Quantitative Structural Activity Relationship Study of Bis-Tetraazacyclic Compounds. A Novel Series of HIV-1 and HIV-2 Inhibitors

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This work describes a study of quantitative structural activity relationships (QSAR) of bis-tetraazamacrocyclic compounds. These compounds represent a novel class of very potent and selective anti-HIV inhibitors, with a new mode of action. The QSAR study correlates structural features of the compounds with anti-HIV activity, resulting in a model which has a high predictive capacity (predictive $r^2 = 0.79$). This predictive model will be of major importance for the design of new anti-HIV inhibitors of this class. Use is made of partial least-squares (PLS) analysis, with the novelty being that structural features derived by inclusion of all sterically allowed conformations for each molecule are included in the analysis. PLS analysis was made of descriptors, including structural parameters, macrocyclic ring size, metal chelating ability, etc., and those features necessary for the observed antiviral activities of these compounds were deduced from the models. Since all sterically allowed conformations are included in the analysis, the flexibility of the molecules is also taken into account. In addition, a correlation is found (indicated by a predictive r^2 value of 0.61) between inhibition of HIV-1 (HIV-2) and syncytium formation inhibition in the presence of bis-cyclam analogues, leading to the suggestion of a common target, namely, gp120, being involved in both inhibition of virus replication and syncytium formation.

Introduction

In the effort to find a suitable drug for the treatment of AIDS, various compounds acting at different sites of the replicative cycle of HIV have been reported. However, the only inhibitors that are formally licensed for clinical use are reverse transcriptase inhibitors. The main drawbacks of the presently reported compounds are their toxicity and/or the rate at which the virus develops resistance to these compounds. Thus, it is vital to identify new drugs with new modes of action. Furthermore, such new drugs could be used in combination therapies in order to circumvent the development of single-drug escape mutants.

A number of bis-tetraazamacrocyclic compounds, mostly consisting of two cyclam (1,4,8,11-tetraazacyclotetradecane) units linked in various ways such as via an aliphatic linker or a linker containing an aromatic moiety, have been shown to be potent and selective inhibitors of HIV-1 and HIV-2 replication.[1,2] This class of compounds represents a novel class of potent antiviral inhibitors with a new mode of action. The most active congener in the series, compound **1** in Table 1, is active against HIV-1 and HIV-2 and has an IC_{50} of 0.0034 μM, while not being toxic at concentrations >500 μM, thus achieving a selectivity index of over 100 000.[2]

There is evidence, based on time-of-addition experiments,[1] that the bis-tetraazamacrocycles interact with a process following virus adsorption but preceding reverse transcription, suggesting virus-cell fusion and/or uncoating as a likely target for mechanistic intervention. It has also been reported[1] that some analogues

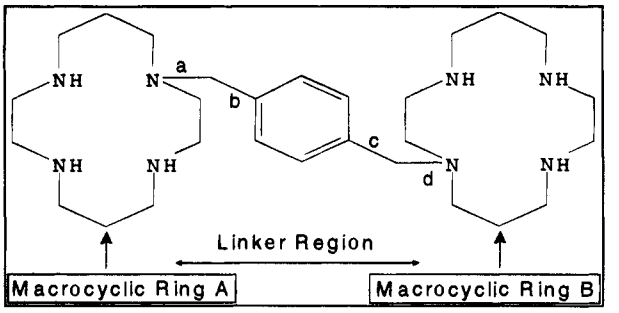

Figure 1. Bis-cyclam analogue JM3100 (compound 1, Table 1), with structural components and rotatable bonds indicated (a–d).

such as compounds **2** and **4** which have aliphatic linkers (Table 1), both show inhibition of HIV-1/-2 replication but fail to inhibit the formation of giant cells in a direct syncytium formation assay. In contrast, analogues which contain an aromatic linker such as compound **1** (Figure 1) inhibit both HIV-1 replication as well as giant cell formation.[2] The mechanism of action of this class of compounds remains unclear. In attempts aimed at deciphering their mode of anti-HIV action, it was shown[2] that the highly active analogue compound **1** failed to inhibit the binding of anti-gp120 monoclonal antibody to persistently HIV-1 infected HUT-78 cells under conditions where dextran sulfate, pentosan polysulfate, and poly(oxometalates) effectively suppressed this binding.[2–4] The bis-tetraazamacrocycles have also proved ineffective in blocking the viral reverse transcriptase or protease in cell-free systems[1,2] and have no effect on virus adsorption[2,5] under conditions where polyanionic substances were found to block HIV-1 binding to the cells.[6] Since the active compounds **1** and **2** protect the viral RNA against degradation while HIV reverse transcriptase inhibitors (AZT, TIBO) or a HIV-protease inhibitor (Ro-31-8959) does not, it has been suggested

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Table 1. HIV-1 and HIV-2 Activities and Cellualr Cytotoxicity Data for 37 Bis-Macrocyclic Analogues

