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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON SOME VIRAL REVERSE TRANSCRIPTASE INHIBITORS ACTING AS ANTI-HIV-1 AGENTS

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The anti-HIV-1 and cytotoxic activities of some viral reverse transcriptase inhibitors, namely the analogues of [1-[2′,5′-bis-*O*-(tert-butyldimethylsilyl)-β-D-xylo- and -ribofuranosyl]]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole 2″,2″-dioxide] (TSAO) pyrimidine and pyrimidine modified-nucleosides, are analysed in relation to their physicochemical and molecular properties. The antiviral activities of the compounds are found to be significantly correlated with hydrophobic and electronic properties of the molecules, but no physicochemical parameters were found to be correlated with the cytotoxic effects of the compounds. This difference is exploited to improve the selectivity of the compounds. It is observed that TSAO can provide potent anti-HIV-1 drugs with a disubstituted thymine ring, in which a substituent may be at the N_3-position. The disubstitution reduces the cytotoxicity, and substituents' hydrophobicity and electron donating character enhance the antiviral activity.

Keywords: Quantitative structure-activity relationship; HIV-1 reverse transcriptase inhibitors; TSAO derivatives.

INTRODUCTION

The human immunodeficiency virus (HIV) is a pathogenic retrovirus and causative agent of Acquired Immunodeficiency Syndrome (AIDS) and its related disorders. Despite advances in the therapy of some viral infections, little has been achieved in the area of chemotherapy of AIDS. This is mainly due to the fact that the retrovirus becomes permanently integrated in the cellular chromosomes in the form of proviral DNA. However, a nucleoside analog, 3′-azido-2′,3′-dideoxy thymidine (AZT or zidovudine) (**1**), was discovered[1] to inhibit the infectivity and cytopathicity of

*Correspondence.

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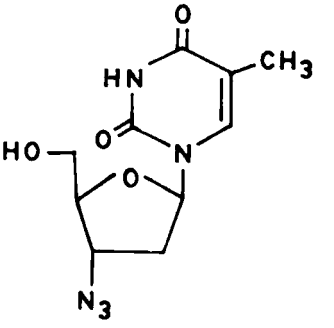

(1)

HIV-1, the most common form of HIV, at a very low concentration, and after many clinical studies and adequate trials, was approved for the treatment of HIV-1 infection. Unfortunately, it was found to suffer from a number of limitations including some side effects and the revelation of the possible emergence of drug resistant mutants of the virus.[2,3] Following AZT, a few more drugs were reported to inhibit the infectivity and cytopathicity of HIV,[4] but their use was more restricted than AZT. Thus the need for new drugs to combat HIV infection.

So far the nucleoside analogues, of which AZT and other recommended drugs are the members, have been prevalent. The common mechanism of action of these drugs, therefore, gives good reason to believe that all members of this structural class could suffer from some or all of the limitations of AZT. Thus clearly a need was felt that some other chemical families should be examined which may inhibit HIV-1 virus possibly by alternative mechanisms. Consequently, some authors[5–8] focussed their attention on [1-[2′,5′-bis-*O*-(tert-butyldimethylsilyl)-β-D-xylo- and -ribofuranosyl]]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole 2″,2″-dioxide] (TSAO) pyrimidine and pyrimidine-modified nucleosides. TSAO derivatives are targeted at the HIV-1 encoded reverse transcriptase (RT) enzyme with which they interact at a non-substrate binding site.[9,10] In this respect they behave like non-nucleoside HIV-1 specific reverse transcriptase inhibitors. However, TSAO derivatives (whose prototype compound is [1-[2′,5′-bis-*O*-(tert-butyldimethylsilyl)-β-D-xylo- or -ribofuranosyl]-thymine]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole 2″,2″-dioxide] (**2**), designated as TSAO-T) are the first HIV-1 specific RT inhibitors, for which a well defined part of molecule (i.e., the 4″-amino group at the 3′-spiro of the ribose moiety) has been identified as an essential pharmacophore, interacting with a well defined moiety of HIV-1 RT.[11]

