounds do not block the cells in a specific checkpoint and do not cause breaks in DNA.

Key words: methanesulfonylhydrazones, *Saccharomyces cerevisiae*, FACS analysis, Comet Assay

INTRODUCTION

Sulfonamide drugs are among the most widely used chemotherapeutic agents with a large spectrum of activity [1]. Cytostatic activity of sulfonamide derivatives attracts much attention in the last years [2–4]. Studies on their mechanism of action have revealed an important role of enzyme inhibition. Among the anticancer sulfonamides there are inhibitors of carbonic anhydrases [3–6], of cyclooxygenase-2 [7, 8], and of topoisomerases [9, 10]. Sulfonylhydrazones combine two pharmacophoric fragments, sulfonamide and hydrazine, and some of their representatives have exhibited strong antileukemic activity [11, 12]. Recently we have studied the crystal structure and conformational features of the prototype compound, methanesulfonylhydrazine (MSH) [13], and firstly synthesized and structurally characterized metal coordination compounds of this ligand [14, 15]. We have also synthesized and studied MSH-derived hydrazones [16, 17] and showed that the parent compound and its hydrazones have antibacterial and cytotoxic activity [16]. Here we wish to present our results showing the cytostatic effect of MSH and its azomethine derivatives: salicylaldehyde methanesulfonylhydrazone (**1**), 2-hydroxyacetophenone methanesulfonylhydrazone (**2**), 2-hydroxy-1-naphthaldehyde methanesulfonylhydrazone (**3**) and acetone methanesulfonylhydrazone (**4**) (Fig. 1) on yeast *Saccharomyces cerevisiae*.

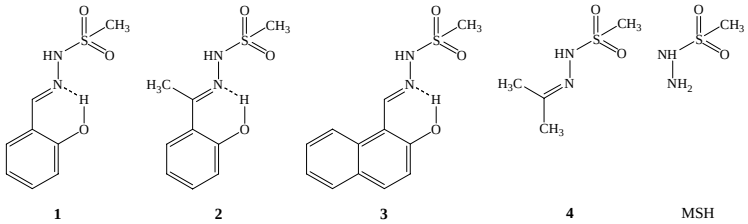

Fig. 1. Structural formulae of MSH and its azomethine derivatives.

MATERIALS AND METHODS

Compounds. MSH was prepared by the method of Powell and Whiting [18], with some modifications [13]. Compound **4** was synthesized according to [18], and **1**–**3** as previously described by us [16].

Yeast strain and cultivation. The strain used in this research was *Saccharomyces cerevisiae* 3A: a/α, gal1, leu 2, ura 3–52. Yeast cultures were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) to a cell density of 10^6 cells/ml and treated by incubation with DMSO solutions of the compounds added in the medium.

Test for viability. Yeast cell cultures were grown to A_{600} = 0.5–0.6 (approx. 10^6 cells/ml). Different con-

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centrations of the compounds, dissolved in DMSO, were added to the media and the cultivation proceeded. After 2 hours of cultivation in the chemicals containing media the cells were collected, washed, resuspended in water and plated in appropriate dilutions on agar plates containing YPD. After 20 hours of incubation at 30 °C the colonies were counted and the percentage viability was calculated – the number of Colony Forming Units of the control cultures (*i. e.* grown without a tested compound) were taken as 100%. Five independent experiments were performed and the results elaborated statistically.

Yeast Comet Assay (YCA). YCA was applied as was developed by us [19].

FACS (Fluorescent Activated Cell Sorter) analysis. Yeast cultures (10^6 cells/ml) were treated by cultivation for 2 hours with 500 μM of the four compounds suspended in the media. The cells were recovered, washed twice, resuspended in fresh YPD media and left to grow at 30 °C. At the times shown in Fig. 3, aliquots were taken from the cultures and subjected to FACS analysis. The analysis was performed as in [20] on a FACS machine FACS Calibur (Beckton Dikinson) equipped with an argon laser operating at λ = 488 nm. The cells in the cultures were analysed by their DNA content according to propidium iodide inclusion. Three independent experiments were performed. An example of one of them is shown in Fig. 3.

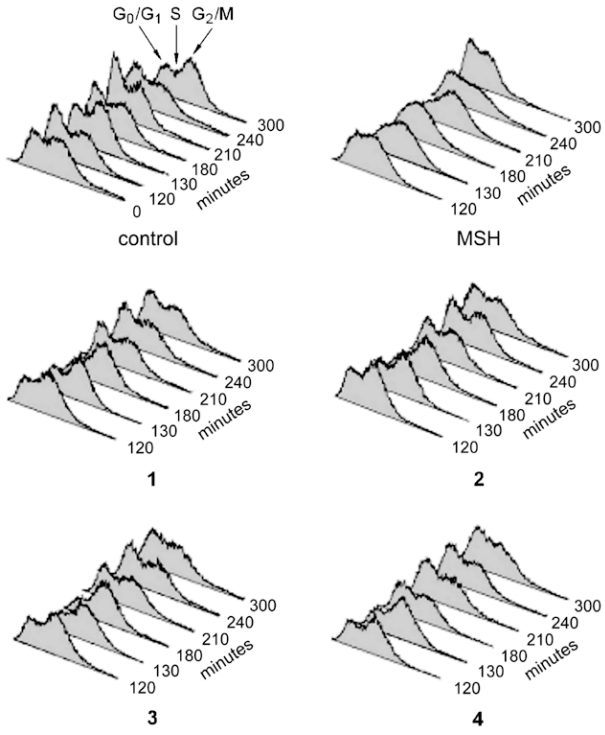

Fig. 3. Dynamics of yeast *Saccharomyces cerevisiae* cell cultures progressing through the cell cycle obtained by means of FACS analysis: control sample and cells treated with the corresponding compound

