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A platform for designing HIV integrase inhibitors. Part 1: 2-Hydroxy-3-heteroaryl acrylic acid derivatives as novel HIV integrase inhibitor and modeling of hydrophilic and hydrophobic pharmacophores

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Abstract—We present a novel series of HIV integrase inhibitors, showing IC_{50}s ranging from 0.01 to over 370 μM in an enzymatic assay. Furthermore, pharmacophore modeling study for the inhibitors was carried out to elucidate the structure–activity relationships. Finally, we found a 3D-pharmacophore model, which is composed of a hydrophilic and a hydrophobic domain, providing valuable information for designing other novel types of integrase inhibitors.

1. Introduction

Anti-HIV drugs with approval for therapeutic use are divided into three categories based on their inhibitory mechanism: nucleoside reverse transcriptase inhibitors (NRTIs), non-nuleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). Combination therapy using the above classes of inhibitors called HAART,[1] highly active antiretroviral therapy, was confirmed to be effective for treatment of a HIV or AIDS carrier, and has been widely used in the clinical setting. Emergence of resistant viruses however is still a limiting factor for long-term therapy. New drugs targeting other steps of the viral replication cycle are expected to enhance therapeutic efficacy. Integrase[2] (IN) is a critical enzyme for HIV replication and catalyzes the integration reaction reverse transcribed double stranded viral DNA into host cell DNA. As no functional homologue of IN is known in human cells it represents an attractive target for new drug design.

Although a significant number of compounds have been identified to possess inhibitory activity against IN,[3] their further development has been delayed because of cytotoxicity, limited potency, and difficulties in structural modification. Simultaneous programs by Shionogi,[5a,b] Merck and Tularik,[6a–c] and Bristol-Myers[7] found 2,4-diketo-butanoic acid derivatives (DKAs)[4] to be inhibitors of HIV integrase and viral replication. Several lead structures are shown in Figure 1 along with biological data. Merck researchers found L-731,988 and L-708,906 to have high inhibitory activities in enzymatic assays and confirmed these are selective inhibitors of the strand transfer reaction.[6a,b] Their SAR efforts further improved the DKA scaffold to give compound **A**.[6c] Investigators at Shionogi synthesized compound **B**[5a] which shows high potency in enzymatic assays and modest potency in antiviral assays. Expanded studies of DKAs presented possibilities to replace the carboxylic group of the DKA moiety with an isosteric heteroaromatic ring[5b] as represented by 5-CITEP.[8] Finally, the series was optimized into S-1360 composed of a 4-fluorobenzylfuran and a diketo-triazole. S-1360 has potent activity not only in enzymatic assays but also in antiviral assays and was the first integrase inhibitor to progress into clinical development.[9]

Keywords: HIV integrase inhibitor; 2-Hydroxy-3-heteroaryl acrylic acid derivative; Pharmacophore modeling.
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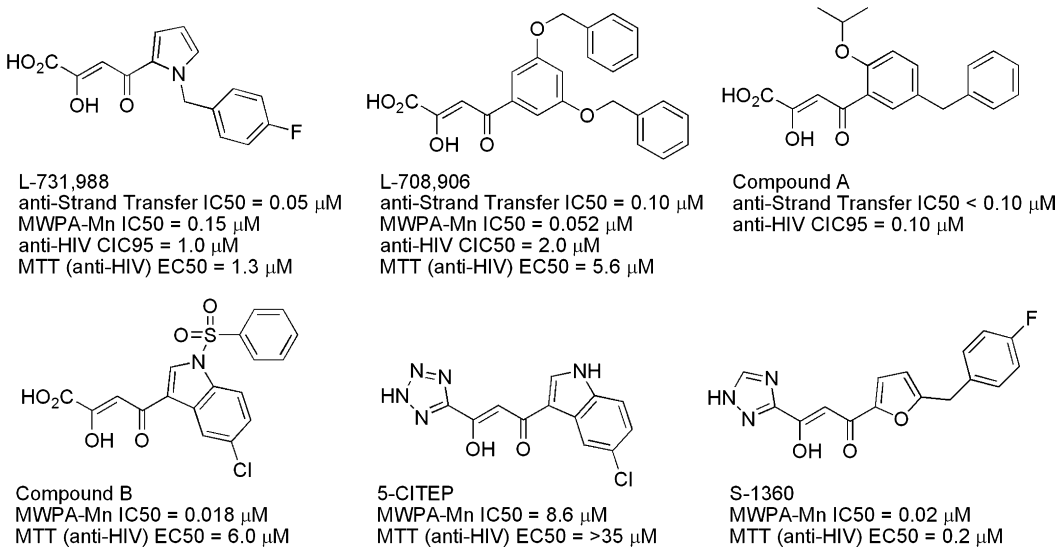


Figure 1. DKAs synthesized by Merck and Shionogi. The 1,3-diketone structures are depicted as a similar enol form. The anti-Strand Transfer and anti-HIV data of Merck's compounds (L-731,988, L-708,906, and compound **A**) are following their literatures.[6a–c] The data of MWPA-Mn enzymatic assay and MTT (anti-HIV) cellar assay were updated in-house.

Currently, DKAs are still attractive leads for designing new IN inhibitors. First, we describe our process of designing structurally novel inhibitors (Fig. 2). The general structure of DKAs can be modeled to consist of a hydrophobic and a hydrophilic domain divided at the junction of the diketo-acid substructure. In the hydrophobic domain, an indole or a benzene ring with one or two arylalkyl substituents is often employed. A variety of other hydrophobic groups have also been shown to give high activity.[4] On the contrary in the hydrophilic domain, the diketo-acid substructure has been very conserved with a few exceptions like the diketo-tetrazole group in 5-CITEP and the diketo-triazole group in S-1360. In particular, a β-diketone substructure contained in the hydrophilic domain is required for potency.

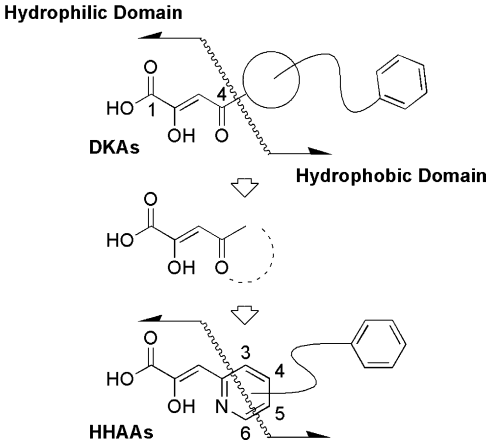


Figure 2. Drug design strategy from DKAs to HHAAs. N-1 nitrogen in the pyridine ring (Prime-Ring) of HHAAs is equivalent of the 4-carbonyl oxygen of DKAs. The bending arrow divides each general structure into a hydrophilic and a hydrophobic domain. The numbering in/on the Prime-Ring is unified to be in order as labeled.

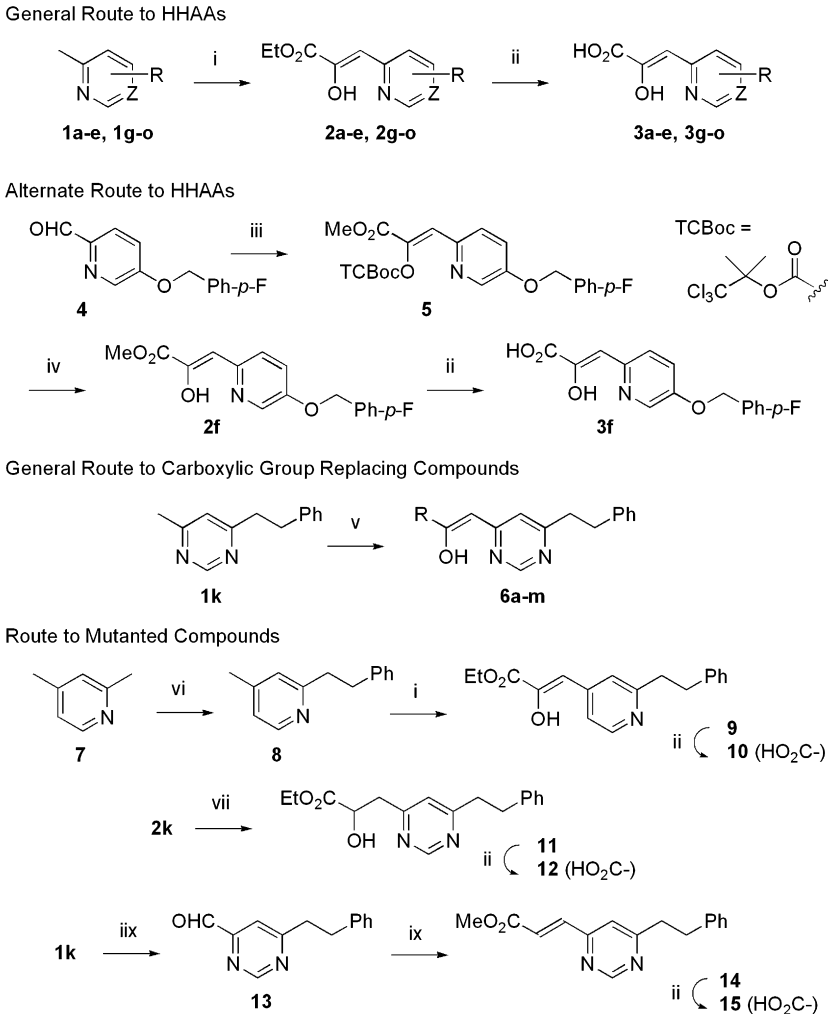
We present 2-hydroxy-3-heteroarylic acrylic acid derivatives (HHAAs) as structurally novel HIV IN inhibitors, which are composed of a 2-hydroxy acrylic acid (HAA) substructure and a nitrogen-containing heteroaromatic ring.[10] The HAA is connected to the heteroaromatic ring at the position adjacent to the nitrogen that is intended to play the role of the 4-carbonyl oxygen in the 2,4-diketo-butanoic acid lead structure. Therefore, a pyridine or a pyrimidine having a lone pair was selected for the heteroaromatic ring. Further, it should be noted that the heteroaromatic ring needs a hydrophobic substituent which would play the role of the hydrophobic domain of the DKAs. Benzyloxy, phenethyl, phenoxy or benzyl have sufficed for this substituent. Examining the combinations of the hydrophobic substituent, the heteroaromatic ring and its connecting architecture was a key feature of this study. Replacement of the carboxylic group of HHAAs with not only some bioisosteres but also heteroaromatic rings was investigated. In this paper, the heteroaromatic ring connecting to the HAA region is referred to as the 'Prime-Ring.' Numbering of positions of the Prime-Ring is unified for all compounds as follows. The position of nitrogen equivalent to the 4-carbonyl oxygen of DKAs is numbered as '1' and the nitrogen is referred to as 'N-1.' The position connecting to a HAA region, next to the N-1, is numbered as '2.' The rest is in order as labeled in Figure 2.