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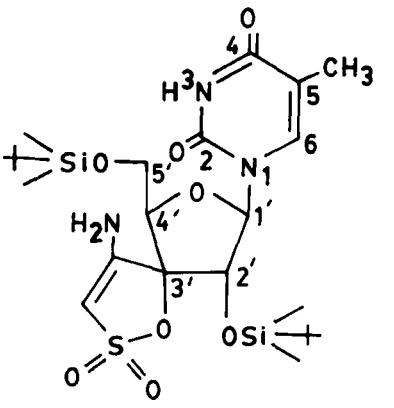
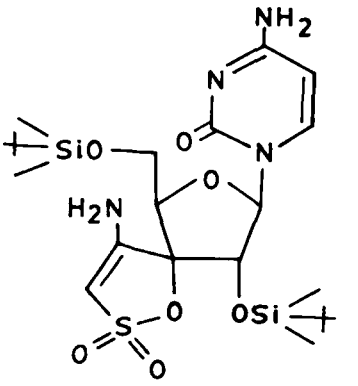


(2)

The initial structure-activity relationship (SAR) studies have shown that the sugar part of the TSAO molecules play a principal and crucial role in the interaction of TSAO compounds with their target enzyme reverse transcriptase. However, the role of the base part in this interaction is as yet unclear. The structure of the base part can be considerably changed while retaining the antiviral activity. The thymine moiety of TSAO-T (**2**) can be replaced by a number of other pyrimidines and purines without marked decrease in antiviral activity, TSAO-T being the most active, and TSAO-C (**3**), where the base is cytosine, being the least cytotoxic



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of TSAO-pyrimidine and pyrimidine-modified nucleoside analogues. In order to obtain TSAO-T and TSAO-C derivatives with enhanced anti-HIV-1 activity and lower toxicity, several derivatives have been prepared, yet no rationale has been provided to reduce the trial-and-error factors in design. It is now desirable to determine the chemical and molecular properties of the molecules that actually govern their viral inhibitory potency, and the way in which the potency depends upon the physicochemical properties. This quantitative SAR (QSAR) study can provide a better understanding of the mechanism of virus inhibition and a rationale for the selection of the substituents.

MATERIALS AND METHOD

The QSAR analysis was made on TSAO derivatives studied by the Camarasa group.[6–8] Their anti-HIV-1 and cytotoxic data were compiled (Tables I–III) and subjected to multiple regression analysis using some physicochemical parameters. The most important physicochemical parameters were found to be the hydrophobic constant π and Hammett's electronic constant σ. Their values were taken directly from the literature.[12] Additionally, some dummy parameters were also used to describe the effect of some specific alterations.

The anti-HIV-1 data refer to the ability of compounds to inhibit the replication of the virus in MT-4 cells. It has been measured in terms of EC_{50}, the effective molar concentration of the compound required to inhibit HIV-1 induced cytopathicity in MT-4 cells by 50%. The cytotoxic data are expressed in terms of CC_{50}, the molar concentration of the compound required to reduce MT-4 cell viability by 50%.

A multiple regression analysis using a least squares method was adopted to derive all QSAR equations.[13]

RESULTS AND DISCUSSIONS

A multiple regression analysis performed, using the least squares method, revealed significant correlations between the inhibition potencies of compounds and their physicochemical and molecular properties. For the compounds of Table I, i.e., the derivatives of TSAO-T (**2**), the correlation obtained was

$$\log(1/EC_{50}) = 0.678(\pm 0.248)\pi_X - 1.385(\pm 0.668)\sigma_X - 0.244(\pm 0.241)\pi_Y + 6.548$$

$$n = 15, \quad r = 0.925, \quad s = 0.25, \quad F_{3,11} = 21.64 \qquad (1)$$

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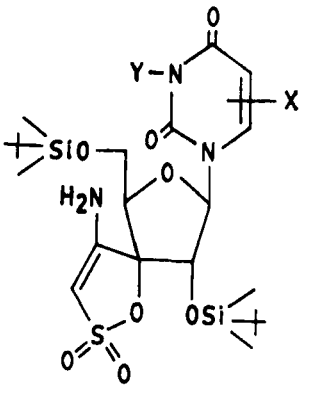
TABLE I TSAO-T derivatives studied by Camarasa *et al.*[6–8] and their anti-HIV-1 and cytotoxic activities and physicochemical parameters.