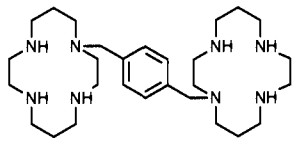
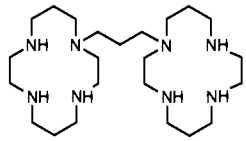
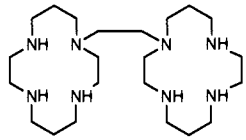
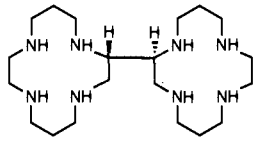
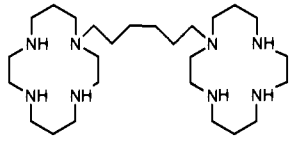
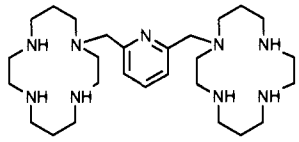
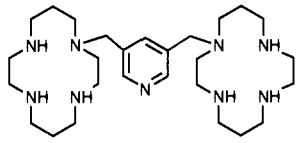
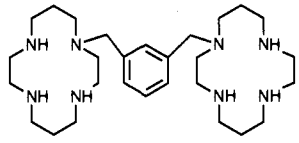
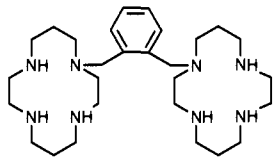
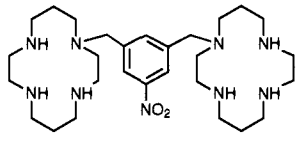
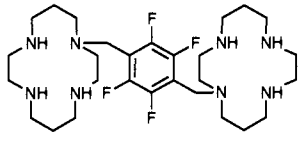
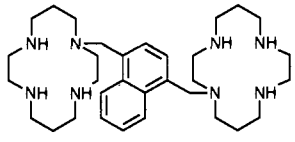
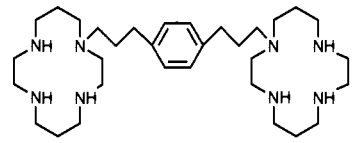
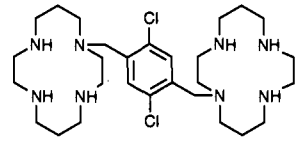
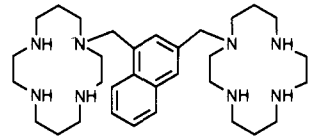
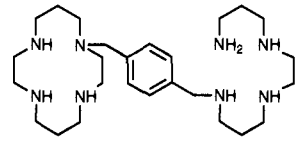
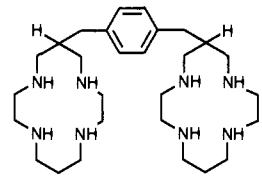
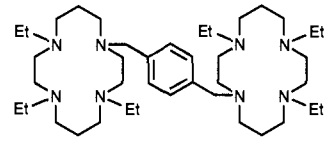
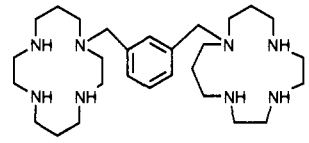
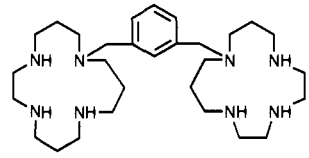
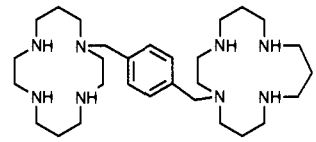
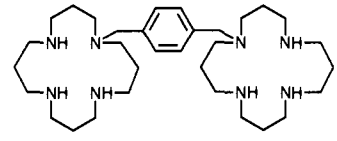
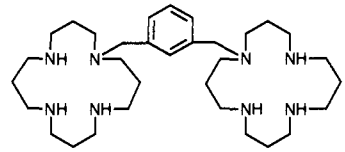
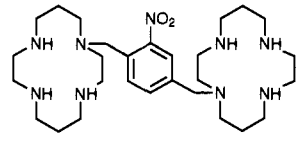
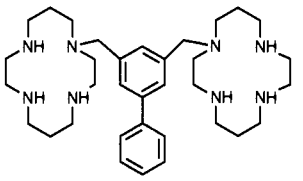
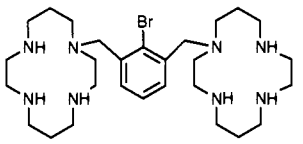
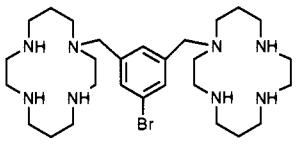
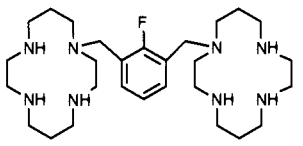
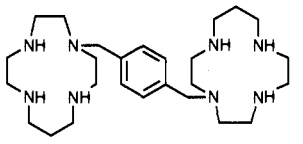
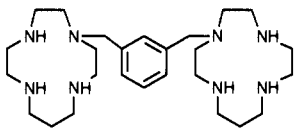
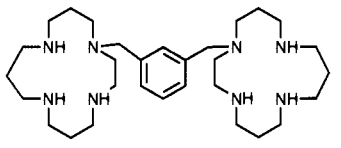
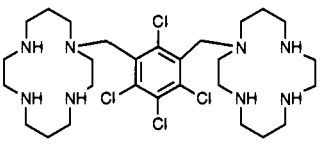
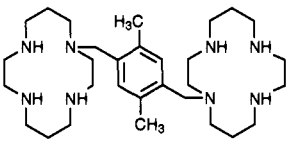
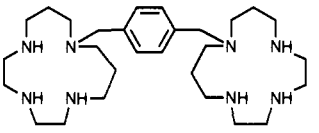
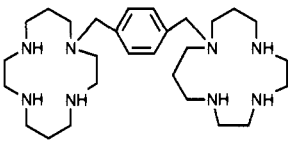
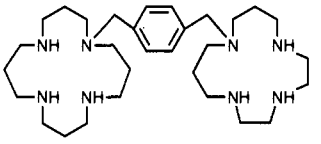
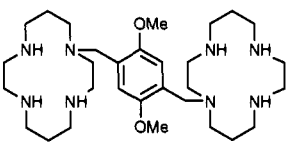
compd no.	structure	log(1/EC$_{50}$) HIV-1	log(1/EC$_{50}$) HIV-2	cytotoxicity CC$_{50}$ (μM)
1		2.40	2.22	241
2		0.86	−0.59	622
3		−1.05	−2.10	404
4		0.80	−0.04	369
5		0.16	0.15	307
6		1.61	1.18	409
7		1.50	1.15	395
8		1.47	1.38	422
9		−0.13	−0.50	169
10		1.39	1.25	45
11		2.10	2.10	48
12		1.26	1.41	56
13		0.40	−1.16	283
14		1.97	2.61	59
15		0.80	1.10	208
16		0.43	0.15	445
17		0.30	0.17	406
18		−0.89	−1.16	342
19		1.07	0.12	422
20		0.49	0.19	403
21		−0.38	−1.06	225
22		−0.96	−1.14	49
23		−1.22	−1.85	193
24		1.19	1.14	203

