binding or to the catalytic site responsible for strand-transfer activity.

To understand in greater detail the substituents responsible for strand-transfer inhibitory activity, we analysed 23 carbazole derivatives, and classified them into three categories according to their levels of inhibition (Table 1). Six compounds were classified as the high-inhibition group, which demonstrated IC_{50} of less than 10 μM, 12 compounds were classified as the intermediate group, which demonstrated IC_{50} of greater than 10 μM and less than 100 μM, and five compounds were classified as the non-inhibition group, in which we did not observe significant inhibition even at the highest concentration tested (100 μM).

Comparing the compounds between and within these three categories, we recognized three factors responsible for strand-transfer inhibition. The first and most important factor is the incidence of a 2-dimethylaminoethyl group at position R2 (Figure 1A).

CA-8, which possesses a 2-dimethylaminoethyl group at position R2, demonstrated high inhibitory activity (IC_{50}: 6.61 ±4.17 μM), but **CA-19** (IC_{50}: >100 μM), which possesses a phenyl ring structure at the same R2 position, did not demonstrate inhibitory activity. Thus, it is clear that the incidence of a 2-dimethylaminoethyl group, which has a basic property, is critical for strand-transfer inhibition activity. Indeed, we recognized that all compounds in the "high-inhibitory group" and "intermediate-inhibitory group" had this basic substituent at position R2 (Table 1A, 1B, Figure 2). In contrast, three of five compounds in the "non-inhibitory group" had the phenyl ring structure at R2 position. It is thought that these compounds might bind to the acidic region on the IN molecule and compete with the target dsDNA.

The second factor is the incidence of a methyl (Me) group at position R5, R6 or R7. We recognized that compounds in the high inhibitory group had at least one Me group at the R5, R6 or R7 position (Table 1A, Figure 2). Comparing **CA-1** (IC_{50}: 7.94 ±4.12 μM), **CA-4** (IC_{50}: 8.99 ±3.39 μM), and **CA-12** (IC_{50}: 5.93 ±3.53 μM) with **CA-15** (IC_{50}: 27.28 ±9.10 μM), it is clear that the incidence of an Me group within the R5 to R7 positions was an important factor for enhanced inhibitory activity. It seems that the position of the substituent may not be critical between R5 and R6, as we did not see significant differences between **CA-1** (IC_{50}: 7.94 ±4.12 μM) and **CA-12** (IC_{50}: 5.93 ±3.53 μM), and also between **CA-8** (IC_{50}: 6.61 ±4.17 μM) and **CA-9** (IC_{50}: 4.42 ±1.87 μM).

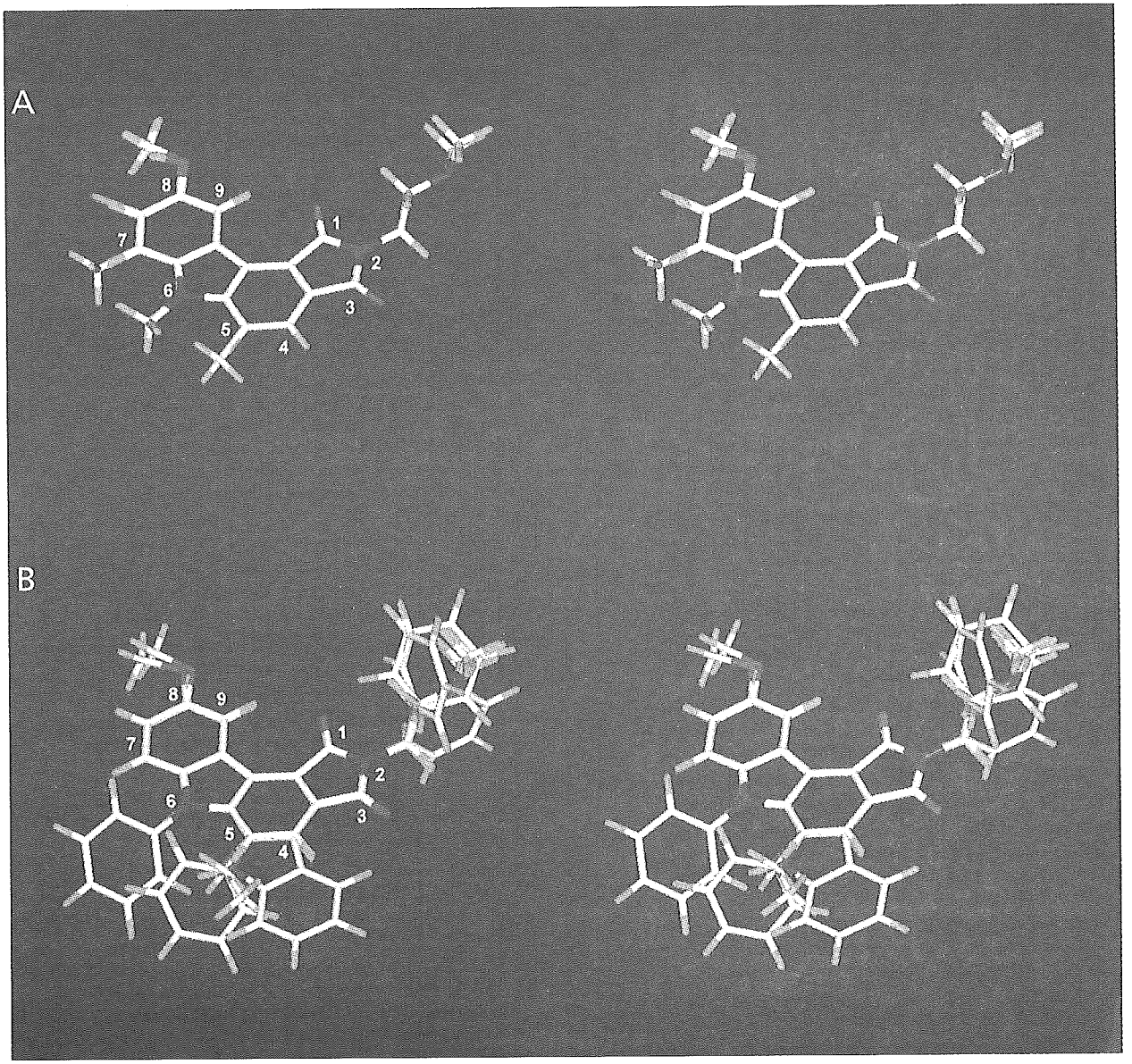
According to the IC_{50} levels of **CA-5** (>100 μM), **CA-6** (>100 μM) and **CA-11** (>100 μM), it appears that bulky substituents at the R5 position have a negative effect on inhibition (Table 1C, Figure 2). Furthermore, the inhibition potential of the three compounds **CA-1** (IC_{50}: 7.94 ±4.12 μM), **CA-16** (IC_{50}: 20.51 ±15.11 μM) and **CA-17** (IC_{50}: 50.64 ±19.02 μM) depended on the molecular size of their R5 substituents. It is probable that the R5 substituents of these compounds were too large and that they interfered with surrounding molecules forming the binding site (Table 1A, 1B, Figure 2). These data indicate that the binding site of carbazole might have a space limitation, and thus the size and shape of the molecules may be important factors for inhibitor activity.