Compound Number	X	Y	π_X^a	σ_X^a	π_Y^a	log(1/EC_{50}) Obsd.[b]	log(1/EC_{50}) Calcd. (Eqn. (3))	log(1/CC_{50}) Obsd.[b]	log(1/CC_{50}) Calcd. (Eqn. (6))
1	5–CH_3	H	0.56	–0.07	0.0	7.24	7.03	4.88	5.02
2	H	H	0.0	0.0	0.0	6.70	6.54	4.84	5.02
3	5–C_2H_5	H	1.02	–0.07	0.0	7.18	7.35	5.26	5.02
4	5–CH_3	CH_3	0.56	–0.07	0.56	7.23	6.89	3.62	3.90
5	5–CH_3	C_2H_5	0.56	–0.07	1.02	6.91	6.80	3.91	3.90
6	5–CH_3	$CH_2CH{=}CH_2$	0.56	–0.07	1.10	6.63	6.76	—	3.90
7	5–CH_3	$CH_2CH{=}C(CH_3)_2$	0.56	–0.07	2.27	6.42	6.47	4.16	3.90
8	H	$CH_2CH{=}CH_2$	0.0	0.0	1.10	6.21	6.27	5.05	5.02
9	5–C_2H_5	H	1.02	–0.07	0.0	7.22	7.35	5.28	5.02
10	5–F	H	0.14	0.34	0.0	6.30	6.17	5.30	5.02
11	5–Br	H	0.86	0.39	0.0	6.50	6.60	5.44	5.02
12	5–I	H	1.12	0.35	0.0	7.05	6.83	4.92	5.02
13	5–CF_3	H	0.88	0.43	0.0	6.09	6.56	4.85	5.02
14	6–CN	H	–0.57	0.66	0.0	5.44	5.22	4.96	5.02
15	6–C(=NH)–OCH_3	H	–0.97	0.0	0.0	5.57	5.87	4.96	5.02

[a]Taken from ref. 12. [b]Taken from ref. 6–8.

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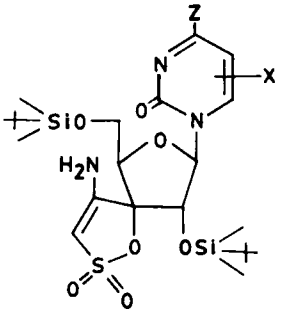
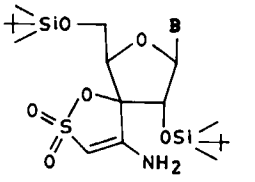
TABLE II TSAO-C derivatives studied by Camarasa *et al.*[6–8] and their anti-HIV-1 and cytotoxic activities and physicochemical parameters.



Sugar Moiety with β-D ribo Configuration



Sugar Moiety with β-D-xylo Configuration[a]

Compound Number	X	Z	σ_Z^a	log(1/EC_{50}) Obsd.[b]	log(1/EC_{50}) Calcd. (Eqn. (3))	log(1/CC_{50}) Obsd.[b]	log(1/CC_{50}) Calcd. (Eqn. (6))
1*	H	$NHCOCH_3$	0.21	—	—	4.94	4.68
2	H	$NHCOCH_3$	0.21	6.80	6.33	4.91	4.68
3	5–CH_3	NH_2	–0.16	6.90	6.82	4.51	4.68
4	H	NH_2	–0.16	6.00	6.33	—	4.68
5	H	$NHCH_3$	–0.30	6.17	6.33	4.92	4.68
6	H	$N(CH_3)_2$	–0.15	6.18	6.33	—	4.68
7	5–CH_3	$NHCH_3$	–0.30	6.92	6.82	4.59	4.68
8	5–CH_3	$N(CH_3)_2$	–0.15	6.80	6.82	4.21	4.68

*Sugar moiety with β-D-xylo configuration. [a]Taken from ref. 12. [b]Taken from ref. 6–8.

where n is the number of data points, r is the correlation coefficient, s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities, and the data within the parentheses are 95% confidence intervals. Equation (1) exhibits a positive coefficient of π_X and a negative coefficient of σ_X, suggesting that a hydrophobic and electron donating X-substituent will be more suitable for the inhibitory activity. The coefficient of π_Y, being negative, indicates that there would be a decrease in the inhibitory activity with an increase in the hydrophobicity of the Y-substituent. Hence the hydrophilicity and not the hydrophobicity of the Y-substituent would be important in virus inhibition. However, π_Y is only marginally significant.

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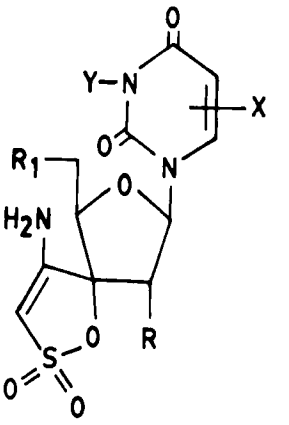
TABLE III TSAO-T derivatives studied by Camarasa *et al.*[6–8] and their cytotoxic activity.