The general structure of HHAAs can be divided into two domains like that of DKAs as depicted in Figure 2. The N-1 and the HAA region are included in the hydrophilic domain, and the rest of the Prime-Ring and the hydrophobic substituent are assigned as the hydrophobic domain. We also introduce some analytical studies to find pharmacophores for each domain. The role of functional groups in the hydrophilic domain was investigated with a strategy in which each of the functional groups was removed or exchanged one by one in a model substrate. Alternatively, systematic

conformational searches and superimposing studies were performed to elucidate behavior of the hydrophobic domain.

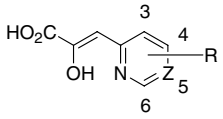
2. Chemistry

Most precursors (**2a**–**e** and **2g**–**o**) of the HHAAs were prepared by a general procedure using a condensation reaction from the corresponding methyl pyridine or methyl pyrimidine derivatives (**1a**–**e** and **1g**–**o**) as generalized in Scheme 1. The starting material was treated with an appropriate base under anhydrous conditions, then was quenched in situ with excess of a diethyloxalate in one portion to suppress generation of dimeric by-products. To prevent the formation of by-product, an alternate route toward the HHAA ester was tried using **2f** as a model study. The Horner–Emmons reaction of the aldehyde **4** with the protected phosphonate gave the corresponding protected enolate product **5**. Deprotection of the TCBoc group gave compound **2f**. Alkaline hydrolysis of esters (**2a**–**o**) gave the corresponding HHAAs (**3a**–**o**). Details of the procedures and preparations of the corresponding methyl pyridine and methyl pyrimidine derivatives (**1a**–**o**) are described in Section 5. Compounds **6a**–**m** in Table 2 which have a heteroaromatic group instead of the carboxylic group of compound **3k** were prepared in a similar manner to the HHAAs in Table 1. Each of the ester reagents having a heteroaromatic group as the R substituent easily reacted with the anion of starting material **1k** to give the corresponding adducts. Deprotection then completed the preparations. Selective benzyl extension of starting material **7** at the 2-position was achieved using *n*-BuLi and benzyl bromide to give compound **8**. Condensation with diethyl oxalate at the 4-position and subsequent basic hydrolysis gave compound **10**. Ester **2k** was reduced with sodium borohydride to give the corresponding secondary alcohol derivative **11**, which led to compound **12** following basic hydrolysis. The aldehyde derivative **13** was obtained from starting material **1k** by oxidation with selenium dioxide. A Horner–Emmons reaction



Scheme 1. Preparations of HHAAs and its mutated compounds. Reagents and conditions: (i) *n*-BuLi or *t*-BuOK, $(CO_2Et)_2$/THF −78 to 0 °C; (ii) LiOH/MeOH–H_2O 60 °C; (iii) (dimethoxy-phosphoryl)-(2,2,2-trichloro-1,1-dimethyl-ethoxycarbonyloxy)-acetic acid methyl ester, LHMDS/THF −78 to 0 °C; (iv) MeONa/MeOH 0 °C; (v) *n*-BuLi or *t*-BuOK, R–CO_2Et or R–CO_2Me (R = heteroaromatic group)/THF −78 to 0 °C; (vi) *n*-BuLi, benzylbromide/THF −78 °C; (vii) $NaBH_4$/EtOH, rt; (iix) SeO_2/1,4-dioxane reflux; (ix) (triphenylphosphoranylidene) acetic acid methyl ester/toluene, rt.

Table 1. Effect of the hydrophobic domain on inhibitory activities in enzymatic and cellular assay



Compound	Z[a]	Position[b]	R	MWPA-Mn[c] (μM)	MTT EC_{50}[d] (μM)
3a	C	3	–OCH_2Ph	332	>147
3b	C	4	–OCH_2Ph	192	>CC_{50}[e]
3c	C	5	–OCH_2Ph	0.059	4.8
3d	C	6	–OCH_2Ph	>369	>74
3e	C	5	–CH_2CH_2Ph	67	>74
3f	C	5	–OCH_2Ph-*p*-F	0.066	46
3g	C	5	–CH_2Ph-*p*-F	0.095	8.8
3h	C	5	–OPh	0.37	>78
3i	C	5	–OPh-*p*-F	0.11	>73
3j	C	4	–CH_2CH_2Ph	4.5	>74
3k	N	4	–CH_2CH_2Ph	0.037	34
3l	N	4	–CH_2CH_2Ph-*p*-F	0.059	27
3m	N	4	–OCH_2Ph	0.034	4.4
3n	N	4	–OCH_2Ph-*p*-F	0.014	7.9
3o	N	4	–OPh	17	>77

[a] 'C' and 'N' indicate carbon and nitrogen, respectively.
[b] A substituted position of R group is indicated as a number labeled by the structure.
[c] Inhibitory activities against the strand transfer. MWPA, Microtiter plate integration assay with preincubation and wash.
[d] Anti-HIV activities.
[e] CC_{50} = 9.2–18.4 μM (cell cytotoxicity).

proceeded to give the corresponding olefin adduct **14**. Alkaline hydrolysis concluded the preparation of compound **15**.

3. Biological results and discussion

3.1. Hydrophobic domain

Inhibitory activities of the HHAAs are summarized in Table 1. Among the series of compounds (**3a**–**d**) having an identical hydrophobic substituent (R = –OCH_2Ph) and an identical Prime-Ring (Z = C: pyridine), the 5-substituted compound **3c** shows the highest activity with three orders of magnitude increase in potency in MWPA-Mn enzymatic assay. Compound **3f** with an extra fluorine is almost equal to **3c**. While a slight inhibitory activity is found in 4-substituted compound **3b**, the other positions show quite low potency. All 5-substituted compounds (**3c** and **3e**–**i**) will be focused on in the following discussion. Despite the fact that the only structural difference is in the atom directly connecting to the Prime-Ring, compounds **3c/e** showed a thousand fold activity gap in the MWPA-Mn assay. The replacement of –OCH_2Ph to –OPh (**3c**–**h**) results in a slight loss of activity, and compound **3i** having an extra fluorine shows some recovery. The activity loss is within one order of magnitude. The shortening of the hydrophobic substituent caused by removing a methylene group from the –OCH_2Ph does not make an enormous impact on their activities. It should be noted that compound **3g** having a –CH_2Ph–*p*-F group possesses nearly three orders of magnitude more activity than **3e**. An extra fluorine would not give a large impact as indicated above, in which case a shortening of the –CH_2CH_2Ph group is highly effective in improving the activity. In other words, in comparison within longer substituents (–OCH_2Ph and –CH_2CH_2Ph), the atom directly connecting with the Prime-Ring has to be oxygen, while within shorter substituents (–OPh and –CH_2Ph), the replacement of an oxygen with a carbon atom does not seem critical.

The results of 4-substituted compounds are described as follows. The activity is improved to a moderate potency by replacing –OCH_2Ph with –CH_2CH_2Ph (**3b**–**j**). It should be noted that comparing each pair of compounds (**3b** vs **3m**, **3j** vs **3k**) having an identical hydrophobic group (–OCH_2Ph, –CH_2CH_2Ph, respectively), the compounds having a pyrimidine ring as the Prime-Ring (Z = N) show two or three orders of magnitude more activity than the corresponding pyridine derivatives (Z = C). Inclusion of an extra fluorine in compounds **3l** and **3n** does not greatly affect activity. Compound **3o** with an –OPh group shows a significant reduction of activity in comparison with **3m**.

Such a strong determinant of activity of the hydrophobic domain is a major point of interest. We classified all 4- or 5-substituted compounds into three active types of compounds and three inactive types of compounds (Fig. 3). Compounds **3c** and **3f** are classified into active type A, and **3e** into inactive type D. They are the 5-substituted type with a longer substituent (–OCH_2Ph or –CH_2CH_2Ph). The atom directly connecting to the Prime-Ring has to be oxygen to show high activities in this case. Compounds **3k**, **3l**, **3m**, and **3n** are classified into active type B, and **3j** and **3b** into inactive type E, 4-substituted type with the longer substituent. The atom directly connecting to the Prime-Ring does not seem to be a key factor, but the atom 'Z' has to be nitrogen

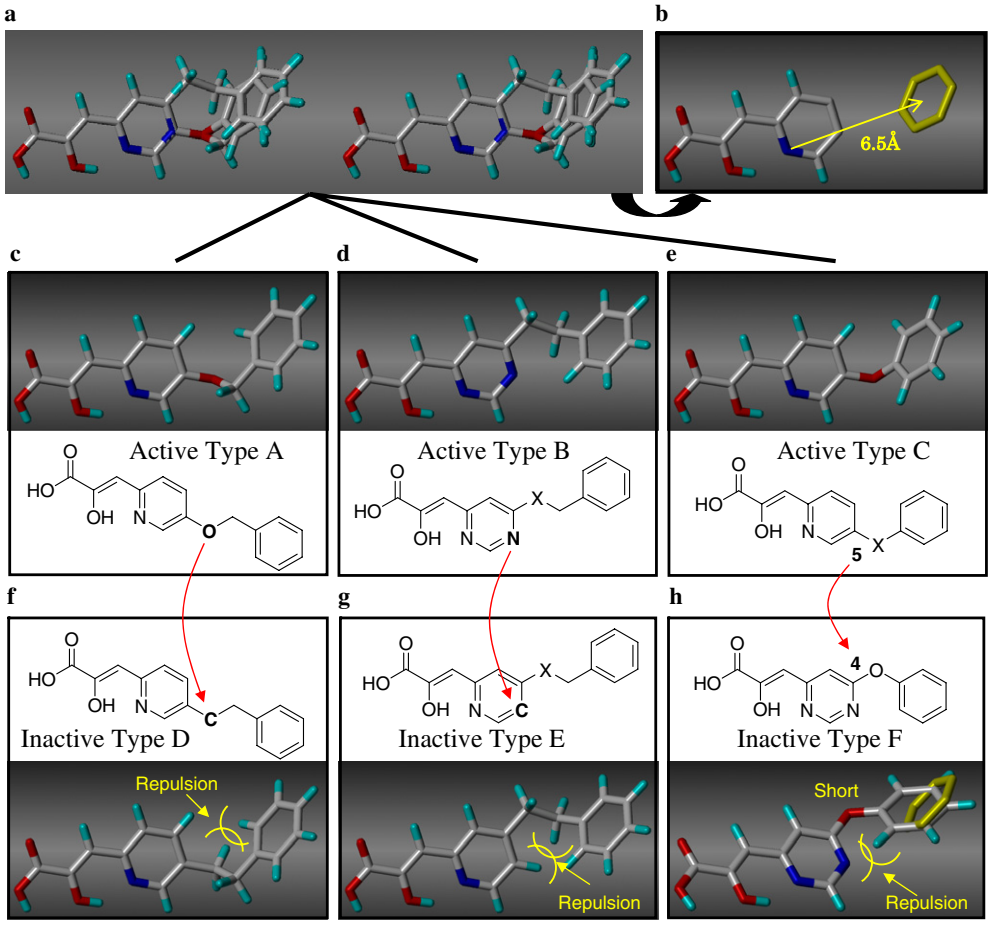


Figure 3. (a) Stereoview of a superimposed model of the molecule **3c**, **3k**, and **3h** representing active types A, B, C, respectively. Those terminal phenyl rings could be placed into a common space. (b) A yellow hexagon indicates the MFB space defined by averaging terminal phenyl rings of the superimposed model. (c–h) Pictorial representations are three types of active compounds and three of inactive, which are classified based on substitution type of the hydrophobic domain. Stick representations of types A–C present 'the active conformers' in the superimposed model. In those of types D–F, each molecule shows the same conformation to its corresponding active type. Type D has a carbon atom at next to the Prime-Ring, and its methylene hydrogen causes serious repulsion with a hydrogen of the terminal phenyl ring. Type E has a carbon atom at the N-5 position of type B, and serious repulsion with a methylene hydrogen was observed. Type F is the 4-substituted analogue of type C. Both types have a shorter substituent in comparison with the other types. The terminal benzene ring of type F cannot be oriented into the MFB space by unfavorable intramolecular repulsion with the Prime-Ring even though the position 5 is nitrogen atom. 'X' indicates a methylene or ether oxygen (X = C or O).

