

# Structure-Cytotoxicity/Antiviral Activity Relationship Studies of Nucleoside Analogs Using Structure-Activity Maps.

Ravi R. Parakulam, Mathew L. Lesniewski, Michael A. Marquis II, and Chun-che Tsai\*

Department of Chemistry, Kent State University, Kent, Ohio 44242-0001 USA  
E-mail: ctsai@kent.edu Phone:(330) 672-2989 Fax:(330) 672-3816

## ABSTRACT

The structure-activity relationships of nucleoside analogs with antiviral activity were studied utilizing structure-activity maps (SAMs). SAMs are chemical structures, quantified by molecular descriptors, plotted against their biological activities. The molecular descriptor number of atoms and bonds of a molecule (NAB) was used to quantify the chemical structures. The cytotoxicity measurement used in this study was the 50% cytotoxic dose ( $CD_{50}$ ) in  $\mu$ M, and the antiviral activity measurement used in this study was the 50% effective dose ( $ED_{50}$ ) in  $\mu$ M. The SAMs were examined for compounds grouped according to NAB. The topoisoer groups (based on NAB) were examined to determine important activity trends utilizing structural orderings and structural transformations. SAMs were used to systematically identify the effects of chemical modification on the antiviral activity of nucleoside analogs and to determine the site and type of modifications for improved activity and reduced toxicity of potential antiviral agents.

## Introduction

Human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS). The enzyme reverse transcriptase is the key enzyme in the conversion of viral RNA into proviral DNA, and is a target for the design and development of inhibitors (Palomino et al. 1990).

In this paper we describe a quantitative structure activity relationship (QSAR) methodology using structure-activity maps (SAMs), and structural orderings (SO) to investigate the correlation of structure and activity for purine nucleoside analogs. A database containing purine nucleoside analogs tested in MT4 cell line was compiled by an extensive search of primary literature sources. Structure-activity maps and structural orderings were used to determine the important types and sites of modification for new nucleoside analogs with reduced toxicities and improved activities.

## Methods

### Biological Activity Data

A computer database of the biological activities was compiled for anti-HIV nucleoside analogs tested in the MT4 cell line. The chemical toxicity measurement used was the cytotoxic dose ( $CD_{50}$ ) in  $\mu$ M, based on the 50% reduction in the viability of the mock-infected host cells. The antiviral activity measurement used was the 50% effective dose ( $ED_{50}$ ) in  $\mu$ M, based on the 50% protection of cells against the cytopathic effect of HIV-1 in  $\mu$ M.