Table 1. (Continued)

compd no.	structure	log(1/EC50) HIV-1	log(1/EC50) HIV-2	cytotoxicity CC50 (μM)
25		0.69	1.61	198
26		0.86	0.61	144
27		1.07	1.27	192
28		1.46	1.13	202
29		0.78	0.63	216
30		1.39	1.21	192
31		−0.44	−1.07	191
32		0.7	2.6	12
33		2.12	2.89	250
34		1.6	1.23	500
35		2.1	1.25	500
36		0.5	−0.5	121
37		2.15	2.10	250

that the bis-tetraazamacrocycles interfere with the viral uncoating process.[2] Based on this suggestion, namely, that these compounds interfere with viral uncoating, both Nucleocapsid protein 7 (NCp7)[7] and viral RNA may be implicated in the mode of action.

Since the bis-macrocyclic analogues are highly flexible compounds, for which both the receptor/target and the bound conformation is unknown, a new quantitative structural activity relationship (QSAR) strategy was applied, in which all the sterically allowed conformations generated for each molecule were included in the study. Part of the aim of the QSAR study was to gain a description of the active conformation, so that more rigid, selective molecules could be designed. The form of the description used, therefore, includes the intrinsic flexibility of the compounds as well as a number of "static" properties. Furthermore, the intention of the QSAR study is to obtain a predictive model correlating structural features with biological activity. Such a model would provide the answer to what structural features are necessary for antiviral activity for this class of compounds. In addition, by definition, a predictive model would be useful in predicting the antiviral activity of new or unsynthesized compounds, without or prior to experimental testing. A further advantage of a predictive model on this class of compounds, with a novel mode of action, is its importance in the design of new anti-HIV inhibitors.

The main descriptors included in the analysis for correlation with antiviral activity are, amongst others chelating properties of the macrocyclic ring, the effect of modification on the macrocyclic ring system, and the effect of modifications in the linker region on three main structural features including (1) the angle and (2) torsion between the planes defined to lie on the face of each of the two macrocyclic rings and (3) the distance between the metal coordination site on each macrocyclic ring over all generated conformations.

The resultant model was analyzed in order to determine rules for putative compounds, with high activity.

Biological activities have been measured for HIV-1, HIV-2, and syncytium inhibition in two different assays for a number of bis-macrocyclic compounds. An investigation of possible correlation between HIV-1/-2 activity and inhibition of syncytium formation has in addition been made in order to shed light on the mechanism of action of these highly potent and selective HIV inhibitors.

Compounds and Biological Data

The bis-macrocyclic compounds were synthesized[8] and analyzed for anti-HIV activity and cell cytotoxicity according to described methods.[1,8] The compounds included in this study, together with their antiretroviral activities (given as $\log(1/EC_{50}$ in $\mu M)$ for HIV-1 and HIV-2 and cellular cytotoxicity (CC_{50} in μM)) are listed in Table 1.

Computational Methods

All the compounds were built using the SYBYL 6.0 molecular modeling software (using standard SYBYL building commands) on a Silicon Graphics IRIS workstation, starting from the template crystallographic X-ray structure of 6,6'-spirobis(1,4,8,11-tetraazacyclotetradecane)dinickel(II) tetraperchlorate,[9] obtained from the Cambridge Structural Database. For each molecule, the starting conformation was energy-minimized using the Tripos standard force field (using the default settings). A dummy atom was defined at the metal coordination center,[9] replacing the metal itself, for both macrocyclic rings. In order to reduce the complexity and computational effort in analyzing the conformations generated for each molecule, planes have been defined to represent the face of each macrocyclic ring, with the angles and torsions between these two planes subsequently being measured (rather than making a full geometrical analysis which would require all internal coordinates to be measured for every conformation generated). These planes were defined (using the SYBYL software commands), for each macrocyclic ring, such that the four nitrogen atoms in each ring would lie approximately in the plane. Also defined was a normal to each plane, passing through the dummy atom and having a length of 2 Å above and below the plane. This allowed measurement of the twist (torsion) between the two planes (referred to as the "plane torsion") by measuring the torsion angle between the end points of the normals for each conformation generated (the shortest distance between the end points of the two normals served as points 2 and 3 in determining the torsion). The angle between the two planes and the distance between the two dummy atoms (located at the metal coordination center of each macrocyclic ring; henceforth referred to as the "plane angle" and "metal–metal distance", respectively) were measured in addition. A systematic conformational search was then made for each compound, in which all rotatable bonds in the linker region of the molecule (see Figure 1) were allowed to rotate in 10-deg increments, starting from the input conformation. Bins were defined to cover the full range of metal–metal distances measured in the conformational search, with each bin covering 0.5 Å (e.g., bin 1 spans the distance range 0–0.5 Å; bin 2 spans the range 0.5 Å $< x \leq$ 1.0 Å, etc.). For the molecule under investigation, a bin was filled with a value of 1 if a conformation having a metal–metal distance in the range defined by the bin existed. If no conformation was observed to meet this requirement, the bin was filled with a zero. Similar bins, each having a range of 10 deg, were defined for plane angles and plane torsions (covering a range of 0–180° and −180 to +180°, respectively). Thus, these indicator variables reflect the distribution of possible conformations that can be taken up by a particular molecule. The 40 bins describing the metal–metal distance, 18 bins describing plane angle, and 36 bins describing plane-torsion distribution were used as descriptor terms and analyzed for multivariate correlation with antiviral activity. The involvement of metal ions in the activity of bis-cyclams is not established, but the high binding affinity for transition metals by azamacrocycles is a well-established physical property of these compounds and therefore metal coordination based mechanisms cannot be ruled out. The binding affinity is therefore a useful descriptor to try and correlate with antiviral activity. Additional descriptors investigated included the affinity of each macrocyclic ring for metal, as obtained from the NIST Critical Stability Constants of Metal Complexes Database[10] (where zinc was chosen as the representative metal; independent binding of metal to each of the rings was assumed) and the size of each macrocyclic ring, defined either by the van der Waals volume (which included the volume of substituents on the ring but excluded the linker) or by the number of atoms included in the ring (excluding substituents and the linker). The van der Waals volume of the linker region alone, as well as that of the whole molecule, presents further descriptor terms.