(Prime-Ring = pyrimidine) in this case. The compounds with a shorter substituent (–OPh or $–CH_2Ph$) are classified into 5-substituted type C (**3g**, **3h**, and **3i**) and into 4-substituted type F (**3o**). Comparing the two types, the substituent has to be connected at the position '5' to be active. To elucidate why types A, B, and C are active and D, E, and F are inactive, we performed a series of superimposing modeling studies including systematic conformational analyses of the hydrophobic substituents as described in Section 5. The study was based on the hypothesis that all terminal phenyl rings of the active types occupy an identical space in HIV IN. Then a potential overlapping model for three molecules (**3c**, **3k**, and **3h**) representing each of the active types was identified as shown in Figure 3a. The molecules are superimposed based on the identical hydrophilic domain, and the model shows the combination of three conformers that best locate their terminal phenyl rings closely in a common space within conformers showing less than 10 kcal/mol energy difference relative to the lowest energy conformer. Taking an average of the terminal phenyl rings we identified a space ca. 6.5 Å from N-1, which is depicted as 'the most favorable benzene (MFB) space' in Figure 3b. We assume that each of the superimposed conformers is 'the active conformer,' which is represented in Figures 3c–e independently. On the contrary, molecule **3e** and **3j** representing inactive types D and E do not fit in the MFB space. Figures 3f and g show the conformers of **3e** and **3j** having the same conformation to **3c** and **3k**, respectively. Energy differences of the conformers relative to the lowest energy conformers are over 100 kcal/mol by repulsion between the methylene directly connecting to the Prime-Ring and the *ortho*-hydrogen of its own terminal phenyl ring in **3e** (Fig. 3f), and between the 5-hydrogen of the Prime-Ring and the methylene directly connecting to its own terminal phenyl ring in **3j** (Fig. 3g). In brief, the methylene in type D and the 5-hydrogen in type B directly affect the formation of active conformations. The nature of the connecting atom to the Prime-Ring does not affect activity whether

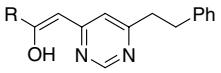
it is carbon as in **3k** and **3l**, or oxygen as in **3m** or **3n** in types B and E, because no possible repulsion is present for either atom. The phenoxy substituent of compound **3o** representing inactive type F is so short that the terminal phenyl ring makes a repulsion with the Prime-Ring, and does not fit in the MFB space closely with the same perpendicular direction (Fig. 3h). The atom directly connecting to the Prime-Ring of type F can be a methylene or an ether oxygen, because no possible repulsion can be expected. Also it does not affect the stability whether the atom directly connecting to the Prime-Ring is a methylene (**3g**: X = C) or an ether oxygen (**3h** and **3i**: X = O) in type C, because no possible intramolecular repulsion is expected. Thus, this modeling study provided a plausible explanation of why types A, B, C are active, and types D, E, F inactive, and suggests the reason why the atom directly connecting to the Prime-Ring is effective in types A, D, why the atom Z has to be nitrogen in types B, E, and why the shorter substituent has to be connecting to the position 5 of the Prime-Ring. The inactive 3- or 6-substituted compounds **3a** and **3d** do not fit their terminal phenyl rings in the MFB space. Thus, the MFB space represents an important hydrophobic pharmacophore as the optimal relative orientation of the terminal phenyl ring.

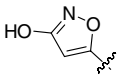
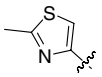
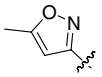
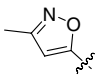
Possible active conformers of some DKAs are modeled in Figure 4. The molecular structures were superimposed into the model based on the hydrophilic domain, and the terminal phenyl rings agree well with the MFB space without serious intramolecular repulsion. Not only these compounds but also many other DKAs[4] seem to be consistent with the model. Our model also supports the observation reported by Wai et. al.[6c] They discussed SAR of the hydrophobic region with an angle of bisection between the benzyl and diketo-acid side chain. Lack of the hydrophobic substituent reaching the MFB space reduces the activity as exemplified by 5-CITEP. This suggests the importance of the hydrophobic pharmacophore.

3.2. Hydrophilic domain

Some bioisosteres and heteroaromatics were applied as a replacement for the carboxylic acid of compound **3k** and the results are summarized in Table 2. While tetrazolyl and triazolyl groups often used in the DKA study[5b] slightly reduced the activity around one order of magnitude (**6a** and **6b**), a 3-hydroxy isoxazolyl (**6c**) group known as a carboxylic acid bioisostere abolishes activity completely. 2-Thiazolyl and 2-pyrimidyl (**6d** and **6i**)

Table 2. Effect of replacements of the carboxylic group to bioisosteres or heteroaromatic rings on inhibitory activities in enzymatic and cellular assay



Compound	R	MWPA-Mn[a] (μM)	MTT EC_{50}[b] (μM)
6a		0.27	>68
6b		0.55	>68
6c		>323	>65
6d		0.21	>CC_{50}[c]
6e		2.3	>CC_{50}[c]
6f		2.3	23
6g		260	23
6h		1.7	>66
6i		0.36	>CC_{50}[c]
6j		1	>CC_{50}[c]
6k		>331	>CC_{50}[c]
6l		>330	>CC_{50}[c]
6m		>330	>CC_{50}[c]

[a] See the footnote of Table 1.
[b] See the footnote of Table 1.
[c] All the CC_{50} values = 5–20 μg/ml.

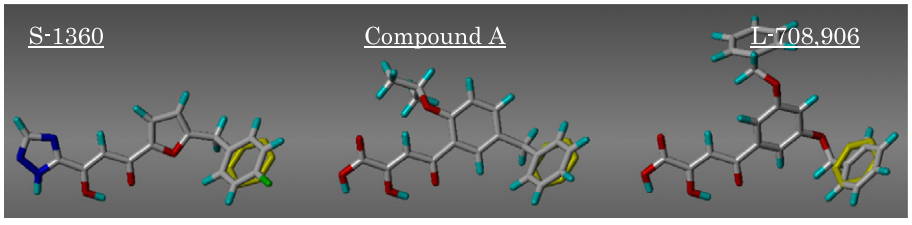


Figure 4. Agreements of DKAs with the hydrophobic pharmacophore model. Each molecule is superimposed over the model based on the hydrophilic domain and represented by stick model with the MFB colored yellow.

groups show activity but some loss around one order of magnitude. The reduction of activity due to 2-methyl-4-thiazolyl, 5-methyl-3-isoxazolyl, pyradyl and 2-pyridyl (**6e**, **6f**, **6h**, and **6j**) groups reach nearly two orders of magnitude as compared with **3k**. It should be noted that the 3-methyl-5-isoxazolyl (**6g**) group, which is a regioisomer of **6f** with opposite arrangement of the nitrogen and oxygen, induces major loss of activity reaching four orders. Also a 3-pyridyl and 4-pyridyl (**6l** and **6m**) group which are regioisomers of **6j** and a phenyl group (**6k**) show no activity. It does not seem important whether the heteroaromatic ring is a five-membered or six-membered system. One of two atoms next to the junction has to be nitrogen as a Lewis base equivalent of the carboxylic acid to possess activity and if another one is nitrogen or sulfur (**6a**, **6b**, **6d**, and **6i**) there is an increase of approximately one order of magnitude of activity observed. Compound **10** of which the N-1 nitrogen was replaced with carbon has no activity. Compound **12** in which the enolic olefin was reduced to a secondary alcohol and compound **15** in which the enolic hydroxy group was removed have no activity.

To emphasize the influence on activity from each functional group in hydrophilic domain, the point mutated compounds are shown in Figure 5 for comparison with reference compound **3k**. Their impact on activity is dramatic resulting in an enormous loss of activity as described above and directly produces a critical rule for the pharmacophore model. (1) The N-1, that potentially plays a role of the 4-carbonyl oxygen of the DKAs, is indispensable for potential activity. (2) The enolic functional group that plays a role similar to that of the 2-carbonyl group of DKAs is also essential. (3) A potentially active compound must have a carboxylic acid or nitrogen containing heteroaromatic ring with the nitrogen next to the junction as a Lewis base equivalent, which could act as a hydrogen bonding acceptor. Generally, aromatic oxygens are not thought to work as a hydrogen bonding acceptor. This idea is in agreement with the fact that compounds **6c** and **6g** did not show any activity.

4. Conclusions

We succeeded in finding the HHAAs as novel inhibitors of HIV integrase. The series of compounds do not have the β-diketone substructure integral to the DKA compounds. Combination of the Prime-Ring, a hydrophobic substituent, and substituted position proved to be fundamental for activity which ranged from 0.01 to 370 μM in enzymatic MWPA-Mn assay. All compounds in Tables 1 and 2 did not show high anti-HIV activities in an MTT/MT-4 assay, even the most active compound **3m** showed only a 4.4 μM EC50. This issue and a further study about the hydrophilic pharmacophore are discussed in the companion paper.[11]

We subsequently obtained the final pharmacophore model (Fig. 6) constructed with the functional groups in the hydrophilic domain and the MFB space for the hydrophobic domain. Both domains are key and should be useful in designing other novel inhibitors and related analogues.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were determined as 300 MHz. All reactions were carried out under a nitrogen atmosphere with anhydrous solvents that had been dried over type 4 Å molecular sieves.

5.1.1. Preparation of methyl pyridine and methyl pyrimidine derivatives (1a–e and 1g–o)

5.1.1.1. 3-Benzyloxy-2-methyl-pyridine (1a). Potassium carbonate (2.76 g, 20 mmol) was added to a mixture

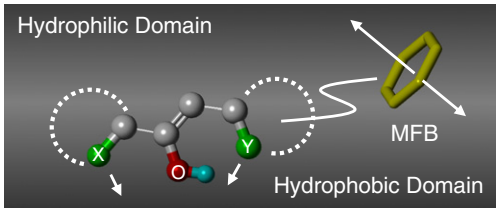


Figure 6. Final pharmacophore model. The atoms 'X' and 'Y' represent possible heteroatoms that serve a lone-pair indicated by the arrow. The double-headed arrow passing through the MFB indicates a favorable direction of the ring plain. The semicircles written in broken line indicate heteroaromatic rings optionally including the 'C = X' or 'C = Y' bond. Two bonds connecting three hydrophilic functional groups are possible to rotate. The winding line indicates any possible linker to connect the hydrophilic domain and a terminal phenyl ring.

