Computational analysis of *in vitro* screening data highlights an atypical cytostatic mechanism of a cytosine derivative

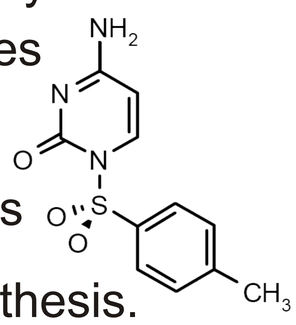
Fran Supek [1], Marijeta Kralj [2], Biserka Žinić [3], Tomislav Šmuc [1]

(1) Division of Electronics, (2) Division of Molecular Medicine, (3) Division of Organic Chemistry and Biochemistry
Rudjer Boskovic Institute, Croatia. Correspondence to: fran.supek@irb.hr

Introduction

1-(p-toluenesulfonyl)cytosine (TsC) is characterized by:

- antiproliferative activity on human tumor cell lines
- selectivity with regard to normal cells
- easy large-scale synthesis.
- induction of a general shutdown in the cellular DNA, RNA and protein biosynthesis [1].



Methods

- growth inhibition assay, after NCI's protocol [2] (10 tumor cell lines + 1 normal fibroblast line)
- **computational methods** applied to cytotoxicity profiles:
  - self-organizing maps
  - permutation tests to assess significance of correlation
  - Random Forest (RF) classifier to determine mechanism-of-action class
- other wet-lab methods (cell cycle analysis, detection of apoptosis by Annexin-V assay)

The NCI Dataset

Cytostatic activity, described by IC_{50} concentration data for 9797 compounds was obtained from the National Cancer Institute's web site [3]. Mechanistic class assignments were kindly provided by dr. David Covell (537 compounds in 13 classes).

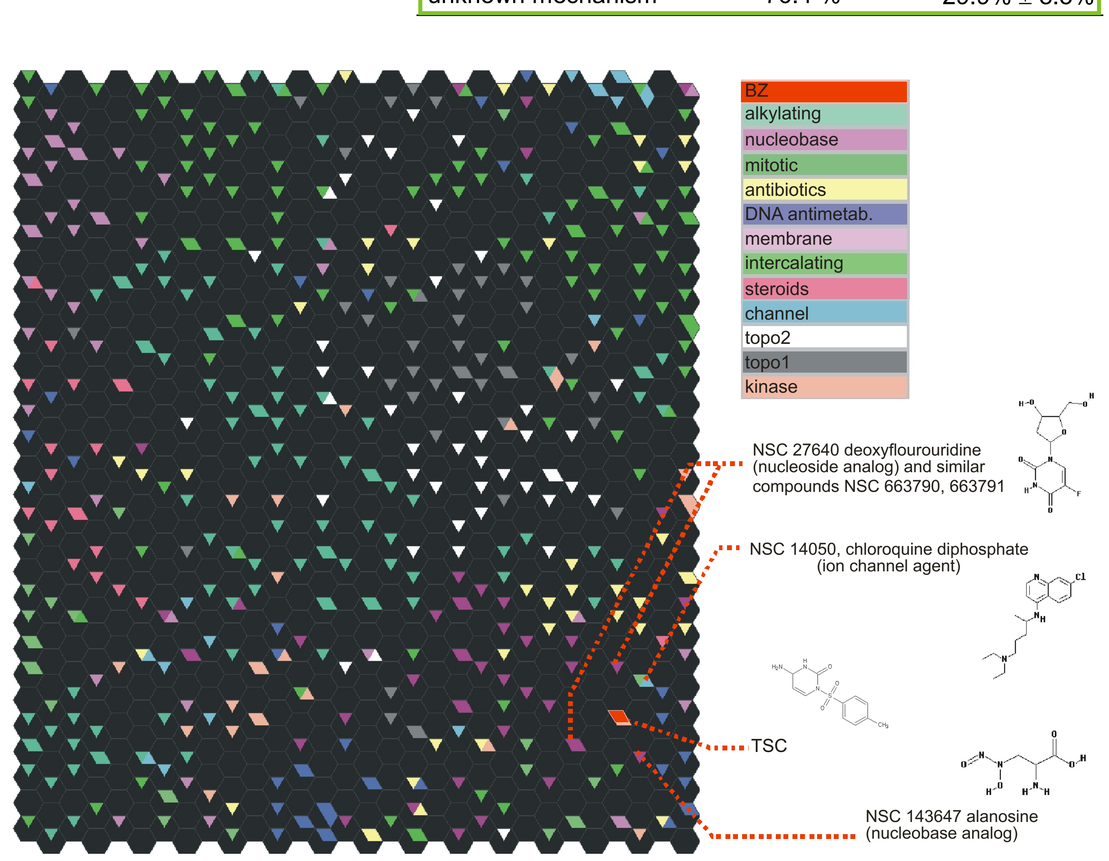
References: **[1]** Glavaš-Obrovac L et al. Anticancer Res 21:1979-1986, 2001
[2] Boyd MR, Kenneth DP. Drug Dev Res 34:91-109, 1995.
[3] http://dtp.nci.nih.gov/docs/cancer/cancer_data.html
[4] Supek F: i2SOM (computer program); http://lis.irb.hr/~fran/i2SOM/
[5] Tusher VG, Tibshirani R, Chu G. Proc Natl Acad Sci USA 98:5116-5121, 2001.

