Automation of cell proliferation assay sulforhodamine B for the screening of stilbene derivatives

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Anne Soikkeli[a,b] Cristina Sempio[a], Erkki Mannila[c], Arto Urtti[b], Jouni Hirvonen[a], Marjo Yliperttula[d]

[a] Division of Pharmaceutical Technology, [b] Centre for Drug Research (CDR), [d] Division of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, University of Helsinki, Finland;
[c] Department of Chemistry, Faculty of Mathematics and Science, University of Jyväskylä, Finland

AIMS

The major aim of the study was to implement sulforhodamine B (SRB) assay on a TECAN Genesis robotic workstation. The critical liquid handling parameters were optimized and the SRB assay was standardized on DU-145 cell line to screen a library of bioactive stilbene derivatives.

INTRODUCTION

Sulforhodamine B (SRB) assay is a colorimetric assay used for the screening of anticancer activity and/or toxicity of drug candidates[1]. The heterogeneous assay protocol with several reagent addition, incubation and washing steps makes the assay laborious when performed manually. For improving the throughput and, thus, the utilization potential, the SRB assay was implemented on a TECAN Genesis workstation.

Stilbene derivatives are known to have chemopreventive and cytotoxic effects[2,3]. To further explore the effects of glucoside and glucoside acetate derivates a series of stilbene compounds was screened with the automated SRB assay.

REFERENCES

1. Skehan et al. (1990) J Nat Cancer Inst 82:1107-1119
2. Orsini & Verotta (1999) Adv Nutr Cancer pp. 169-186
3. Mannila et al. (1993) Phytochem 33:813-816

Contact info:

anne.soikkeli@helsinki.fi

www.pharmtech.helsinki.fi/english/frontpage.htm

RESULTS AND DISCUSSION

On TECAN Genesis workstation, the critical liquid handling steps and optimized parameters for automated SRB assay were the following:

- Process 1: drug addition (cell detachment) →optimized dispense 200 µl/s
- Process 2: TCA addition (improper fixation, precipitation in tubing), →optimized dispense 25 µl/s, cleaning step
- Process 2: wash (protein detachment / basal signal↑) →optimized aspiration 10 µl/s and tip height

Process 3: wash (basal signal↑) →optimized tip height

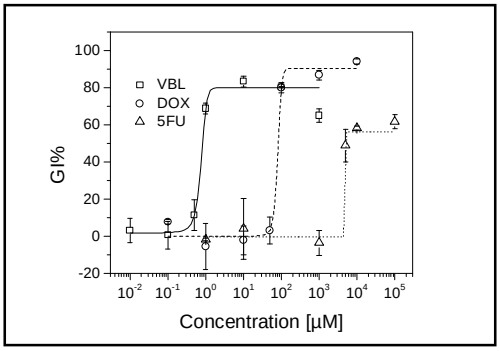


Fig. 2. Standardization of the SRB assay with DU-145 cells was performed with vinblastine (VBL), doxorubicin (DOX) and 5-fluorouracil (5-FU).

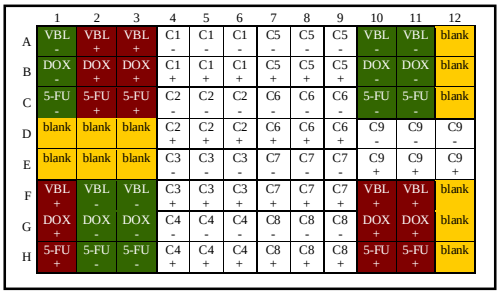
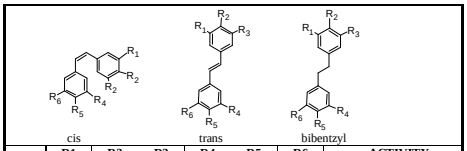


Fig. 3. Plate map for the SRB on/off screening. C1-C9 refer to the compounds 1-9, while (+) and (-) the max and min concentrations, respectively.

Table 1. Structures and anticancer activities of the screened stilbene derivatives. Due to the lack of activity in the structures 11-31, only the structure of the aglycone form is shown here. However, also the glucose and glucose acetate derivatives were screened.