### Molecular Descriptors

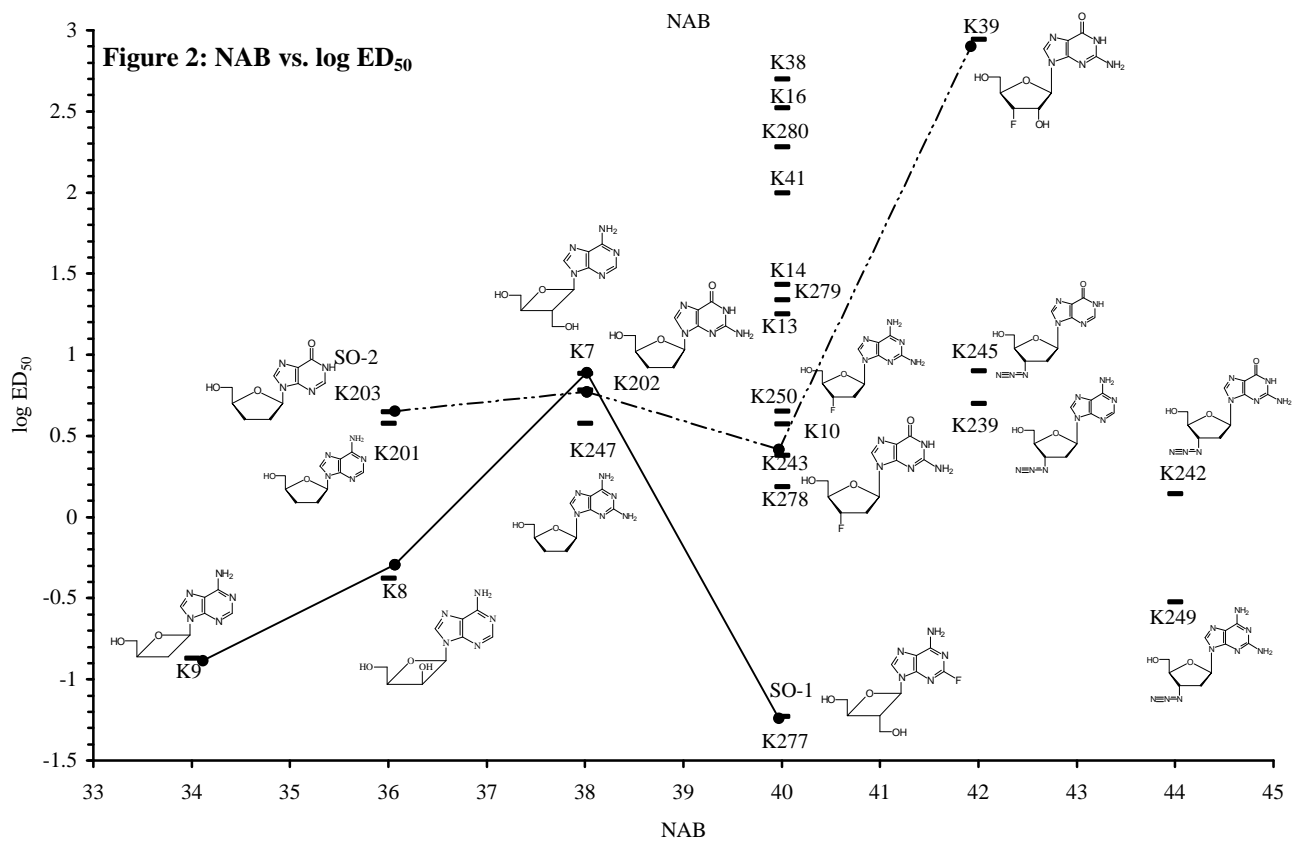
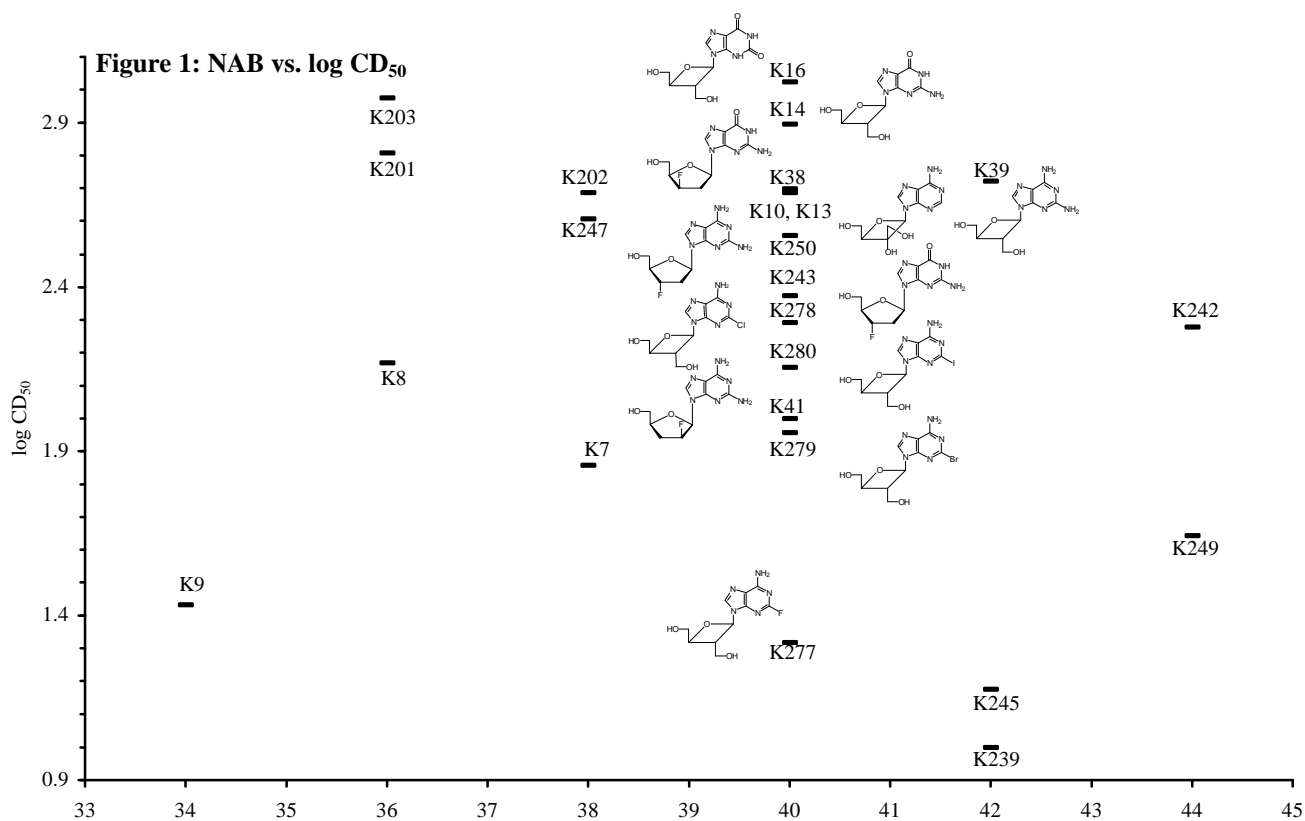
Quantitative structure activity relationship (QSAR) requires quantification of a compound's activity and its chemical structure. A simple descriptor to quantify the chemical structure is the integer value NAB that denotes the number of non-hydrogen atoms and bonds in a molecule (Tsai et al. 1987). NAB classifies compounds into topological isomer groups (topoisomers), and is the foundation of the other topological descriptors used. Maximum common substructure,  $MaCS_{(X, Y)}$ , is defined as a substructure of molecule X and molecule Y such that no other common substructure of X and Y has a greater value of NAB (Tsai et al. 1987). The  $MaCS_{(X, Y)}$  is allowed to have isolated atoms and structural fragments. Each compound in the database is compared in a pairwise manner to all the compounds in the database in order to calculate each pair of compounds'  $MaCS_{(X, Y)}$  using the program TOPSIM (Durand 1996).