The third factor is the substituent at position R9. Comparing **CA-20** (IC_{50}: >100 μM), **CA-21** (IC_{50}: 25.01 ±10.60 μM) and **CA-22** (IC_{50}: 16.92 ±7.32 μM), these three compounds were identical, with the exception of the substituent at position R9 (Table 1B, 1C, Figure 2). **CA-21** and **CA-22** have hydroxyl residue and a methoxy group at position R9, respectively. We noticed a significant difference in inhibitory activity between **CA-20** and **CA-21**, and between **CA-20** and **CA-22**, suggesting the possibility that both the hydroxyl group and the methoxy group at R9 formed hydrogen bonds with the amino acid molecules forming the binding sites, as these two substituents have the potential to be hydrogen bond acceptors. It appears that hydroxyl and methoxy groups have similar effects on strand-transfer inhibitory activities. In addition to the above three factors, we found that molecular interaction between R8 and R9 substituents, and their arrangement, are also important determinants for efficient inhibitory activity. **CA-3**, with two methoxy groups at R8 and R9, appears to have a bulky arrangement of the two side chains, and demonstrated an IC_{50} of 72.69 ±5.44 μM, whereas **CA-14** and **CA-18**, which were expected to have horizontal arrangements, demonstrated lower IC_{50} values of 17.37 ±1.79 μM and 10.68 ±8.88 μM, respectively (Table 1B, Figure 2).

To summarize these structural elements, and to understand the common structure of molecules that demonstrated strand-transfer inhibitory activity, we superposed inhibitor structures having significant strand-transfer inhibition (**CA-0**, **CA-1**, **CA-4**, **CA-8**, **CA-9**, **CA-12** and **CA-13**) (Figure 5A), and the structures of compounds with no inhibition (**CA-5**, **CA-6**, **CA-10**, **CA-19** and **CA-20**) (Figure 5B). In comparing these two overlapped figures, we found that the compounds with inhibitory activity share a largely identical structure and similar molecular size. In contrast, the non-inhibitory compounds had larger and more uneven-shaped side chains. Overall, the superposed structures indicate that the molecules should be planar and have basic diethylaminoethyl groups to demonstrate strand-transfer inhibitory activity.

In conclusion, we have identified a small molecular weight compound with a carbazole scaffold, which can be the lead compound for developing novel IN inhibitors. Furthermore, analysing the IN inhibitory mechanisms of

Figure 5. A structural comparison between high/intermediate inhibitory compounds and non-inhibitory compounds



Superposed structures of **(A)** five non-inhibitory compounds, **CA-5, 6, 10, 19** and **20**, and **(B)** seven inhibitory compounds, **CA-0, 1, 4, 8, 9, 12** and **13**, are demonstrated in stereo-view images. In both figures, residue numbers are indicated beside the structures. Red, dark blue and light blue indicate oxygen, nitrogen and hydrogen molecules, respectively. Green indicates chlorine or fluorine molecules. SYBYL software Version 6.9.1 running on an SGI Fuel workstation was used to construct the figures.

carbazole derivatives may yield more detailed information regarding HIV-1 IN structure and function.

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