Results

The IC_{50} profile of TSC over 8 cell lines was compared to IC_{50} profiles in the NCI dataset.

Self-organizing Map

A self-organizing map was trained using i2SOM software [4] to gain a rough estimate of TSC's mechanistic class, and find similar compounds by the IC_{50} activity profile.



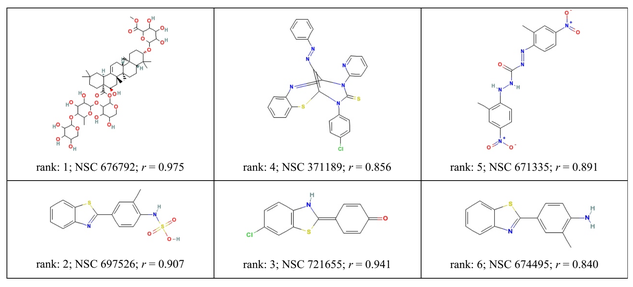
Random Forests

RF classification was repeated with different samples of the compounds in "unknown" mechanistic class. Out-of-bag accuracy was **54.7% ± 1.0%.**

mechanistic class	model precision	TSC (probability of belonging to class)
alkylating agent	81.6 %	0.6% ± 0.2%
antineoplastic antibiotic	45.5 %	3.1% ± 0.7%
ion channel agent	35.8 %	17.4% ± 4.1%
DNA antimetabolite	63.4 %	3.3% ± 0.9%
intercalating agent	84.0 %	1.6% ± 0.3%
kinase inhibitor	53.2 %	3.3% ± 1.1%
membrane agent	68.6 %	3.6% ± 0.8%
mitotic agent	58.0 %	20.3% ± 3.4%
nucleobase analog	55.9 %	14.6% ± 2.5%
steroid	46.6 %	1.0% ± 0.5%
topoisomerase I poison	54.1 %	0.2% ± 0.2%
topoisomerase II poison	49.9 %	1.1% ± 0.4%
unknown mechanism	76.4 %	29.9% ± 8.3%

Permutation test

The *Significance Analysis of Microarrays* (SAM 2.33) software [5] was used to search for similar IC_{50} profiles among the NCI compounds.



This list is estimated (by SAM) to contain 2-3 false positives.

Conclusion

Our results point to an unusual mechanism of cytostatic action, a combination of **nucleic acid antimetabolite activity** and a novel molecular mechanism, possibly similar to activity of **benzothiazoles,** previously described as involving the aryl hydrocarbon receptor, activation of CYP1A1 and CYP1B1 genes and a DNA damage response.