For each conformation generated, the energy was calculated and all sterically disallowed conformations were rejected. A linear model was determined using the partial least-squares (PLS) analysis algorithm in conjunction with the cross-validated procedure using the QSAR option of SYBYL. The "best" model was chosen on the basis of which model led to the highest cross-validated r_{cv}^2 (predictive r^2),[11] corresponding to the lowest sum of squares of the difference in activity between the predicted and observed values (PRESS), using predictions made from a leave-one-out cross-validation test. This calculation also establishes the optimum number of latent variable (components) to be used in the final model. The conventional r^2 value is then obtained after repeating the PLS analysis of the descriptors with the optimum number of components but without cross-validation. The importance of each descriptor in the analysis is given by the modeling power[12] (obtainable in the QSAR analysis using the SYBYL COMFA Research Initiative module). The modeling power is defined as the fraction of the variance of a given descriptor used in the final model (where no cross-validation is used).

Results and Discussion

QSAR. PLS analysis of the whole data set comprising 37 compounds (Table 1) for correlation of $\log(1/EC_{50})$ for HIV-1 and HIV-2 with the descriptors plane-angle distribution, plane-torsion distribution, metal–metal distance range, metal affinity for ring A and ring B (Figure 1), number of atoms defining the size of ring A and ring B, and van der Waals volumes of (i) ring A, (ii) ring B, (iii) the linker, and (iv) the whole molecule are summarized in Table 2. The best analysis correlates HIV-1 and HIV-2 activity with (a) metal–metal distance, (b) plane-angle distribution, (c) plane-torsion distribution, (d) metal affinity for ring A and (e) ring B, and (g) ring size (atoms in the ring). Inclusion of the remaining parameters (van der Waals volumes of (i) ring A, (ii) ring B, (iii) the linker, and (iv) the whole molecule) does not contribute to improving the predictiveness of the model. It is seen that van der Waals volumes can replace ring size (=number of atoms in the ring) to give very similar r_{cv}^2 values (compare models 9 and 14, Table 2). The inclusion of both ring size A and ring size B (or volumes of ring A and ring B) descriptor terms does not lead to an improvement in the r_{cv}^2 value, probably due to the limited variation in ring size A over the full set of compounds (see molecules sketched in Table 1). The best model determined for the set of 37 compounds has a $r_{cv}^2 = 0.74$ (model 14 in Table 2). Application of model 14 to an expanded set of 80 compounds gives a still better r_{cv}^2 of 0.79 (model 15, Table 2). The same descriptors used in model 14 could also be used to predict both HIV-1 and HIV-2 activities simultaneously, with r_{cv}^2 values of 0.76 and 0.70, respectively (Table 2, model 19), indicating that the same underlying properties responsible for HIV-1 activity are also responsible for the HIV-2 activity. These cross-validated r^2 values show the considerable predictive capacity of the PLS-derived models as is evident from plots of experimental (actual) versus predicted HIV-1 and HIV-2 activities for the 37 compounds presented in Table 1 (Figure 2A–D) as well as for an extended set of 80 compounds (Figure 3; data and

Table 2. Descriptors Used To Predict Antiviral Activity for 37 Compounds, with the Associated r^2 Values for Each Model

model no.	descriptors used in PLS analysis	no. of components	r^2 cross-validated	r^2 conventional
	Prediction of HIV-1 Activity			
1	a	2	0.21	0.63
2	b	2	0.06	0.40
3	c	2	−0.15	0.34
4	d,e	2	0.18	0.33
5	f,g	2	0.15	0.24
6	a,b,c	4	0.25	0.74
7	a,b,c,i	4	0.42	0.91
8	a,b,c,d,e	5	0.72	0.93
9	a,b,c,d,e,h	5	0.71	0.95
10	a,b,c,d,e,f,g	5	0.69	0.94
11	a,b,c,d,e,g,i	6	0.74	0.96
12	a,b,c,d,e,h,i	6	0.70	0.95
13	a,b,c,d,e,g[a]	6	0.61	0.94
14	a,b,c,d,e,g	5	0.74	0.94
15	a,b,c,d,e,g[b]	6	0.79	0.92
16	a,b,c,d,e,g,k	6	0.80	0.96
	Prediction of HIV-2 Activity			
17	a,b,c,d,e,g	6	0.71	0.98
18	a,b,c,d,e,g,j	6	0.75	0.98
	Simultaneous Prediction of HIV-1 and HIV-2 Activity			
19	(as for model 14)	6	HIV-1 = 0.76; HIV-2 = 0.70	HIV-1 = 0.95; HIV-2 = 0.98

[a] This model (number 13) is applied to a data set of 30 compounds randomly selected from the full data set of 37 compounds (Table 1). This model is subsequently used to predict the activities of the remaining 7 compounds (see Table 3). [b] This model (number 15) is applied to an extended data set of 80 bis-macrocyclic analogues. a: Metal–metal distance distribution, covering the range 2.5–16.5 Å (bins 6–34). b: Plane-torsion distribution, covering the range −180 to +180° (bins 41–76). c: Plane-angle distribution, covering the range +20 to +180° (bins 79–94). d: Metal affinity for ring A; e: Metal affinity for ring B; f: Ring size, as defined by the number of atoms in the ring, for ring A. g: Ring size, defined as for *f*, for ring B. h: van der Waals volume of ring B. i: van der Waals volume of the whole molecule. j: HIV-1 activity (taking $\log(1/EC_{50}(\mu M))$. k: HIV-2 activity (taking $\log(1/EC_{50}(\mu M))$).

structures for additional compounds in the extended set to be published separately).