### Structure-Activity Maps

Structure-activity maps (SAMs) are chemical structures, quantified by molecular descriptors, plotted against their biological activities. The SAMs provide a method for grouping compounds in order to determine important trends in activity and sites of modification.

### NAB-Activity Maps

We have found that NAB is a simple and useful topological descriptor for ordering, grouping, and comparing chemical structures. Figure 1 describes



NAB-log CD<sub>50</sub> SAM with the NAB 40-topoisomer group labeled. Since in a NAB-Activity SAM neither the atoms nor the bonds in a compound are weighted, the effects of small topochemical changes on biological activity are observed.

The NAB 40 Group is composed of twelve topoisomers: eight compounds contain an adenine base or its analog (K277, K10, K278, K250, K13, K279, K41, K280), three have a guanosine base (K243, K14, K38), and one contains a xanthine base (K16). This group contains two classes of sugars. Eight of the compounds are oxetanocin derivatives (K277, K10, K278, K13, K14, K16, K279, K280). The remaining four compounds contains either 2' or 3' fluoro-dideoxyribose sugar. Analysis of the group indicates that compounds with a xanthine or inosine derivative are some of the least cytotoxic compounds in the group (Balzarini and DeClercq 1979; Herdewijn and DeClercq 1979; Nasr, Craddock, and Johnston 1992a and 1992b; Masuda et. al. 1993).

An examination of the OXT-A analogs (K277, K10, K278, K250, K13, K279, K280) illustrates the effects of various functional groups at the C2 position of the adenine base. The following OXT-A analogs are listed in order of increasing toxicity: K13 (NH<sub>2</sub>) < K278 (Cl) < K280 (I) < K279 (Br) < K277 (F). The functional group listed in parenthesis is attached at the 2 position of the OXT-A analog. K13 has the highest CD<sub>50</sub> value, but is not a very active compound. The remaining compounds in the sequence exhibit a steady increase in toxicity, but only K277 showed a dramatic increase in anti-HIV activity (Figure 2).

A comparison of compounds K243 (3'-fluoro ddG) and K250 (2-amino, 3'-fluoro ddA) shows the only case where a guanine derivative of a nucleoside analog has a lower CD<sub>50</sub> value than an adenine nucleoside analog. Yet, K243 is more active than K250, where as, K13 is more active against HIV than K14 (Figure 2).

An examination of K250 and K38 reveals the effects of the three dimensional position of the 3' fluorine on toxicity and antiviral activity. When the fluorine is in the up position (K38) the compound is inactive, however if the fluorine is in the down (K250) position the compound is active.

## Structural Ordering

A structural ordering (SO) is a set of compounds, L, if every triplet of the structures in L is collinear. Three compounds (X, Y, Z) are collinear if compound Y lies between compounds X and Z such that:  $TD_{(X,Z)} = TD_{(X,Y)} + TD_{(Y,Z)}$ . The topological distance,  $TD_{(X,Y)}$ , between two molecules X and Y is defined as:  $TD_{(X,Y)} = NAB_{(X)} + NAB_{(Y)} - 2 MaCS_{(X,Y)}$ .