	R1	R2	R3	R4	R5	R6	ACTIVITY cis	trans	bibenzyl
1	OMe	OMe	OMe	OH	OMe	H	+	+	+
2	OMe	OMe	OMe	Glc	OMe	H	+	+	-
3	OMe	OMe	OMe	GlcAc	OMe	H	+	+	-
4	OMe	H	OMe	OH	OMe	H	+	+	+
5	OMe	H	OMe	Glc	OMe	H	+	+	-
6	OMe	H	OMe	GlcAc	OMe	H	-	-	-
7	OMe	H	OMe	H	OH	H	-	-	-
8	OMe	H	OMe	H	Glc	H	+	-	-
9	OMe	H	OMe	H	GlcAc	H	+	-	-
10	OMe	H	OMe	H	OMe	H	+	+	nd.
11-13	OMe	OMe	OMe	H	OH*	OMe	-	-	-
14-16	OMe	OMe	OMe	H	OH*	H	-	-	-
17-19	H	H	H	OH*	H	H	-	-	-
20-22	H	OMe	H	OH*	H	H	-	-	-
23-25	OMe	H	OMe	H	OH*	OMe	-	-	-
26-28	H	OX**	H	OH*	H	OH	-	-	-
29-31	H	H	H	OH*	H	OH	-	-	-

*these series included also the compounds having functional groups of glucoside (Glc) and glucoside acetate (GlcAc) as in the case of compounds 1-3 and 4-6.
**X=$Si(CH_3)_2C(CH_3)_3$

The automated SRB assay was optimized (linearity of cell growth, cell number) and standardized (Fig. 2) on the DU-145 cell line using cytotoxic drugs vinblastine (Z´=0.6, S/B=2.0), doxorubicin (Z´=0.8, S/B=2.4) and 5-fluorouracil (Z´=0.4, S/B=1,5).

For the on/off screening of the library of stilbene derivatives, a plate map was designed (Fig. 3). To be able to take into account the border effect (15% lower total protein amount in the outermost wells), standards were located in the corners of the plate.

The automated SRB assay was used for the screening of the antiproliferative activity of a series of stilbene compounds (Table 1). Of the active compounds, the structures 1 and 4 had lower GI50%-values (~50 nM) compared to that of 2-3 and 5-6 (>500 nM).

MATERIALS AND METHODS

The SRB assay was automated on TECAN Genesis RSP 150/8 workstation by creating four processes (Fig. 1).

The TECAN workstation was equipped with eight standard tips, pipetting carrier, hotels, TECAN incubator, tailor-made carrier for incubations at +4ºC, beaker holder and reagent troughs.

The library of stilbene derivatives derived from the bark of *Picea Abis* were screened at concentrations 1 nM (low) and 10 µM (high) with the DU-145 prostata cancer cell line.

The stock solutions of compounds were done at 40 mM in pure DMSO and serial dilutions were prepared into complete culture medium keeping the DMSO concentration at 0.25%.

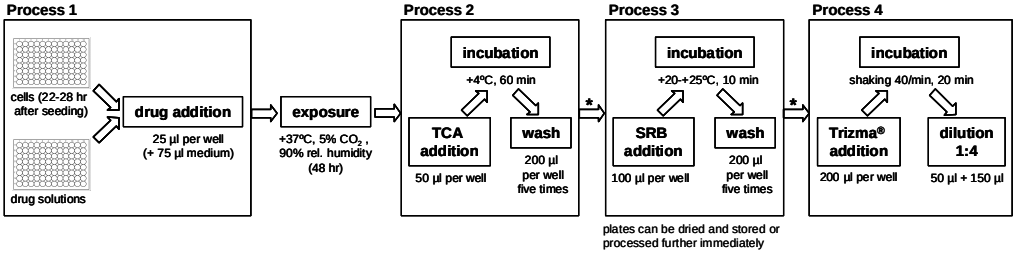


Fig. 1. The sulforhodamine B (SRB) assay was initiated by transferring the compounds from the drug solution plate (Nunc, 96-well, #267245) to the test plate (Costar, 96-well, #3599). After the exposure, the cells were fixed using 50% (w/v) TCA. The supernatant including TCA, growth medium and cell remnants were removed. The fixate was washed with mQ-water and stained with 0.4% SRB in 1% acetic acid. The protein unbound dye was removed by washing with 1% acetic acid. Finally, the protein bound dye was dissolved into Trizma® base and diluted prior to absorbance measurements at 520 nm on Varioskan (Thermo Fisher, MA).

CONCLUSIONS

The SRB assay was succesfully automated on a TECAN Genesis workstation (Z´ ≥ 0.4). From the screened stilbene compounds, the aglycone derivatives showed higher anticancer activity than the glycoside or glycoside acetate derivatives.

Acknowledgements:

- The Graduate School of Pharmaceutical Reserch (Finland)
- The National Technology Agency Finland (40186/04)
- European Comission (FP6, LIINTOP, LSH-2005-1.2.3-4)