It is also noted that, if the HIV-2 activity is included as an additional descriptor in model 14 for the prediction of HIV-1 activities, the accuracy of the prediction is significantly improved, as reflected by the increase in the value of r_{cv}^2 from 0.74 to 0.80 (Table 2, model 16). This indicates that the descriptors used in model 14 cover most, but not all, of the properties required for activity. Furthermore, measuring only HIV-2 (or HIV-1) activities and including this data in the model result in an improvement in the predictability of HIV-1 (or HIV-2) activities.

In order to test the predictive ability of the model, the 37 compounds in Table 1 were divided randomly into two sets: a larger set (consisting of 30 compounds, used to define the model) and a smaller set which acts as a test set (consisting of 7 compounds), for which the activities are to be predicted using the model based on the 30 compounds. The descriptors used in defining the best model (model 14, Table 2) were used to generate the new model (model 13 in Table 2) for the set of 30 compounds, with a resultant r_{cv}^2 value of 0.61 being achieved for the prediction of HIV-1 activity. This model was subsequently used to predict the activities of the 7 compounds in the test set. The experimental activities (which have an associated error of a factor of $\sim\pm5$)[8] of all 7 analogues were accurately predicted

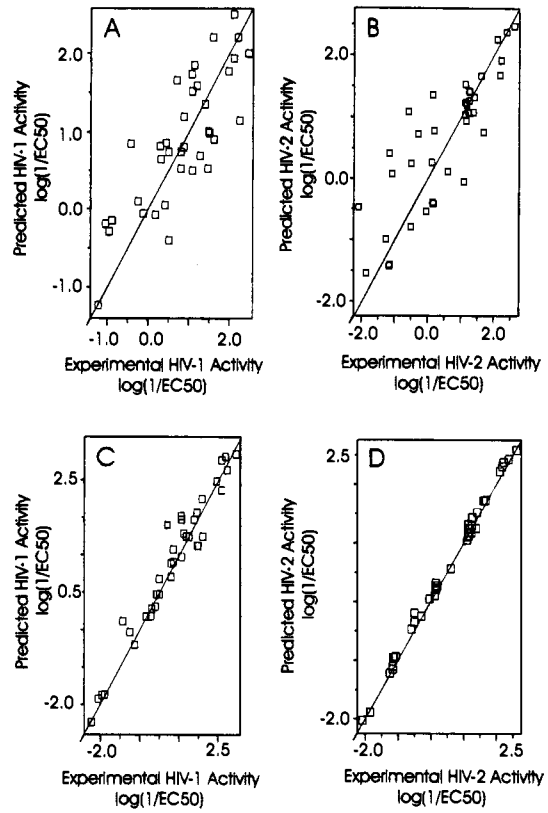


Figure 2. Plot of predicted versus experimental activities ($\log(1/EC_{50})$): (A) HIV-1 ($r_{cv}^2 = 0.74$), (B) HIV-2 ($r_{cv}^2 = 0.71$), (C) HIV-1 ($r^2 = 0.96$), (D) HIV-2 ($r^2 = 0.98$), for 37 bis-macrocyclic analogues using the best derived PLS model (model 14 in Table 2).

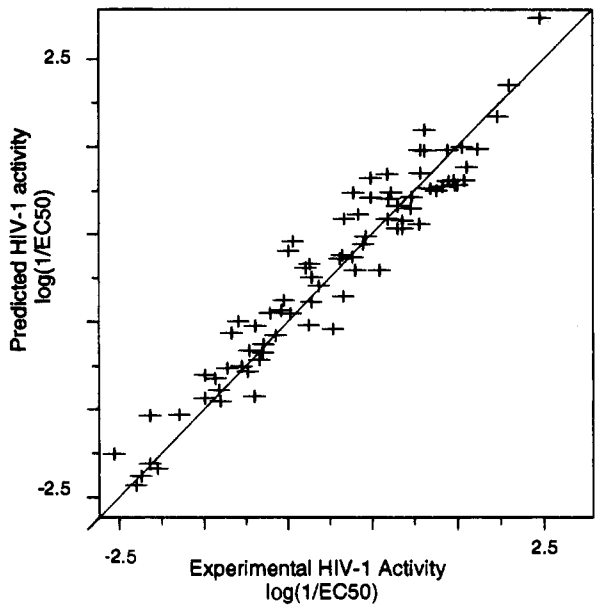


Figure 3. Plot of predicted versus experimental HIV-1 activity ($\log(1/EC_{50})$) obtained with the cross-validated procedure using the best derived PLS model as applied to a larger data set of 80 bis-macrocyclic analogues ($r_{cv}^2 = 0.79$).

(within the experimental error margins) and are compared in Table 3.

Examination of the magnitude and sign of the coefficients relating the contribution of each descriptor to HIV-1 (HIV-2) activity, multiplied by the modeling power for the descriptor in question, reveals a number of properties necessary for the compounds to be active. First, the optimal distance range between the two

Table 3. Comparison of Predicted and Experimental Activities for HIV-1, Using Model 13 (Table 2)

compound	HIV-1 activity (μM)[a] experimental	predicted
27	0.08	0.05
29	0.08	0.02
17	0.50	0.21
32	0.52	0.24
19	0.09	0.14
22	9.12	2.75
23	16.6	3.38

[a] Predicted and experimentally measured activities have an associated error of a factor of ~±5.

metal-binding centers in the bis-macrocyclic compounds is 9.5–11.5 Å. Second, all compounds which are unable to take up a conformation fulfilling this requirement are observed to have significantly reduced activities. Since, in contrast to the bis-cyclams (e.g., compound 1), the monocyclam (JM1498) is inactive,[1] the separation between the two metal binding centers of 9.5–11.5 Å reflects the distance between two binding sites on the receptor.