Structural ordering is utilized to examine the effect of systematic modification of compounds on the biological

activity. Two structural orderings are illustrated in Figure 2. SO-1 (K9 – K8 – K7 – K277), and SO-2 (K203 – K202 – K243 – K39). In the structural ordering SO-1, K9 (Balzarini, and De Clercq 1990) of NAB 34 group is transformed to K8 (Nasr, and Tuck 1992b) (NAB 36) with a hydroxyl attached to the 2' position of the sugar ring, which decreases the activity yet increases the SI<sub>50</sub> value. K8 becomes K7 (Balzarini, and De Clercq 1990; Nasr, and Tuck 1992b) (NAB 38) by substituting the hydroxyl group with a methanol group at the 2' position, which decreases the activity and increases the toxicity. K7 is then transformed into K277 (Masuda, et. al. 1993) (NAB 40) by attachment of a fluorine to the C2 position of the adenine base. The attachment of the fluorine produces a highly active compound. An examination of the SO-2 structural ordering illustrates that 3' fluoro-guanosine is an inactive compound but, 3' fluoro-dideoxyguanosine has higher toxicity and anti-HIV activity than ddG.

## Conclusions

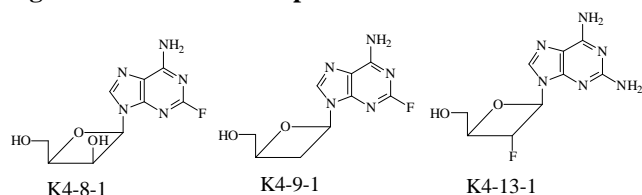
Structure-activity maps were used to determine the sites and types of modifications for reduced cytotoxicity and improved biological activity of new compounds. A set of guidelines, for the development of less toxic and/or active compounds, was elucidated. Compounds with the inosine nucleoside analogs were generally less cytotoxic. Where as, adenosine analogs had the lowest ED<sub>50</sub> values. The compound ddA was generally more active than the OXT-A analogs. There were important exceptions to this observation. OXT-A analogs containing either a fluorine attached at the C2 position, a hydroxy group at the C2' position, or a hydrogen at the C2' position demonstrated a marked increase in activities and values.

Compounds with a fluorine or an azido attached at the 3' position of the dideoxyribose reduces the activity and increases toxicity for both adenosine and inosine. However if the compound is either 2-amino adenosine or guanosine with the azido group attached at the 3' position of the dideoxyribose have an improved activity and reduced toxicity. Dideoxyguanosine or 2-amino dideoxyadenosine with a fluorine attached at the 3' position also has improved anti-HIV activity, but has a marginal increase in toxicity.

Based upon the observations from the NAB-activity SAMs and the structural orderings a set of rules for developing new lead compounds was determined. Using these rules three new compounds (Figure 3) were designed by modify compounds K8, K9 and K13. The first compound (K4-8-1) is based on K8 and contains a fluorine attached at the C2 position of the adenine base. The second compound (K4-9-1) based on K9 and has a fluorine attached at the C2 position of the adenine base. The third compound (K4-13-1) uses compound K13 as the parent compound and has a fluorine attached to the 2'

position of four-member ring sugar instead of the methanol group.

**Figure 3: New Lead Compounds**



## References

Balizarini, J., and De Clercq, E., 1990. *Acyclic and Carbocyclic Nucleoside Analogues as Inhibitors of HIV Replication*. In *Design of Anti-AIDS Drugs*, 175-194, De Clercq, E. ed. New York, N.Y.: Elsevier.

Durand, P.J. 1996. An Improved Program for Topological Similarity Analysis of Molecules. Masters Thesis, Dept. of Mathematics and Computer Science, Kent State Univ.

Herdewijn, P. and De Clercq, E., 1990. *Dideoxynucleoside Analogues as Inhibitors of HIV Replication*. *Design of Anti-AIDS Drugs*. 141-174, De Clercq, E. ed. New York, NY: Elsevier.

Masuda, A et. al.. 1993. Synthesis and Antiviral Activity of Adenosine Deaminase-Resistant Oxetanocin A Derivatives: 2-Halogeno-Oxetanocin A. *J. Antibiotics*. 46(6): 1034-1037

Nasr, M.; Cradock, J.; and Johnston, M. I.; 1992a. . *Structure-Activity Correlation of Natural Products with Anti-HIV Activity*. In *Natural Products as Antiviral Agents*, 31-56. Chu, C.K., and Cutler, H.G. eds. New York, N.Y.: Plenum Press

Nasr, M., and Turk, S. R. 1992b Computer-Assisted Structure-Activity Correlation's of Halodideoxynucleoside Analogs as Potential Anti-HIV Drugs. *AIDS Research and Human Retroviruses*. 8: 135-144

Palomino, E., et. al.. 1990. Synthesis and In Vitro Evaluation of Some Modified 4-Thiopyrimidine Nucleoside for Prevention or Reversal of AIDS-Associated Neurological Disorders. *J. Med. Chem.* 33: 258-263

Tsai, C.-c.; Johnson, M.; Nicholson, V.; and Naim, M.; 1987. A Topological Approach to Molecular Similarity Analysis and Its Application. *Studies in Physical and Theoretical Chemistry*. 51: 231-236