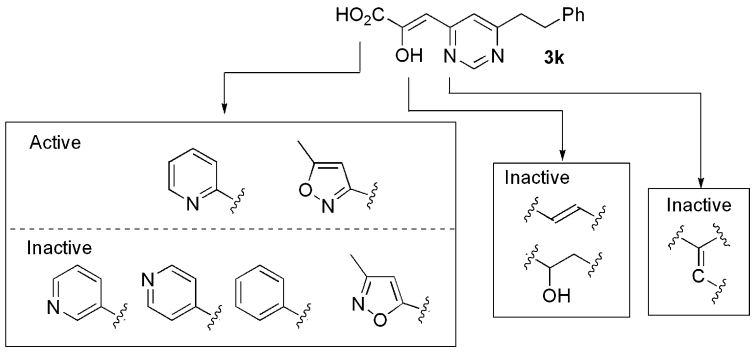


Figure 5. Methodical representation and results of the compounds mutated for each hydrophilic functional group of the test compound **3k**.

of benzyl bromide (3.42 g, 20 mmol) and 2-methyl-pyridin-3-ol (2.18 g, 20 mmol) in DMF (50 mL) at room temperature. The reaction mixture was stirred for 3 h, then diluted with a saturated aqueous solution of NH_4Cl and ether. The organic layer was washed with water and brine, and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 1:1 as eluent to give the product as a colorless oil (1.60 g, 40% yield). ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H), 5.40 (s, 2H), 7.07 (dd, J = 5.9, 2.9 Hz, 1H), 7.13 (dd, J = 5.9, 1.2 Hz, 1H), 7.30–7.46 (m, 5H), 8.09 (dd, J = 2.9, 1.2 Hz, 1H).

5.1.1.2. 4-Benzyloxy-2-methyl-pyridine (1b). Benzyl alcohol (1.62 g, 15 mmol) was added to a suspension of NaH (400 mg, 60% oil suspension, 15 mmol) in DMF (10 mL) at 0 °C, then the reaction mixture was warmed to room temperature and stirred for 10 min. 4-Chloro-2-methyl-pyridine (1.27 g, 10 mmol) was added to the mixture then warmed to 60 °C. After additional stirring for 3 h the reaction mixture was diluted with a saturated aqueous solution of NH_4Cl and ether. The organic layer was washed with water and brine, then dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 1:1 as eluent to give the product as a colorless oil (1.60 g, 80% yield). ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H), 5.09 (s, 2H), 6.70 (dd, J = 4.2, 1.5 Hz, 1H), 6.75 (d, J = 1.5 Hz, 1H), 7.32–7.42 (m, 5H), 8.31 (d, J = 4.2 Hz, 1H).

5.1.1.3. 5-Benzyloxy-2-methyl-pyridine (1c). This compound was prepared by a similar method to that described for **1a** from 6-methyl-pyridin-3-ol (colorless oil, 35% yield). ^1H NMR ($CDCl_3$) δ 2.48 (s, 3H), 5.09 (s, 2H), 7.05 (d, J = 5.3 Hz, 1H), 7.17 (dd, J = 5.3, 2.2 Hz, 1H), 7.30–7.45 (m, 5H), 8.26 (d, J = 2.2 Hz, 1H).

5.1.1.4. 2-Benzyloxy-6-methyl-pyridine (1d). This compound was prepared by a similar method to that described for **1c** from 2-chloro-6-methyl-pyridine (colorless oil, 80% yield). ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H), 5.35 (s, 2H), 6.59 (d, J = 5.7 Hz, 1H), 6.73 (d, J = 5.7 Hz, 1H), 7.28–7.50 (m, 6H).

5.1.1.5. 2-Methyl-5-phenethyl-pyridine (1e). This compound was prepared by a similar method to that of Vanier.[12] A solution of 9-BBN (11.1 mL, 0.5 M in THF, 5.54 mmol) was added to a solution of 2-methyl-5-vinyl-pyridine[13] (330 mg, 2.77 mmol) in THF (2 mL) at room temperature. After stirring for 3 h at 60 °C iodobenzene (1.70 g, 8.31 mmol), NaOH (2.77 mL, 3 M in water, 8.31 mmol), and $PdCl_2$(dppf) (226 mg, 0.28 mmol) was added to the solution at room temperature. After stirring for 3 h at 50 °C the mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water and brine, and then dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 4:1 as eluent to give the product as a colorless oil (540 mg, 99% yield). ^1H NMR ($CDCl_3$) δ 2.52 (s, 3H), 2.89 (s, 4H), 7.04 (d, J = 7.8 Hz, 1H), 7.14–7.34 (m, 6H), 8.30 (d, J = 2.4 Hz, 1H).

5.1.1.6. 5-(4-Fluoro-benzyl)-2-methyl-pyridine (1g). Trifluoromethanesulfonic acid anhydride (18.5 mL, 120 mmol) was added to a solution of 6-methyl-pyridin-3-ol (10.9 g, 100 mmol) and pyridine (12.2 mL, 150 mmol) in CH_2Cl_2 (100 mL) at 0 °C. After stirring for 1.5 h MeOH (2 mL) and a saturated aqueous solution of $NaHCO_3$ were added to the mixture. The organic layer was washed with water and brine, and then dried over $MgSO_4$. The solvent was removed under in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 9:1 to 4:1 as eluent to give trifluoromethanesulfonic acid 6-methyl-pyridin-3-yl ester as a colorless oil (23.0 g, 95% yield). ^1H NMR ($CDCl_3$) δ 2.61 (s, 3H), 7.26 (d, J = 8.4 Hz, 1H), 7.52 (dd, J = 9.0, 3.0 Hz, 1H), 8.47 (d, J = 3.0 Hz, 1H). To a solution of this compound (10.4 g, 43.2 mmol) in THF (130 mL) were added 4-fluorobenzyl zinc bromide (65 mmol in THF 50 mL) prepared according to the manner of Takai,[14] and $Pd(PPh_3)_4$ (2.4 g, 2.1 mmol), then the mixture was refluxed for 5 h. After evaporation of the solvent, water and EtOAc were added to the residue, then the insoluble precipitate was removed by filtration. The filtrate was extracted with EtOAc, washed with 1 M aqueous solution of HCl. The aqueous layer was basified with 2 M aqueous solution of NaOH, then extracted with EtOAc. The organic layer was washed with water and brine, then dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 2:1 as eluent to give the desired compound as a colorless oil (5.42 g, 60% yield). ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 3.91 (s, 2H), 6.95–7.01 (m, 2H), 7.06–7.14 (m, 3H), 7.33 (dd, J = 8.1, 2.1 Hz, 1H), 8.36 (d, J = 1.5 Hz, 1H).

5.1.1.7. 2-Methyl-5-phenoxy-pyridine (1h). This compound was prepared by a similar method to that of Marcoux.[15] A mixture of 6-methyl-pyridin-3-ol (6.0 g, 55.0 mmol), iodo-benzene (10.2 g, 50.0 mmol), copper powder (635 mg, 10.0 mmol), and *t*-BuOK (6.73 g, 60.0 mmol) in DMF (20 mL) was stirred for 6 h at 140 °C. MeOH (100 mL) was added to the mixture, and the precipitate was filtered off. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 4:1 as eluent to give the desired compound as a colorless oil (6.26 g, 68% yield). ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 6.97–7.01 (m, 2H), 7.10–7.15 (m, 2H), 7.22 (dd, J = 8.4, 2.7 Hz, 1H), 7.31–7.38 (m, 2H), 8.30 (d, J = 2.7 Hz, 1H).

5.1.1.8. 5-(4-Fluoro-phenoxy)-2-methyl-pyridine (1i). This compound was prepared by a similar method to that described for **1h** (colorless oil, 68% yield). ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 6.94–7.07 (m, 4H), 7.11 (d, J = 8.4 Hz, 1H), 7.18 (dd, J = 8.4, 2.8 Hz, 1H), 8.26 (d, J = 2.7 Hz, 1H).

5.1.1.9. 2-Methyl-4-phenethyl-pyridine (1j). This compound was prepared according to a manner of

Hamana.[16] ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H), 2.91 (s, 4H), 6.94 (d, J = 4.8 Hz, 1H), 7.13–7.23 (m, 5H), 8.38 (d, J = 5.1 Hz, 1H).

5.1.1.10. 4-Methyl-6-phenethyl-pyrimidine (1k). A solution of *n*-BuLi (129 mL, 1.55 M in *n*-hexane, 200 mmol) was added to a solution of 4,6-dimethyl-pyrimidine (21.6 g, 200 mmol) in THF (150 mL) at −78 °C. Then a solution of benzyl bromide (34.2 g, 200 mmol) in THF (50 mL) was added to the mixture at −78 °C. The mixture was diluted with a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, and dried over $MgSO_4$. The solvent was removed in vacuo to give the product as a colorless oil (39.2 g, 99% yield). ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H), 2.96–3.10 (m, 4H), 6.96 (s, 1H), 7.15–7.31 (m, 5H), 9.02 (s, 1H).

5.1.1.11. 4-[2-(4-Fluoro-phenyl)-ethyl]-6-methyl-pyrimidine (1l). This compound was prepared by a similar method to that described for **1k** (colorless oil, quantitative yield). ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H), 2.96–3.10 (m, 4H), 6.92–6.99 (m, 3H), 7.09–7.16 (m, 2H), 9.02 (d, J = 1.2 Hz, 1H).

5.1.1.12. 4-Benzyloxy-6-methyl-pyrimidine (1m). This compound was prepared by a similar method to that described for **1b** from 4-chloro-6-methyl-pyrimidine, which was prepared in a similar manner to that of Butters[17] (colorless oil, 93% yield). ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 5.42 (s, 2H), 6.64 (s, 1H), 7.30–7.48 (m, 5H), 8.69 (s, 1H).

5.1.1.13. 4-(4-Fluoro-benzyloxy)-6-methyl-pyrimidine (1n). This compound was prepared by a similar method to that described for **1m** (colorless oil, 87% yield). ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 5.38 (s, 2H), 6.62 (s, 1H), 7.03–7.10 (m, 2H), 7.39–7.44 (m, 2H), 8.67 (s, 1H).

5.1.1.14. 4-Methyl-6-phenoxy-pyrimidine (1o). This compound was prepared by a similar method to that described for **1m** (colorless oil, 93% yield). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H), 6.72 (s, 1H), 7.10–7.18 (m, 2H), 7.25–7.32 (m, 1H), 7.41–7.49 (m, 2H), 8.68 (s, 1H).