RESULTS AND DISCUSSION

Test for viability. The growth-inhibitory effect of MSH and its hydrazones is illustrated in Fig. 2. All compounds exhibited a moderate and similar anti-yeast activity, the percentage of viability at concentrations of 100 μmol l^{-1} being between 80 ± 20 and

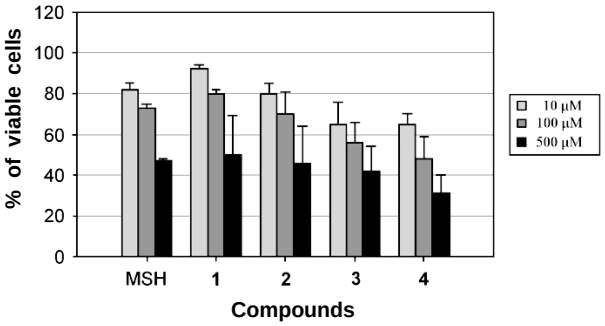

Fig. 2. Percentage of survival of yeast cells in cultures grown for 2 hour in the presence of the corresponding compound

48 ± 11. The most active was the acetone derivative **4** (31 ± 9% viability at 500 mM).

FACS analysis. The genome integrity of the cell depends on the specific control mechanisms named checkpoints. These are surveillance mechanisms monitoring the status of the cell. In yeast the checkpoints are required to reinitiate DNA replication after a transient mediated arrest by certain chemicals [21]. In order to investigate a possible checkpoint arrest of the cells caused by the tested compounds, we applied FACS analysis of yeast cultures after treatment. The results from one representative experiment are shown in Fig. 3. One can see that all five compounds block culture growth after their addition to the media. According to the curves of the FACS analysis, the cell cycle of the control culture lasts 120 minutes (curves 120 to 240 of the diagram "control", Fig. 3), which is in good agreement with our previous observations for this yeast strain (unpublished observation). The block of the growth in the treated cells was released after removing the respective compound, but the culture continued to grow more slowly (approx. 180 min/cell cycle). It should be supposed that the cultures continue to grow because of the cells that have survived the treatment. Since the cells do not accumulate in a particular phase of the cell cycle, the main conclusion from these experiments is that the studied compounds do not block cells in a specific checkpoint.

Yeast Comet Assay (YCA). It is known [18, 23–25] that in alkaline medium sulfonylhydrazones, *via* the reaction of Bamford–Stevens, undergo elimination of sulfinate anion giving diazoalkanes, highly

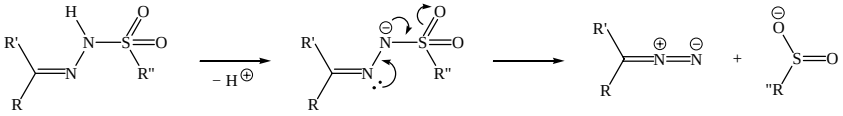

Fig. 4. Mechanism of the alkaline splitting of sulfonylhydrazones into diazoalkanes and alkyl- (aryl-) sulfinate anion

reactive alkylating species (Fig. 4). It could be suggested that methanesylfonylhydrazones studied here follow such a decomposition scheme in the cell, and diazoalkane intermediates further could alkylate the nucleophilic centres of biologically important macromolecules and in particular DNA. Moreover, a similar mechanism of action, leading to DNA single-strand breaks and responsible for the cytotoxicity of some arylsulfonylhydrazones, has previously been suggested by Sartorelli et al. [11, 12]. To check a possible DNA damage, cells treated with different concentrations of the compounds (10 μM, 100 μM, 500 μM and 1 μM) were subjected to the alkaline variant of the Yeast Comet Assay – a technique that has been developed in our laboratory [19]. This technique reveals double- and single-strand breaks and alkali labile sites in the DNA helix [22]. We showed that YCA is 100 times more sensitive than the respective technique applied on higher eukaryotic cells [19]. However, regardless of the high concentrations of the tested compounds (up to 1 mM), we could not detect appearance of comets (not shown), which indicates that they do not cause DNA damage.

CONCLUSION

The obtained results show that MSH and its azomethine derivatives exert a moderate inhibitory effect on yeast *Saccharomyces cerevisiae*, thus confirming our previous data about the cytotoxic activity of these compounds. On the other hand, the appearance of DNA fragmentation – expected by analogy with the mechanism of action of other compounds of this class – was not observed. This finding implies that the molecular mechanism of action of the compounds tested might differ from that of the common cytostatically active sulfonylhydrazones, and deserve further attention.

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