Compound Number	X	Y	R	R_1	$\log(1/CC_{50})$ Obsd.[a]	$\log(1/CC_{50})$ Calcd. (Eqn. (6))
1*	5–CH_3	H	[Si–][b]	[Si–]	4.52	5.02
2*	H	H	[Si–]	[Si–]	5.27	5.02
3*	H	H	OH	OH	3.04	3.15
4*	H	H	[Si–]	OH	3.32	3.89
5	5–CH_3	H	OH	OH	3.52	3.15
6	5–CH_3	H	OH	[Si–]	4.03	4.29
7	5–CH_3	H	H	[Si–]	5.49[c]	5.02
8	5–CH_3	H	[Si–]	OH	3.64	3.89
9	5–CH_3	H	[Si–]	BzO	4.44	3.89

*See Table II. [a]Taken from ref. 6–8. [b]*O*-tert-butyldimethylsilyl. [c]Not used in derivation of Equation (6).

A slight variation in the *Z*-substituent of TSAO-C (**3**) (Table II) was also studied and a significant correlation was obtained between the electronic characteristic of this substituent and the activity as shown by Equation (2). The dummy parameter I_X was used in place of π_X, as there were no variations in the *X*-substituent — it was either 5-CH_3 or H, which could be described by I_X with a value of unity and zero, respectively. Equation (2) exhibits a positive coefficient of σ_Z, indicating that an electron withdrawing *Z*-substituent will favour the activity. Also the positive coefficient of I_X indicates that a methyl group at 5-position will have an added effect.

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$$\log(1/EC_{50}) = 1.265(\pm 1.260)\sigma_Z + 0.717(\pm 0.402)I_X + 6.414$$
$$n = 7, \quad r = 0.931, \quad s = 0.18, \quad F_{2,4} = 13.03 \qquad (2)$$

TSAO-T (Table I) and TSAO-C (Table II) derivatives were merged, using an indicator parameter D with a value of unity for the former and zero for the latter, to study which ring is more favourable. A significant correlation was obtained for this combination (Equation (3)), which suggests that while, in both the series, a hydrophobic and electron donating X-substituent and a hydrophilic Y-substituent would be favourable, there is no significant advantage of a thymine ring over the cytosine ring, as the parameter D is insignificant at 95% confidence level. Equation (3) is capable of accounting for 81% of the variance in the activity ($r^2 = 0.81$). Its F-value is highly significant at 99% level [$F_{4,17}(0.01) = 4.67$] and so is the case with Equation (1) [$F_{3,11}(0.01) = 6.22$]. However, in Equation (2) it is significant only at the 95% level [$F_{2,4}(0.05) = 6.94$].

$$\log(1/EC_{50}) = 0.696(\pm 0.229)\pi_X - 1.396(\pm 0.644)\sigma_X$$
$$- 0.247(\pm 0.233)\pi_Y + 0.213(\pm 0.296)D + 6.330$$
$$n = 22, \quad r = 0.900, \quad s = 0.25, \quad F_{4,17} = 18.20 \qquad (3)$$

The analysis of CC_{50} data for TSAO-T derivatives (Tables I and III) revealed the following correlation,

$$\log(1/CC_{50}) = 0.726(\pm 0.436)I_{2'} + 1.056(\pm 0.372)I_{5'}$$
$$- 1.169(\pm 0.409)I_{X,Y} + 0.319(\pm 0.372)I_{\text{sug}} + 2.965$$
$$n = 22, \quad r = 0.929, \quad s = 0.30, \quad F_{4,17} = 26.81 \qquad (4)$$

in which parameter $I_{2'}$ has been used for an O-tert-butyldimethylsilyl group at the 2′-position and parameter $I_{5'}$ for the same at the 5′-position of the sugar ring each with a value of 1. The parameter I_{sug} has been used for the configuration of the sugar ring. It is equal to 1 for the β-D-ribo configuration and zero for the β-D-xylo configuration. Finally, the parameter $I_{X,Y}$ has been used with a value of unity for a thymine ring that has both X- and Y-substituents. For a thymine ring that has only one substituent either X or Y or no substituent at all, this parameter is zero.