5.1.2. General procedure for the preparation of 2-hydroxy-3-heteroaryl acrylic acid ethyl ester derivatives (2a–k)

5.1.2.1. 2-Hydroxy-3-(6-phenethyl-pyrimidin-4-yl)-acrylic acid ethyl ester (2k). A solution of *n*-BuLi (9.75 mL, 1.54 M in *n*-hexane, 15 mmol) was added to a solution of compound **1k** (2.97 g, 15 mmol) in THF (60 mL) at −78 °C. The reaction mixture was stirred for 30 min and then diethyl oxalate (11.0 g, 75 mmol) was added in one portion. The mixture was then warmed to 0 °C and stirred for 30 min. After dilution with a saturated aqueous solution of NH_4Cl, the mixture was extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. The solvent was removed in vacuo, then the precipitate was filtered and washed with *n*-hexane. Removal of the solvent in vacuo gave the product as pale yellow crystals (2.70 g, 60% yield). Mp 135–137 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 3H), 3.06 (s, 4H), 4.35 (q, J = 7.1 Hz, 2H), 6.39 (s, 1H), 6.86 (s, 1H), 7.12–7.35 (m, 6H), 8.95 (s, 1H). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.34; H, 5.94; N, 9.46.

5.1.2.2. 3-(3-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid ethyl ester (2a). This compound was prepared by a similar method to that described for **2k** from the compound **1a** (pale yellow crystals, 45% yield). Mp 112–113 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), 5.16 (s, 2H), 7.03 (s, 1H), 7.08 (dd, J = 8.2, 5.0 Hz, 1H), 7.21 (dd, J = 8.2, 1.2 Hz, 1H), 7.31–7.46 (m, 5H), 8.01 (dd, J = 5.0, 1.2 Hz, 1H). Anal. Calcd for $C_{17}H_{17}N_1O_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.02; H, 5.77; N, 4.60.

5.1.2.3. 3-(4-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid ethyl ester (2b). This compound was prepared by a similar method to that described for **2k** from the compound **1b** (pale yellow crystals, 48% yield). Mp 156–158 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.3 Hz, 3H), 4.35 (q, J = 7.3 Hz, 2H), 5.14 (s, 2H), 6.47 (s, 1H), 6.70–6.80 (m, 2H), 7.30–7.50 (m, 5H), 8.16 (d, J = 6.0 Hz, 1H). Anal. Calcd for $C_{17}H_{17}N_1O_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.02; H, 5.52; N, 4.73.

5.1.2.4. 3-(5-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid ethyl ester (2c). This compound was prepared by a similar method to that described for **2k** from the compound **1c** (pale yellow crystals, 6% yield). Mp 115–117 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.0 Hz, 3H), 4.35 (q, J = 7.0 Hz, 2H), 5.14 (s, 2H), 6.58 (s, 1H), 7.18–7.43 (m, 7H), 8.24 (d, J = 2.4 Hz, 1H). Anal. Calcd for $C_{17}H_{17}N_1O_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.22; H, 5.56; N, 4.69.

5.1.2.5. 3-(6-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid ethyl ester (2d). This compound was prepared by a similar method to that described for **2k** from the compound **1d** (pale yellow crystals, 21% yield). Mp 55–57 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 3H), 4.36 (q, J = 7.1 Hz, 2H), 5.33 (s, 2H), 6.57 (s, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 7.30–7.50 (m, 5H), 7.65 (dd, J = 8.0, 7.8 Hz, 1H). Anal. Calcd for $C_{17}H_{17}N_1O_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.21; H, 5.71; N, 4.76.

5.1.2.6. 2-Hydroxy-3-(5-phenethyl-pyridin-2-yl)-acrylic acid ethyl ester (2e). This compound was prepared by a similar method to that described for **2k** from the compound **1e** (yellow crystals, 21% yield). Mp 108–110 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 2.94 (s, 4H), 4.35 (q, J = 7.1 Hz, 2H), 6.54 (s, 1H), 7.10–7.31 (m, 6H), 7.47 (dd, J = 8.1, 2.4 Hz, 1H), 8.20 (d, J = 2.4 Hz, 1H). Anal. Calcd for $C_{18}H_{19}N_1O_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.91; H, 6.30; N, 4.82.

5.1.2.7. 3-[5-(4-Fluoro-benzyl)-pyridin-2-yl]-2-hydroxy-acrylic acid ethyl ester (2g). This compound was prepared by a similar method to that described for **2k**

from the compound **1g** (pale yellow crystals, 45% yield). Mp 94–96 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 3.96 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 6.56 (s, 1H), 6.98–7.04 (m, 2H), 7.11–7.18 (m, 2H), 7.51 (dd, J = 8.3, 2.0 Hz, 1H), 8.29 (d, J = 2.0 Hz, 1H). Anal. Calcd for $C_{17}H_{16}F_1N_1O_3$: C, 67.76; H, 5.35; F, 6.31; N, 4.65. Found: C, 67.83; H, 5.21; F, 6.13; N, 4.63.

5.1.2.8. 2-Hydroxy-3-(5-phenoxy-pyridin-2-yl)-acrylic acid ethyl ester (2h). This compound was prepared by a similar method to that described for **2k** from the compound **1h** (pale yellow crystals, 26% yield). Mp 73–75 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.0 Hz, 3H), 4.36 (q, J = 7.0 Hz, 2H), 6.58 (s, 1H), 7.04–7.08 (m, 2H), 7.17–7.22 (m, 2H), 7.35–7.43 (m, 3H), 8.24 (d, J = 2.7 Hz, 1H). Anal. Calcd for $C_{16}H_{15}N_1O_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.37; H, 5.28; N, 4.91.

5.1.2.9. 3-[5-(4-Fluoro-phenoxy)-pyridin-2-yl]-2-hydroxy-acrylic acid ethyl ester (2i). This compound was prepared by a similar method to that described for **2k** from the compound **1i** (pale yellow crystals, 26% yield). Mp 99–101 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.0 Hz, 3H), 4.36 (q, J = 7.0 Hz, 2H), 6.58 (s, 1H), 7.01–7.13 (m, 4H), 7.21 (d, J = 8.9 Hz, 1H), 7.33 (dd, J = 8.5, 2.7 Hz, 1H), 8.22 (d, J = 2.7 Hz, 1H). Anal. Calcd for $C_{16}H_{14}F_1N_1O_4$: C, 63.36; H, 4.65; F, 6.26; N, 4.62. Found: C, 63.43; H, 4.60; F, 5.98; N, 4.62.

5.1.2.10. 2-Hydroxy-3-(4-phenethyl-pyridin-2-yl)-acrylic acid ethyl ester (2j). This compound was prepared by a similar method to that described for **2k** from the compound **1j** (pale yellow crystals, 53% yield). Mp 120–122 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.0 Hz, 3H), 2.98 (s, 4H), 4.38 (q, J = 7.0 Hz, 2H), 6.65 (s, 1H), 7.01 (d, J = 5.7 Hz, 1H), 7.13 (d, J = 7.2 Hz, 1H), 7.20–7.34 (m, 5H), 8.29 (d, J = 5.4 Hz, 1H). Anal. Calcd for $C_{18}H_{19}N_1O_3{\cdot}0.1H_2O$: C, 72.27; H, 6.47; N, 4.86. Found: C, 72.30; H, 6.31; N, 4.95.

5.1.3. General procedure for the preparation of 2-hydroxy-3-heteroaryl acrylic acid ethyl ester derivatives (2l–o).

5.1.3.1. 3-{6-[2-(4-Fluoro-phenyl)-ethyl]-pyrimidin-4-yl}-2-hydroxy-acrylic acid ethyl ester (2l). Diethyl oxalate (5.12 g, 35 mmol) was added to a mixture of the compound **1l** (1.51 g, 7 mmol) and *t*-Bu-OK (1.57 g, 14 mmol) in THF (20 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 3 h. After dilution with a saturated aqueous solution of NH_4Cl, the mixture was extracted with EtOAc. The organic layer was washed with water and brine then dried over Na_2SO_4. The solvent was removed in vacuo, then the precipitate was filtered and washed with *n*-hexane. The solvent was removed in vacuo to give the product as pale yellow crystals (923 mg, 42% yield). Mp 139–141 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.0 Hz, 3H), 3.04 (s, 4H), 4.36 (q, J = 7.0 Hz, 2H), 6.39 (s, 1H), 6.86 (s, 1H), 6.93–6.99 (m, 2H), 7.10–7.15 (m, 2H), 8.95 (s, 1H). Anal. Calcd for $C_{17}H_{17}F_1N_2O_3$: C, 64.55; H, 5.42; F, 6.01; N, 8.86. Found: C, 64.50; H, 5.58; F, 5.85; N, 8.84.

5.1.3.2. 3-(6-Benzyloxy-pyrimidin-4-yl)-2-hydroxy-acrylic acid ethyl ester (2m). This compound was prepared by a similar method to that described for **2l** from the compound **1m** (pale yellow crystals, 77% yield). Mp 136–137 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.2 Hz, 3 H), 4.36 (q, J = 7.2 Hz, 2H), 5.46 (s, 2H), 6.42 (s, 1H), 6.57 (d, J = 1.1 Hz, 1H), 7.35–7.46 (m, 5H), 8.69 (d, J = 1.1 Hz, 1H). Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.95; H, 5.25; N, 9.31.

5.1.3.3. 3-[6-(4-Fluoro-benzyloxy)-pyrimidin-4-yl]-2-hydroxy-acrylic acid ethyl ester (2n). This compound was prepared by a similar method to that described for **2l** from the compound **1n** (pale yellow crystals, 70% yield). Mp 107–108 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), 5.42 (s, 2H), 6.42 (s, 1H), 6.56 (d, J = 0.9 Hz, 1H), 7.05–7.11 (m, 2H), 7.40–7.46 (m, 2H), 8.69 (s, 1H). Anal. Calcd for $C_{16}H_{15}F_1N_2O_4$: C, 60.37; H, 4.75; F, 5.97; N, 8.80. Found: C, 60.40; H, 4.68; F, 5.74; N, 8.77.

5.1.3.4. 2-Hydroxy-3-(6-phenoxy-pyrimidin-4-yl)-acrylic acid ethyl ester (2o). This compound was prepared by a similar method to that described for **2l** from the compound **1o** (pale yellow crystals, 73% yield). Mp 122–124 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 3H), 4.37 (q, J = 7.1 Hz, 2H), 6.46 (s, 1H), 6.64 (s, 1H), 7.14–7.18 (m, 2H), 7.29–7.34 (m, 1H), 7.44–7.49 (m, 2H), 8.69 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.67; H, 4.82; N, 9.72.