It is also evident from the analysis that conformations having plane torsions of 70–110° should be avoided, while those of +30° to −60° satisfy requirements for activity. In accordance with this, those molecules which take up a conformation in which the planes (which are roughly in the plane of the cyclam ring) are perpendicular to each other are inactive. Active molecules take up conformations in which the plane angles are in the range of 40–70° and 110–140°, while conformations in which angles of 0–35° and 160–180° occur lead to low activity.

Based on the above deduced structural requirements for antiviral activity, it is possible to explain the observed activity of different macrocyclic compounds. For example, examination of the conformational data generated for compound **9** shows that this molecule can have a distance separation between the metal binding centers in the range 6.15–6.65 Å, with plane angles ranging from 15 to 30° and plane torsions from −45 to −70° and 70–160° (with most conformations having a plane angle of 80–120°). The nonoptimal distance between the metal binding centers together with the observed plane and torsion angles falling into ranges identified to be unfavorable for activity is consistent with the low antiviral activity observed for this compound (EC_{50} = 1.35 μM). A similar analysis of the conformations generated for compound **4** (unique to the set of bis-macrocycles investigated due to the direct carbon–carbon linkage between the rings) gives metal–metal distances in the range 5.2–5.5 Å, plane angles of 65–69°, and plane torsions of 55–65° and 80–110°. In this case, violation of the torsion and metal–metal distance requirements can explain the relatively low activity of this compound (EC_{50} = 0.15 μM) relative to compound **1** (EC_{50} = 0.0042 μM). The highly active compound **1** can take up conformations in which the metal–metal distance is 11.3 ± 0.2 Å, the plane angles are in the range 20–60° and 150–180°, and the plane torsions are predominantly in the range −30 to +30°. These structural features all favor high activity. It is evident that substituents on the aromatic ring of compound **1** (Figure 1) can have a pronounced effect on the degree of conformational freedom of the molecule and thus on the observed allowable plane angles, plane

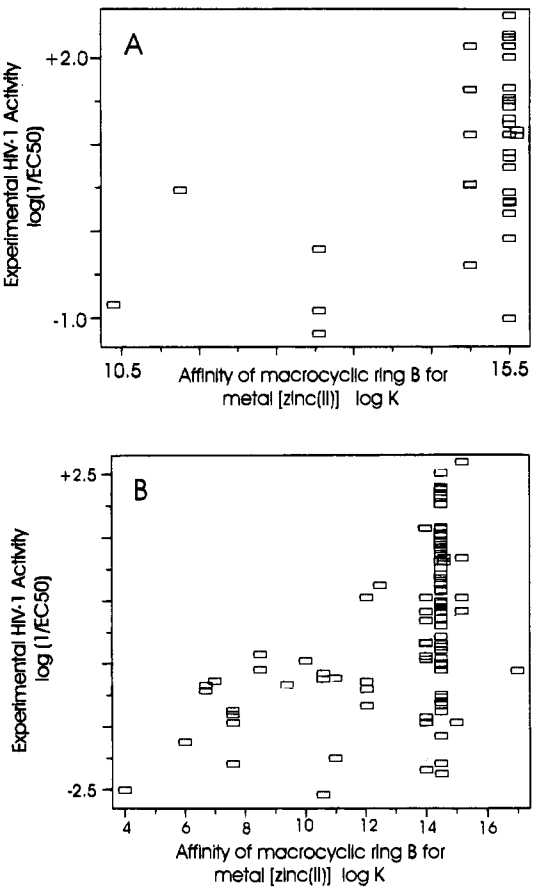


Figure 4. HIV-1 activity ($\log(1/EC_{50})$) is plotted against the binding affinity ($\log K$) of macrocyclic ring B in each compound for metal (Zn^{2+}): (A) 37 compounds included in Table 1; (B) extended to a larger data set of 80 compounds.

torsions, and metal–metal distances for the molecule in question, with a resultant direct impact on activity. Compound **13**, which has an additional $-(CH_2)_2-$ in the linker region on either side of the aromatic ring when compared to compound **1**, shows only moderate inhibition of HIV-1/-2 (EC_{50} = 0.40 μM); this is 100-fold weaker than the inhibition of HIV-1 by compound **1**. Compound **13** has 8 rotatable bonds in the linker region, resulting in a high degree of flexibility, as is further reflected by the conformational search results: metal–metal distances span 7.45–16 Å; plane angles range from 20 to 180°, with most conformations taking up plane torsions in the ranges −100 to −80°, −45 to +45°, and 80–180°. The majority of the conformations taken up by this molecule are observed to have structural features which are not compatible with the requirements for high activity.

Those bis-macrocyclic analogues which have significantly reduced affinity for metal binding are also observed to have significantly lower HIV-1 (HIV-2) activity (Figure 4A,B). A maximum activity of 0.2 μM is observed for bis-macrocycles in which the macrocyclic rings have log K's equal to 12 or less for zinc binding (cf. JM3100: log K = 15.5; EC_{50} = 0.0042 μM). Replacement of a nitrogen atom in the macrocyclic ring by a sulfur, oxygen, or carbon atom results in a decrease in the affinity of the cyclam for Zn^{2+} binding (with log K's of ~12, ~10, and ~8.8, respectively).[10] This drop in affinity for the metal ion on substitution of nitrogen might therefore be expected to be paralleled by a drop in HIV-1 (HIV-2) activity. The importance of the

chelating properties of the bis-macrocycles for antiviral activity have also been highlighted by Kimura *et al.*[13] and is further emphasized when a comparison is made between JM3118, JM3117, and JM3158. These three compounds are the Zn^{2+}, Cu^{2+}, and Pd^{2+}-bound forms of compound **1**, respectively. JM3117, the zinc-bound form, has the same activity as JM3100 (EC_{50} = 0.0042 μM). The Cu^{2+} form, JM3117, has a reduced activity of 0.018 μM, while the Pd^{2+} form has still worse activity (37.4 μM). An inverse relationship is observed between these activities and the affinity of the cyclam in these compounds binding the respective metal ions (log K = 15.5, 27.2, and >40 for Zn^{2+}, Cu^{2+}, and Pd^{2+}, respectively). The above findings suggest that the chelation of bis-macrocycles to their receptor is mediated either through a low-affinity metal-bound (e.g., zinc-bound) or metal-free form of the molecule.