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The use of any physicochemical parameters for these substituents was found to be of no consequence. In this correlation, however, the parameter I_{sug} also does not appear to be statistically so significant, though the correlation on the whole is highly significant. If this parameter is dropped, the significance of the correlation is hardly affected (Equation (5)). Equation (5) is found to accommodate well all the TSAO-C derivatives (Table II) also with the parameter D as defined in Equation (3) to differentiate between TSAO-T and TSAO-C derivatives. The resulting equation

$$\log(1/CC_{50}) = 0.734(\pm 0.460)I_{2'} + 1.135(\pm 0.381)I_{5'} - 1.124(\pm 0.428)I_{X,Y} + 3.152$$
$$n = 22, \quad r = 0.915, \quad s = 0.32, \quad F_{3,18} = 30.78 \qquad (5)$$

for the combine (Equation (6)) is exactly parallel to Equation (5) and the parameter D in it, unlike in Equation (3) obtained for antiviral activity, is statistically significant, suggesting that TSAO-T derivatives will be slightly more cytotoxic than TSAO-C derivatives. But since the coefficient of $I_{X,Y}$

$$\log(1/CC_{50}) = 0.734(\pm 0.446)I_{2'} + 1.135(\pm 0.369)I_{5'} - 1.124(\pm 0.415)I_{X,Y} + 0.341(\pm 0.319)D + 2.811$$
$$n = 28, \quad r = 0.900, \quad s = 0.31, \quad F_{4,23} = 24.19 \qquad (6)$$

used for the disubstitution at the thymine ring is negative and very large as compared to that of D, a disubstituted thymine ring will highly reduce the cytotoxic effect of compounds in the TSAO-T series. Thus the disubstitution seems to be an important factor for providing good selectivity in the compounds. The presence of both X- and Y-substituents in the thymine ring can not only lead to a drastic reduction in the cytotoxicity of the compounds, but their suitable physicochemical parameters can increase their antiviral activity also (Equation (3)). There seem to be no other factors that can reduce the cytotoxicity of the compound without producing any adverse effect on its antiviral potency, except that the thymine ring can be replaced by a cytosine ring but that does not lead to a very significant effect. Although Equations (4–6) suggest that the replacement of O-tert-butyldimethyl group at 2′- and 5′-positions of the ring by an OH group for which both $I_{2'}$- and $I_{5'}$-parameters are equal to zero would lead to a reduced cytotoxic effect, it cannot be so because the presence of the silyl group at both the 2′- and 5′-positions has been found to be essential for the antiviral activity of the

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TABLE IV Mutual correlations (r-values) of the variables used in Equation (3).

	π_X	σ_X	π_Y	D
π_X	1.0	0.09	0.09	0.16
σ_X		1.0	0.28	0.31
π_Y			1.0	0.32
D				1.0

TABLE V Mutual correlations (r-values) of the variables used in Equation (6).

	$I_{2'}$	$I_{5'}$	$I_{X,Y}$	D
$I_{2'}$	1.0	0.44	0.12	0.18
$I_{5'}$		1.0	0.16	0.24
$I_{X,Y}$			1.0	0.18
D				1.0

compounds. Its complete absence at any of these two positions seems to be more disastrous as exhibited by compound (7) in Table III, which is the only compound in the entire set studied that has no silyl group at its 2′-position and possesses very high cytotoxic activity. This compound, therefore, showed an aberrant behaviour in our regression analysis and hence was excluded in the derivation of all the equations related to cytotoxic effects (Equations (4–6)).

In the derivation of Equations (4–6), only dummy parameters could be used as no physicochemical parameters for any substituents were found to be relevant. All the dummy parameters used were found to be mutually orthogonal (Table V). Similarly, the physicochemical parameters and the dummy parameters, if any, used in the derivation of Equations (1–3) were also shown to be mutually orthogonal to each other (Table IV).

Based on the present study the following conclusions can be drawn:

(1) In the TSAO-T series, hydrophobic and electron donating X-substituent at the 5-position and a hydrophilic Y-substituent at the N_3-position of the thymine ring would be important for the antiviral activity of the compounds and the presence of these two X- and Y-substituents on the ring will reduce the cytotoxic effect of the compounds providing them with high selectivity to act as effective anti-HIV-1 drugs.

(2) In the TSAO-C series, an electron withdrawing Z-substituent at the 4-position of the cytosine ring will favour the antiviral activity. A cytosine ring will have

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less cytotoxic effect than a thymine ring, but a disubstituted thymine ring proves much better for the selectivity.

(3) The presence of an *O*-tert-butyldimethylsilyl group at both the 2′- and 5′-positions of the sugar ring is essential for the antiviral activity of the compounds.

(4) The configuration of the sugar ring whether it is β-D-ribo or β-D-xylo has little effect on the cytotoxic activity of the compounds. Its effect on antiviral activity has been least studied.

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Acknowledgements

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