5.1.4. Alternate route toward HHAA ester: 3-[5-(4-Fluoro-benzyloxy)-pyridin-2-yl]-2-(2,2,2-trichloro-1,1-dimethyl- ethoxycarbonyloxy)-acrylic acid methyl ester (5). A solution of lithium bis (trimethylsilyl)amide (5.62 mL, 1 M in THF, 5.62 mmol) was added to a solution of (dimethoxy-phosphoryl)-(2,2,2-trichloro-1,1-dimethyl-ethoxycarbonyloxy)- acetic acid methyl ester[18] (1.91 g, 4.76 mmol) in THF (50 mL) at −78 °C. After stirring for 30 min, 5-(4-fluoro-benzyloxy)-pyridine-2-carbaldehyde (4) (1.00 g, 4.3 mmol), which was prepared according to a manner of Jen,[19] was added to the mixture at −78 °C. After stirring for 15 min, the reaction mixture was warmed to 0 °C, then stirred for 30 min. The mixture was diluted with a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, then dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc = 1:1 as eluent to give the product (1.10 g, 50% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 6H), 3.77 (s, 3H), 5.09 (s, 2H), 6.99 (s, 1H), 7.06–7.12 (m, 2H), 7.23 (dd, J = 8.7, 3.0 Hz, 1H), 7.38–7.43 (m, 2H), 7.68 (d, J = 9.0 Hz, 1H), 8.36 (d, J = 2.7 Hz, 1H).

5.1.4.1. 3-[5-(4-Fluoro-benzyloxy)-pyridin-2-yl]-2-hydroxy-acrylic acid methyl ester (2f). A solution of MeO-Na (0.45 mL, 28 w/v% in MeOH, 2.36 mmol) was added to a solution of **5** in MeOH (25 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature. The

mixture was diluted with a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, then dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using EtOAc as eluent to give the product as pale yellow crystals (233 mg, 32% yield). Mp 131–132 °C, ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 5.10 (s, 2H), 6.56 (s, 1H), 7.10 (t, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.4, 2.7 Hz, 1H), 7.36–7.45 (m, 2H), 8.13 (d, J = 2.7 Hz, 1H). Anal. Calcd for $C_{16}H_{14}F_1N_1O_4$: C, 63.36; H, 4.65; F, 6.26; N, 4.62. Found: C, 63.63; H, 4.57; F, 6.06; N, 4.66.

5.1.5. General procedure for the preparation of 2-hydroxy-3-heteroaryl acrylic acid derivatives (3a–o)

5.1.5.1. 2-Hydroxy-3-(6-phenethyl-pyrimidin-4-yl)-acrylic acid (3k). A solution of LiOH (28.4 mL, 1 M in water, 28.4 mmol) was added to a solution of **2k** (4.23 g, 14.2 mmol) in MeOH (100 mL) at room temperature. The reaction mixture was warmed to 60 °C and stirred for 2 h. Concentration of the mixture to 50 ml was followed by acidification to pH 5–6 with citric acid. The precipitate was filtered and washed with water, then dried up in vacuo to give the desired compound as pale yellow crystals (3.65 g, 95% yield). Mp 199–201 °C, ^{1}H NMR (DMSO-d_6) δ 3.00 (s, 4H), 6.29 (s, 1H), 7.15–7.40 (m, 6H), 8.92 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.61; H, 5.17; N, 10.35.

5.1.5.2. 3-(3-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid (3a). This compound was prepared by a similar method to that described for **3k** from compound **2a** (pale yellow crystals, 77% yield). Mp 67–69 °C, ^{1}H NMR (DMSO-d_6) δ 5.25 (s, 2H), 6.74 (s, 1H), 7.28–7.52 (m, 6H), 7.64 (dd, J = 8.5, 1.1 Hz, 1H), 8.12 (dd, J = 5.1, 1.1 Hz, 1H). Anal. Calcd for $C_{15}H_{13}N_1O_4$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.06; H, 4.73; N, 5.34.

5.1.5.3. 3-(4-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid (3b). This compound was prepared by a similar method to that described for **3k** from compound **2b** (pale yellow crystals, 65% yield). Mp 142–144 °C, ^{1}H NMR (DMSO-d_6) δ 5.22 (s, 2H), 6.35 (s, 1H), 6.91 (dd, J = 6.0, 2.4 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.32–7.53 (m, 5H), 8.29 (d, J = 6.0 Hz, 1H). Anal. Calcd for $C_{15}H_{13}N_1O_4 \cdot 0.2H_2O$: C, 65.54; H, 4.91; N, 5.10. Found: C, 65.57; H, 4.58; N, 5.12.

5.1.5.4. 3-(5-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid (3c). This compound was prepared by a similar method to that described for **3k** from compound **2c** (pale yellow crystals, 78% yield). Mp 176–177 °C, ^{1}H NMR (DMSO-d_6) δ 5.23 (s, 2H), 6.66 (s, 1H), 7.30–7.65 (m, 7H), 8.38 (d, J = 3.3 Hz, 1H). Anal. Calcd for $C_{15}H_{13}N_1O_4 \cdot 0.3H_2O$: C, 65.12; H, 4.95; N, 5.06. Found: C, 65.27; H, 4.77; N, 4.97.

5.1.5.5. 3-(6-Benzyloxy-pyridin-2-yl)-2-hydroxy-acrylic acid (3d). This compound was prepared by a similar method to that described for **3k** from compound **2d** (pale yellow crystals, 55% yield). Mp 155–156 °C, ^{1}H NMR (DMSO-d_6) δ 5.35 (s, 2H), 6.53 (s, 1H), 6.87 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 7.30–7.50 (m, 5H), 7.82 (dd, J = 8.1, 7.9 Hz, 1H). Anal. Calcd for $C_{15}H_{13}N_1O_4$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.28; H, 4.73; N, 5.24.

5.1.5.6. 2-Hydroxy-3-(5-phenethyl-pyridin-2-yl)-acrylic acid (3e). This compound was prepared by a similar method to that described for **3k** from compound **2e** (yellow crystals, 80% yield). Mp 162–165 °C, ^{1}H NMR (DMSO-d_6) δ 2.92 (s, 4H), 6.52 (s, 1H), 7.15–7.31 (m, 5H), 7.42 (d, J = 8.4 Hz, 1H), 7.77 (dd, J = 7.8, 2.1 Hz, 1H), 8.36 (d, J = 2.1 Hz, 1H). Anal. Calcd for $C_{16}H_{15}N_1O_3 \cdot 0.3H_2O$: C, 69.96; H, 5.72; N, 5.10. Found: C, 69.94; H, 5.38; N, 5.13.

5.1.5.7. 3-[5-(4-Fluoro-benzyloxy)-pyridin-2-yl]-2-hydroxy-acrylic acid (3f). This compound was prepared by a similar method to that described for **3k** from compound **2f** (pale yellow crystals, 92% yield). Mp 201–203 °C, ^{1}H NMR (DMSO-d_6) δ 5.21 (s, 2H), 6.57 (s, 1H), 7.20–7.27 (m, 2H), 7.50–7.56 (m, 3H), 7.62 (dd, J = 8.9, 2.9 Hz, 1H), 8.38 (d, J = 2.9 Hz, 1H). Anal. Calcd for $C_{15}H_{12}F_1N_1O_4$: C, 62.28; H, 4.18; F, 6.57; N, 4.84. Found: C, 62.50; H, 4.17; F, 6.30; N, 4.94.

5.1.5.8. 3-[5-(4-Fluoro-benzyl)-pyridin-2-yl]-2-hydroxy-acrylic acid (3g). This compound was prepared by a similar method to that described for **3k** from compound **2g** (auburn crystals, 70% yield). Mp 139–141 °C, ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 2H), 6.48 (s, 1H), 7.02–7.18 (m, 5H), 7.58 (dd, J = 8.5, 2.1 Hz, 1H), 7.96 (s, 1H). Anal. Calcd for $C_{15}H_{12}F_1N_1O_3$: C, 65.93; H, 4.43; F, 6.95; N, 5.13. Found: C, 66.12; H, 4.44; F, 6.77; N, 5.19.

5.1.5.9. 2-Hydroxy-3-(5-phenoxy-pyridin-2-yl)-acrylic acid (3h). This compound was prepared by a similar method to that described for **3k** from compound **2h** (pale yellow crystals, 82% yield). Mp 152–154 °C, ^{1}H NMR (DMSO-d_6) δ 6.62 (s, 1H), 7.10–7.13 (m, 2H), 7.19–7.24 (m, 1H), 7.42–7.47 (m, 2H), 7.58 (s, 1H), 7.59 (s, 1H), 8.37–8.40 (m, 1H). Anal. Calcd for $C_{14}H_{11}N_1O_4$: C, 65.37; H, 4.31; N, 5.44. Found: C, 65.33; H, 4.30; N, 5.39.

5.1.5.10. 3-[5-(4-Fluoro-phenoxy)-pyridin-2-yl]-2-hydroxy-acrylic acid (3i). This compound was prepared by a similar method to that described for **3k** from compound **2i** (pale yellow crystals, 88% yield). Mp 173–175 °C, ^{1}H NMR (DMSO-d_6) δ 6.61 (s, 1H), 7.16–7.31 (m, 4H), 7.55 (dd, J = 8.7, 2.6 Hz, 1H), 7.59 (dd, J = 8.7, 0.9 Hz, 1H), 8.37 (dd, J = 3.3, 0.9 Hz, 1H). Anal. Calcd for $C_{14}H_{10}F_1N_1O_4$: C, 61.09; H, 3.66; F, 6.90; N, 5.09. Found: C, 61.00; H, 3.49; F, 6.59; N, 5.01.

5.1.5.11. 2-Hydroxy-3-(4-phenethyl-pyridin-2-yl)-acrylic acid (3j). This compound was prepared by a similar method to that described for **3k** from compound **2j** (pale yellow crystals, 58% yield). Mp 131–133 °C, ^{1}H NMR (DMSO-d_6) δ 2.94 (s, 4H), 6.40 (s, 1H), 7.15–7.33 (m, 7H), 8.35 (d, J = 5.4 Hz, 1H). Anal. Calcd for

$C_{16}H_{15}N_1O_3 \cdot 0.1H_2O$: C, 70.89; H, 5.65; N, 5.17. Found: C, 70.89; H, 5.50; N, 5.19.

5.1.5.12. 3-{6-[2-(4-Fluoro-phenyl)-ethyl]-pyrimidin-4-yl}-2-hydroxy-acrylic acid (3l). This compound was prepared by a similar method to that described for **3k** from compound **2l** (pale yellow crystals, 91% yield). Mp 193–195 °C, ^{1}H NMR (DMSO-d_6) δ 2.99 (s, 4H), 6.30 (s, 1H), 7.06–7.12 (m, 2H), 7.23–7.28 (m, 2H), 7.33 (s, 1H), 8.91 (s, 1H). Anal. Calcd for $C_{15}H_{13}F_1N_2O_3$: C, 62.50; H, 4.55; F, 6.59; N, 9.72. Found: C, 62.24; H, 4.65; F, 6.46; N, 9.66.

5.1.5.13. 3-(6-Benzyloxy-pyrimidin-4-yl)-2-hydroxy-acrylic acid (3m). This compound was prepared by a similar method to that described for **3k** from compound **2m** (pale yellow crystals, 90% yield). Mp 184–186 °C, ^{1}H NMR (DMSO-d_6) δ 5.44 (s, 2H), 6.46 (s, 1H), 7.06 (s, 1H), 7.35–7.48 (m, 5H), 8.82 (s, 1H). Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.63; H, 4.36; N, 10.09.