A further property deduced from the model is that a 14-membered ring affords the optimal size in regards to achieving high antiviral activity for this series of analogues. Smaller 9- or 12-membered macrocyclic rings, containing only three nitrogen atoms in the ring, have very low affinities for binding a single metal ion (log K in the range 8.6–11.6)[10] and preferentially form "sandwich" (cyclam–metal–cyclam) Metal(Ligand)$_2$ complexes in which the metal is very tightly bound.[10] Bis-cyclic compounds forming such complexes are unable to meet the structural requirements for activity as determined from the model (see above) and would therefore be expected to have low antiviral activity. The antivirally active compounds containing larger 14-membered macrocyclic rings with four nitrogen atoms form cyclam–metal (1:1) complexes with high affinity[10] (e.g., compound **1**, log K = 15.5 for complexation of zinc to the cyclam ring; EC_{50} = 0.0042 μM for HIV-1). However, compounds with 14-membered rings containing only three nitrogen atoms also form 1:1 cyclam–metal complexes, but with reduced metal affinity (log K in the range 10.5–12.5).[10] Based on the requirement of high metal affinity for high antiviral activity, as deduced from the model, the latter class of molecules would be expected to have lower activities than the tetraaza compounds. Comparison of the metal affinities for 9- and 12-membered macrocyclic rings containing three nitrogens with those observed for 14-membered triaza macrocyclic rings would suggest that compounds falling into this latter class possess higher activity. It is clear from the above discussion that ring size has a distinct affect on the macrocyclic ring's affinity for metal binding and its ligand–metal coordination. However, analysis of the model shows that this is not a linear relationship. Both the descriptors, ring size and metal-binding affinity, are therefore necessary for correlation with HIV-1/-2 activity.

PLS analysis failed to show any correlation between the descriptors listed in Table 2 and cell cytotoxicity. The implication of this is that completely different parameters are required to describe cytotoxicity compared with antiviral activity.

Inhibition of syncytium formation (giant cell inhibition) has been measured (HIV-1 IIIB strain) for a number of bis-macrocyclic analogues[1,2] (see Table 4 and Figure 5). A PLS analysis, in which an attempt was made to predict the giant cell inhibition (taken as log(1/EC_{50} (μM))), using only the anti-HIV-1 (or HIV-2) activities (taken as log(1/EC_{50} (μM))) as a descriptor resulted in a good model, as indicated by an r_{cv}^2 value of 0.62. The direct correlation between inhibition of HIV replication and syncytium formation is further illustrated by plots (Figure 5A,B) of (a) experimental versus predicted giant cell inhibition activities and (b) giant cell inhibition versus HIV-1 activity for these compounds (which includes compounds **1** and **2**). This is in contrast to earlier reports by De Clercq and co-workers[2] that compound **2** had no inhibitory effect on syncytium formation, while compound 1 did (cf. Figure 5). This is probably due to analysis of a more complete data set here. The experimental EC_{50} values for syncytium inhibition by compounds **1** and **2** are 0.048 and 12.3 μM, respectively (10–100 times lower than the EC_{50} for

Table 4. Summary of the Inhibition of Syncytium Formation by Bis-Macrocyclic Analogues

compound	log(1/EC_{50}) giant cell inhibition	compound	log(1/EC_{50}) giant cell inhibition
1	1.32	**2**	−1.09
6	−0.91	**8**	−0.62
32	−0.78	**10**	1.19
11	1.50	**12**	1.20
16a[a]	−1.73	**16b**[a]	−1.43
33	1.48	**46c**[a]	0.49
14	0.48	**35**	1.90
18	−1.53	**24**	−0.51
37	1.88	**25**	0.50

[a] Compound structures and numbering according to ref 8.

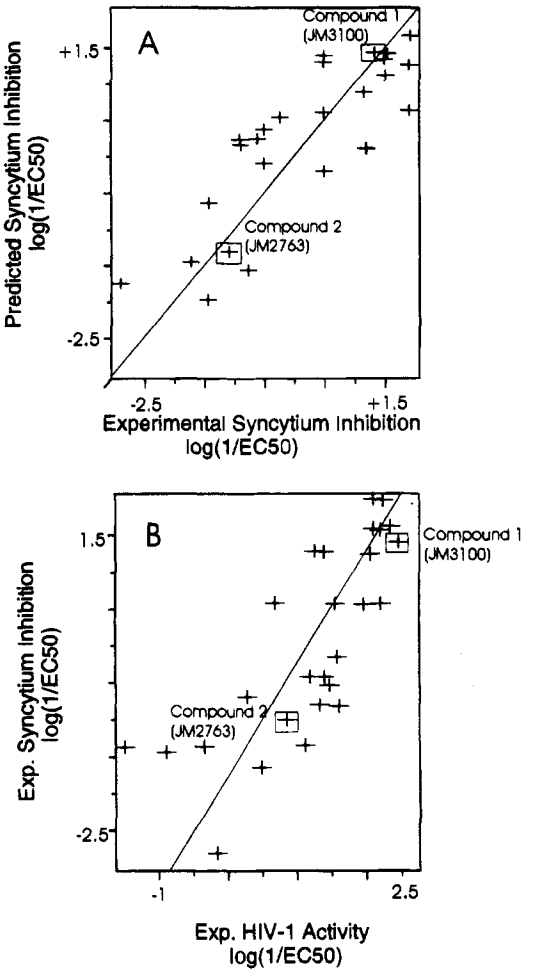


Figure 5. (A) Predicted versus experimental syncytium inhibition (log 1/CC_{50})). (B) Experimental syncytium inhibition (log(1/EC_{50})) plotted against HIV-1 activity (log(1/EC_{50})).