5.1.5.14. 3-[6-(4-Fluoro-benzyloxy)-pyrimidin-4-yl]-2-hydroxy-acrylic acid (3n). This compound was prepared by a similar method to that described for **3k** from compound **2n** (pale yellow crystals, 77% yield). Mp 178–179 °C, ^{1}H NMR (DMSO-d_6) δ 5.43 (s, 2H), 6.43 (s, 1H), 6.51 (d, J = 1.2 Hz, 1H), 7.05–7.11 (m, 2H), 7.40–7.45 (m, 2H), 8.62 (s, 1H). Anal. Calcd for $C_{14}H_{11}F_1N_2O_4 \cdot 0.1H_2O$: C, 57.58; H, 3.87; F, 6.51; N, 9.59. Found: C, 57.50; H, 3.95; F, 6.14; N, 9.35.

5.1.5.15. 2-Hydroxy-3-(6-phenoxy-pyrimidin-4-yl)-acrylic acid (3o). This compound was prepared by a similar method to that described for **3k** from compound **2o** (pale yellow crystals, 84% yield). Mp 201–204 °C, ^{1}H NMR (DMSO-d_6) δ 6.51 (s, 1H), 7.18 (s, 1H), 7.22–7.33 (m, 3H), 7.45–7.50 (m, 2H), 8.74 (s, 1H). Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.90; N, 10.85. Found: C, 60.42; H, 3.79; N, 10.69.

5.1.6. General procedure for preparing the compounds 6a–m

5.1.6.1. 2-(6-Phenethyl-pyrimidin-4-yl)-1-(2*H*-tetrazol-5-yl)-ethenol (6a). The protected precursor (2-(6-phenethyl-pyrimidin-4-yl)-1-(2-trityl-2*H*-tetrazol-5-yl)-ethenol) was prepared by a similar method to that described for **2k** from 2-trityl-2*H*-tetrazole-5-carboxylic acid ethyl ester[5b] with a modification of the equivalent ester to be used in 1.0 for compound **1k** (pale yellow crystals, 59% yield). Mp 175–177 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.08 (m, 4H), 6.47 (s, 1H), 6.78 (s, 1H), 7.12–7.38 (m, 20H), 8.81 (s, 1H). Then a solution of the precursor (200 mg, 0.37 mmol) in 1,4-dioxane (5 mL) and 3 M aqueous solution of HCl (1 mL) was stirred for 30 min at 60 °C. After dilution with a saturated aqueous solution of $NaHCO_3$ the mixture was acidified with a solid citric acid then extracted with EtOAc. The organic layer was washed with water and brine then dried over Na_2SO_4. The solvent was removed in vacuo then the precipitate was washed with ether. The solvent was removed in vacuo to give the product as yellow crystals (76 mg, 70% yield). Mp 224–226 °C, ^{1}H NMR (DMSO-d_6) δ 2.85–3.05 (m, 4H), 6.27 (s, 1H), 7.07 (s, 1H), 7.16–7.35 (m, 5H), 8.70 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_6O_1 \cdot 0.1H_2O$: C, 60.84; H, 4.83; N, 28.38. Found: C, 61.09; H, 4.85; N, 27.99.

5.1.6.2. 2-(6-Phenethyl-pyrimidin-4-yl)-1-(1*H*-[1,2,4]-triazol-3-yl)-ethenol (6b). This compound was prepared by a similar method to that described for **6a** using 1-trityl-1*H*-[1,2,4]triazole-3-carboxylic acid ethyl ester.[5b] The protected precursor (2-(6-phenethyl-pyrimidin-4-yl)-1-(1-trityl-1*H*-[1,2,4]triazol-3-yl)- ethenol) was obtained as yellow crystals in 56% yield. Mp 126–128 °C, ^{1}H NMR ($CDCl_3$) δ 2.98–3.08 (m, 4H), 6.39 (s, 1H), 6.74 (s, 1H), 7.05–7.40 (m, 20H), 7.98 (s, 1H), 8.81 (s, 1H). The desired compound as yellow crystals in 58% yield. Mp 212–214 °C, ^{1}H NMR (DMSO-d_6) δ 2.90–3.05 (m, 4H), 6.29 (s, 1H), 7.08 (s, 1H), 7.18–7.35 (m, 5H), 8.35 (br s, 1H), 8.75 (s, 1H). Anal. Calcd for $C_{16}H_{15}N_5O_1$: C, 65.52; H, 5.15; N, 23.88. Found: C, 65.27; H, 5.11; N, 23.58.

5.1.6.3. 5-[1-Hydroxy-2-(6-phenethyl-pyrimidin-4-yl)-vinyl]-isoxazol-3-ol (6c). This compound was prepared by a similar method to that described for **6a** using 3-methoxymethoxy-isoxazole-5-carboxylic acid methyl ester, which was prepared from 3-hydroxy-isoxazole-5-carboxylic acid methyl ester by a general protecting procedure. The protected precursor (1-(3-methoxymethoxy-isoxazol-5-yl)-2-(6-phenethyl-pyrimidin-4-yl)-ethenol) was obtained as yellow crystals in 45% yield. Mp 121–123 °C, ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 4H), 3.57 (s, 3H), 5.36 (s, 2H), 6.08 (s, 1H), 6.42 (s, 1H), 6.80 (s, 1H), 7.17–7.30 (m, 5H), 8.83 (s, 1H). The desired compound as a yellow crystal with 50% yield. Mp 254–256 °C, ^{1}H NMR (DMSO-d_6) δ 2.91–3.01 (m, 4H), 6.01 (s, 1H), 6.41 (s, 1H), 7.05 (s, 1H), 7.18–7.28 (m, 5H), 8.72 (s, 1H). Anal. Calcd for $C_{17}H_{15}N_3O_3 \cdot 0.1H_2O$: C, 65.63; H, 4.92; N, 13.51. Found: C, 65.58; H, 4.89; N, 13.30.

5.1.6.4. 2-(6-Phenethyl-pyrimidin-4-yl)-1-thiazol-2-yl-ethenol (6d). This compound was prepared by a similar method to that described for **6a** using thiazole-2-carboxylic acid ethyl ester.[20] (yellow crystals, 54% yield). Mp 165–168 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.07 (m, 4H), 6.44 (s, 1H), 6.70 (s, 1H), 7.18–7.29 (m, 5H), 7.53 (d, J = 3.0 Hz, 1H), 7.92 (d, J = 3.0 Hz, 1H), 8.67 (s, 1H). Anal. Calcd for $C_{17}H_{15}N_3O_1S_1$: C, 66.00; H, 4.89; N, 13.58; S, 10.36. Found: C, 65.90; H, 4.85; N, 13.37; S, 10.22.

5.1.6.5. 1-(2-Methyl-thiazol-4-yl)-2-(6-phenethyl-pyrimidin-4-yl)-ethenol (6e). This compound was prepared by a similar method to that described for **6a** using 2-methyl-thiazole-4-carboxylic acid ethyl ester[21] (yellow crystals, 46% yield). Mp 98–100 °C, ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H), 2.96–3.10 (m, 4H), 6.37 (s, 1H), 6.76 (s, 1H), 7.15–7.32 (m, 5H), 7.70 (s, 1H), 8.82 (s, 1H). Anal. Calcd for $C_{18}H_{17}N_3O_1S_1$: C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.60; H, 5.23; N, 12.83; S, 9.94.

5.1.6.6. 1-(5-Methyl-isoxazol-3-yl)-2-(6-phenethyl-pyrimidin-4-yl)-ethenol (6f). This compound was prepared by a similar method to that described for **6a** using

5-methyl-isoxazole-3-carboxylic acid ethyl ester[22] (yellow crystals, 62% yield). Mp 162–164 °C, ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H), 2.95–3.08 (m, 4H), 6.16 (s, 1H), 6.34 (s, 1H), 6.71 (s, 1H), 7.18–7.35 (m, 5H), 8.77 (s, 1H). Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.25; H, 5.81; N, 13.51.

5.1.6.7. 1-(3-Methyl-isoxazol-5-yl)-2-(6-phenethyl-pyrimidin-4-yl)-ethenol (6g). This compound was prepared by a similar method to that described for **6a** using 3-methyl-isoxazole-5-carboxylic acid ethyl ester[23] (yellow crystals, 62% yield). Mp 161–163 °C, ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 3.00–3.08 (m, 4H), 6.11 (s, 1H), 6.56 (s, 1H), 6.79 (s, 1H), 7.18–7.36 (m, 5H), 8.82 (s, 1H). Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.26; H, 5.87; N, 13.58.

5.1.6.8. 2-(6-Phenethyl-pyrimidin-4-yl)-1-pyrazin-2-yl-ethenol (6h). This compound was prepared by a similar method to that described for **6a** using pyrazine-2-carboxylic acid ethyl ester (yellow crystals, 23% yield). Mp 108–110 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.12 (m, 4H), 6.67 (s, 1H), 6.84 (s, 1H), 7.16–7.32 (m, 5H), 8.59 (s, 1H), 8.62 (s, 1H), 8.90 (s, 1H), 9.21 (s, 1H). Anal. Calcd for $C_{18}H_{16}N_4O_1 \cdot 0.1H_2O$: C, 70.62; H, 5.33; N, 18.30. Found: C, 70.90; H, 5.70; N, 18.00.

5.1.6.9. 2-(6-Phenethyl-pyrimidin-4-yl)-1-pyrimidin-2-yl-ethenol (6i). This compound was prepared by a similar method to that described for **6a** using pyrimidine-2-carboxylic acid ethyl ester (yellow crystals, 41% yield). Mp 187–189 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.06 (m, 4H), 6.89 (s, 1H), 6.90 (d, J = 1.5 Hz, 1H), 7.18–7.35 (m, 6H), 8.86 (d, J = 5.1 Hz, 2H), 8.99 (s, 1H). Anal. Calcd for $C_{18}H_{16}N_4O_1 \cdot 0.1H_2O$: C, 70.62; H, 5.33; N, 18.30. Found: C, 70.81; H, 5.34; N, 17.94.

5.1.6.10. 2-(6-Phenethyl-pyrimidin-4-yl)-1-pyridin-2-yl-ethenol (6j). This compound was prepared by a similar method to that described for **6a** using pyridine-2-carboxylic acid ethyl ester (yellow crystals, 89% yield). Mp 104–106 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.06 (m, 4H), 6.70 (s, 1H), 6.83 (d, J = 1.2 Hz, 1H), 7.08–7.40 (m, 6H), 7.81 (dt, J = 7.8, 1.5 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 8.64 (d, J = 3.6 Hz, 1H), 8.86 (d, J = 1.5 Hz, 2H). Anal. Calcd for $C_{19}H_{17}N_3O_1$: C, 75.23; H, 5.65; N, 13.85. Found: C, 75.43; H, 5.64; N, 13.94.