Table 5. Structural Requirements of Antivirally Active Bis-Macrocycles

Features Necessary
(i) molecules require two chelating macrocyclic rings for high activity
(ii) distance between metal-binding centers must be 9.5–11.5 Å
(iii) plane torsions of −60 to 30° and 120–140° are allowed
(iv) plane angles of 40–70° and 110–140° are allowed
(v) maximize metal affinity for each macrocyclic ring
(vi) optimum ring size for cyclam rings: 14 atoms
Features To Avoid
(i) plane torsions of 70–110°
(ii) plane angles 0–35° and 160–180°

inhibition of HIV-1 replication). For comparison, the predicted syncytium inhibition EC_{50} values for compounds **1** and **2** are 0.03 and 4.4 μM, respectively.

Conclusions

A reliable predictive QSAR model has been developed for the bis-macrocyclic compounds, a novel class of highly potent HIV-1/-2 inhibitors. Using this model the activities for inhibition of HIV-1/-2 replication and syncytium formation can be accurately predicted, to within experimental error, for all the compounds studied. The model is therefore capable of predicting activities of compounds prior to their synthesis and can as a result considerably reduce synthetic chemical effort. A further asset of the model is that it enabled the identification of structural properties essential for activity. Such information provides an important basis for the design of new potential antiviral drugs with chelator–linker–chelator structures.

The inclusion of all sterically allowed conformations, generated from a systematic search of conformational space, for each molecule in the QSAR study, proved to be a new and successful modeling approach, as confirmed by the predictive capacity of the model given by an $r_{cv}^2 = 0.79$. A further advantage of this approach is that it enabled analysis of the model to lead to a description of the active conformation of these compounds. The most important features necessary for potent antiviral activity are summarized in Table 5. The optimum separation between the two macrocyclic rings, as given by the distance between the metal coordination sites in each ring, is found to be 9.5–11.5 Å. Conformational properties necessary for high as well as poor antiviral activity are further described by plane angles and plane torsions between the macrocyclic rings (see Table 5). Compounds in which bulky substituents are attached to the aromatic ring in the linker result in reduced flexibility of the molecule and consequently in reduced antiviral activity. It is noted that *N*-(4-methylbenzyl)cyclam (compound **41** in ref 8), which is missing a second macrocyclic ring, also possesses antiviral activity ($EC_{50} = 1.4$ μM), while removal of the aromatic moiety to give cyclam results in an inactive compound ($EC_{50} = 399$ μM). It is therefore evident that both macrocyclic rings are required for high antiviral activity and that the aromatic moiety in the linker plays an important role in the interaction of these compounds with their receptor. Furthermore, it is clear from the model that the macrocyclic ring size as given by the van der Waals volumes, and independent of its effects on chelating properties of the macrocyclic ring, plays an important role in dictating the antiviral activity. This could either be a result of substituents causing a steric hinderance in the interaction of the bis-macrocycles with the target or be due to their restriction on conformational freedom. It is also noted that high antiviral activity is only observed for compounds with strong chelating ability (see Figure 4).

In light of the published data suggesting that these compounds interfere with viral uncoating[2] (NCp7/viral RNA interaction), one might postulate NCp7, containing two zinc fingers which participate in several nucleic acid interactions such as specific recognition of the viral genome during budding,[13-17] packaging of RNA in mature virions, and initiation of reverse transcription,[14,16-19] to be a possible target for this class of compounds. Further, other aromatic C-nitroso compounds such as 3-nitrosobenzamide (NOBA) and 6-nitroso-1,2-benzopyrone (NOBP) have been shown to inhibit infection of HIV-1 in human lymphocytes by extracting zinc via an oxidative mechanism from the zinc fingers of NCp7.[20] Independent of the mechanism involved, if extraction of zinc from the zinc fingers by bis-macrocycles occurred, this would result in a similar inhibition profile to the C-nitroso compounds. However, NMR studies have shown that the highly active compound **1** does not bind or extract zinc from the double zinc finger peptide of NCp7 (data to be published separately) under conditions where 3-nitrosobenzamide was observed to extract zinc. The biological activity of compound **1** is therefore very unlikely to be due to an interaction with NCp7.

The direct correlation observed between HIV-1 (HIV-2) activity and giant cell inhibition in the present study suggests that the interaction of bis-macrocyclic compounds with a common target may be involved in both inhibition of virus replication and syncytium formation. Possible targets may be present in the envelope glycoproteins of HIV, namely, gp41 and gp120, or their putative cellular receptor. This hypothesis is also shared by Inouye *et al.*[21]

Recently, De Clercq and co-workers have shown that a monoclonal antibody (9284) which binds to the V-3 loop of native gp120 is no longer able to bind to the compound **1**-resistant mutant gp120 (HIV-1 (NL43) strain).[22] Evidence supporting the suggestion of gp120 as a target for the bis-macrocyclic analogues comes from the analysis of mutant virus, resistant to bicyclam compound **1**, in which the mutations have been shown to reside in gp120, predominantly in the V3-loop, and not NCp7.[23] The involvement of gp120 in the mechanism of action of the bis-macrocyclic compounds is consistent with the time-of-addition experiments[1] and the fact that dextran sulfate, which shows cross-resistance with bis-cyclam compound **1** and inhibits virus adsorption to the host cell, binds to gp120. Studies to clarify the mode of action are ongoing. The model, taken together with the known experimental data, therefore strongly suggests gp120 as the target for this novel class of potent, selective antiviral inhibitors.

In conclusion, this QSAR study on a new class of antiviral inhibitors, which includes compound **1** which is destined for clinical trials, has led to a reliable predictive model. This model is being used to design new drugs for the treatment of AIDS.

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a program to automatically calculate the plane-torsion and plane-angle distributions from the systematic conformational searches generated using SYBYL.

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