5.1.6.11. 2-(6-Phenethyl-pyrimidin-4-yl)-1-phenyl-ethenol (6k). This compound was prepared by a similar method to that described for **6a** using benzoic acid methyl ester (yellow crystals, 42% yield). Mp 97–99 °C, ^{1}H NMR ($CDCl_3$) (enol/keto 2:1) δ 3.00–3.12 (m, 4H), 4.40 (s, 2/3H), 5.95 (s, 2/3H), 6.73 (s, 2/3H), 7.14 (s, 1/3H), 7.14–7.32 (m, 5H), 7.40–7.61 (m, 3H), 7.80–7.87 (m, 4/3H), 8.02–8.05 (m, 2/3H), 8.82 (s, 2/3H), 9.10 (s, 1/3H). Anal. Calcd for $C_{20}H_{18}N_2O_1$: C, 79.44; H, 6.00; N, 9.26. Found: C, 79.21; H, 5.91; N, 9.29.

5.1.6.12. 2-(6-Phenethyl-pyrimidin-4-yl)-1-pyridin-3-yl-ethenol (6l). This compound was prepared by a similar method to that described for **2l** using nicotinic acid ethyl ester with a modification of the equivalent ester to be used in 1.0 for compound **1k** (yellow crystals, 64% yield). Mp 86–88 °C, ^{1}H NMR ($CDCl_3$) δ 2.98–3.10 (m, 4H), 5.97 (s, 1H), 6.76 (s, 1H), 7.12–7.41 (m, 6H), 8.13 (d, J = 7.8 Hz, 1H), 8.65 (br s, 1H), 8.83 (s, 1H), 9.06 (br s, 1H). Anal. Calcd for $C_{19}H_{17}N_3O_1$: C, 75.23; H, 5.65; N, 13.85. Found: C, 75.20; H, 5.50; N, 13.84.

5.1.6.13. 2-(6-Phenethyl-pyrimidin-4-yl)-1-pyridin-4-yl-ethenol (6m). This compound was prepared by a similar method to that described for **6l** using isonicotinic acid ethyl ester (yellow crystals, 77% yield). Mp 99–101 °C, ^{1}H NMR ($CDCl_3$) δ 3.00–3.11 (m, 4H), 6.05 (s, 1H), 6.80 (s, 1H), 7.18–7.32 (m, 5H), 7.67 (d, J = 6.1 Hz, 2H), 8.70 (br s, 2H), 8.89 (s, 1H). Anal. Calcd for $C_{19}H_{17}N_3O_1$: C, 75.23; H, 5.65; N, 13.85. Found: C, 75.32; H, 5.50; N, 13.82.

5.1.6.14. 4-Methyl-2-phenethyl-pyridine (8). This compound was prepared by a similar method to that described for **1k** from 2,4-dimethyl-pyridine (**7**). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 3.04 (s, 4H), 6.93–6.95 (m, 2H), 7.16–7.31 (m, 5H), 8.41 (d, J = 4.8 Hz, 1H).

5.1.6.15. 2-Hydroxy-3-(2-phenethyl-pyridin-4-yl)-acrylic acid ethyl ester (9). This compound was prepared by a similar method to that described for **2k** from compound **8** (pale yellow crystals, 36% yield). Mp 151–152 °C, ^{1}H NMR ($CDCl_3$) (enol/keto = 2:1) δ 1.36 (t, J = 7.2 Hz, 3/3H), 1.41 (t, J = 7.2 Hz, 6/3H), 3.50–3.18 (m, 4H), 4.09 (s, 2/3H), 4.32 (q, J = 7.2 Hz, 2/3H), 4.39 (q, J = 7.2 Hz, 4/3H), 6.38 (s, 2/3H), 6.97 (s, 1/3H), 7.02 (d, J = 4.8 Hz, 2/3H), 7.16–7.31 (m, 5H), 7.44 (s, 1/3H), 7.50 (d, J = 5.7 Hz, 2/3H), 8.52–8.55 (m, 3/3H).

5.1.6.16. 2-Hydroxy-3-(2-phenethyl-pyridin-4-yl)-acrylic acid (10). This compound was prepared by a similar method to that described for **3k** from compound **9** (pale yellow crystals, 63% yield). Mp 255–258 °C, ^{1}H NMR (DMSO-d_6) δ 6.17 (br s, 1H), 7.15–7.31 (m, 5H), 7.52 (s, 1H), 7.56 (d, J = 6.0 Hz, 1H), 8.30 (br s, 1H). Anal. Calcd for $C_{16}H_{15}N_1O_3 \cdot 0.1H_2O$: C, 70.89; H, 5.65; N, 5.17. Found: C, 70.89; H, 5.65; N, 5.17.

5.1.6.17. 2-Hydroxy-3-(6-phenethyl-pyrimidin-4-yl)-propionic acid ethyl ester (11). $NaBH_4$ (15 mg, 0.4 mmol) was added to a solution of **2k** (80 mg, 0.27 mmol) in EtOH (3 mL) and stirred for 10 min. The mixture was diluted with water and extracted with chloroform, the organic layer was then dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc 1:1 as eluent to give the product as a colorless oil (79 mg, 98% yield). ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.1 Hz, 3H), 3.05 (s, 4H), 3.07 (dd, J = 15.3, 7.2 Hz, 1H), 3.22 (dd, J = 15.3, 3.9 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 4.63 (dd, J = 7.2, 3.9 Hz, 1H), 7.01 (s, 1H), 7.16–7.31 (m, 5H), 9.04 (s, 1H).

5.1.6.18. 2-Hydroxy-3-(6-phenethyl-pyrimidin-4-yl)-propionic acid (12). This compound was prepared by a similar method to that described for **3k** from compound

11 (colorless crystals, 79% yield). Mp 125–127 °C, ^{1}H NMR (DMSO-d_6) δ 2.87 (dd, J = 14.2, 8.7 Hz, 1H), 2.99 (s, 4H), 3.06 (dd, J = 14.2, 4.3 Hz, 1H), 4.02 (br s, 2H), 4.42 (dd, J = 8.7, 4.3 Hz, 1H), 7.16–7.33 (m, 6H), 8.98 (s, 1H). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 65.95; H, 6.01; N, 10.23.

5.1.6.19. 6-Phenethyl-pyrimidine-4-carbaldehyde (13). A mixture of compound **3k** (160 mg, 0.81 mmol) and SeO_2 (179 mg, 1.62 mmol) in 1,4-dioxane (3 mL) was refluxed for 2 h, then insoluble precipitate was filtered off and the solvent was removed in vacuo. The residue (183 mg) was used for the next step without purification.

5.1.6.20. 3-(6-Phenethyl-pyrimidin-4-yl)-acrylic acid methyl ester (14). A (triphenylphosphoranylidene) acetic acid methyl ester (300 mg, 0.90 mmol) was added to a solution of **13** (crude 183 mg) in toluene (3 mL) at room temperature, and the mixture was stirred for 1 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water and brine then dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography using *n*-hexane/EtOAc 2:1 as eluent to give the product (40 mg, 18% yield from **3k**). The stereo chemistry was decided to be 'trans' for the reason of a large coupling constant (15.6 Hz) between two hydrogens on each side of the olefin. ^{1}H NMR ($CDCl_3$) δ 3.09 (s, 4H), 3.83 (s, 3H), 7.08 (d, J = 15.6 Hz, 1H), 7.08 (d, J = 1.2 Hz, 1H), 7.16–7.31 (m, 5H), 7.50 (d, J = 15.6 Hz, 1H), 9.15 (d, J = 1.2 Hz, 1H).

5.1.6.21. 3-(6-Phenethyl-pyrimidin-4-yl)-acrylic acid (15). This compound was prepared by a similar method to that described for **3k** from compound **14** (pale yellow crystal, 65% yield). Mp 219–224 °C, ^{1}H NMR (DMSO-d_6) δ 3.05 (s, 4H), 6.99 (d, J = 15.7 Hz, 1H), 7.17–7.31 (m, 5H), 7.44 (d, J = 15.7 Hz, 1H), 7.71 (d, J = 1.2 Hz, 1H), 9.11 (d, J = 1.2 Hz, 1H). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 65.95; H, 6.01; N, 10.23.

5.2. Assay

As previously reported[8,9] the assay method used included pre-formation of IN-substrate DNA complex on a microtiter plate and washing out of the unbound IN protein (MWPA, Microtiter plate integration Assay with Preincubation and Wash). In brief, after formation of the IN–DNA complex and washing out of unbound IN, a test compound was added to integration reaction buffer (10 mM dithiothreitol, 5% glycerol, 100 μg/mL bovine serum albumin, and 30 mM Mops, pH 7.2), and the plate was incubated at 30 °C for 30 min. in order to let the compound bind to the IN–DNA complex. Then, digoxigenin (Dig) labeled target DNA was added to the reaction mixture to initiate the strand transfer reaction. The amount of strand transfer products was estimated by a standard enzyme linked immune assay with anti-Dig antibody. When we use manganese (Mn^{2+}, 15 mM) as a cofactor through all steps, we name it MWPA with Mn assay (MWPA-Mn).

Anti-HIV activity in vitro of each compound was assayed by inhibition of virus replication in the cells with a modification of the reported procedure.[24] In brief, MT-4 cells were incubated with the serial 2-fold dilution of the compounds for 1 h and were infected with HIV-1 IIIB strain. After 4 days incubation, the antiviral effect of the compounds to HIV replication was monitored by cell viability using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

5.3. Molecular modeling studies

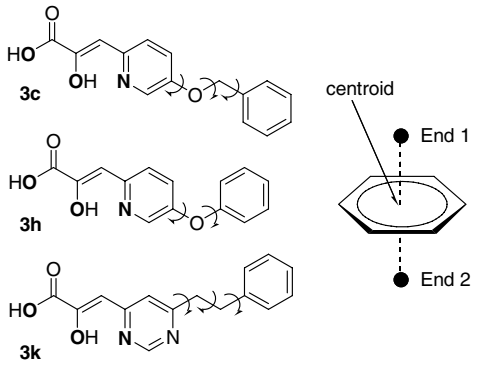
All modeling studies were performed using the SYBYL 6.9 software package.[25] Energy minimizations and evaluations were done with the Tripos force field with charges calculated by Gasteiger–Huckel method. Each 3D molecular structure was generated using the Sketch Molecule command of SYBYL, then it was relaxed using the Minimization command of SYBYL. Systematic conformational analysis was achieved using the Systematic Search module of SYBYL. An extra in-house program was used to identify the MFB space. Schematic representations of the models were prepared with SYBYL.

5.3.1. Identifying the hydrophobic pharmacophore model from the compounds 3c, 3k and 3h. Modeling was carried out with the hydrophilic domain in the enolic form, with the enolic hydroxyl group making an intramolecular hydrogen bond with the N-1 nitrogen. The carboxylic group was modeled as the protonated form making intramolecular hydrogen bonding with the enolic oxygen.

5.3.1.1. Superimposing the structures. The N-1, the enolic oxygen, and the hydroxyl oxygen of carboxylic group were defined as 'Anchor-Atoms' as illustrated in Figure 7. Then the molecules **3k** and **3h** were, respectively, superimposed over the **3c** using a rigid-body least-squares fit based on the Anchor-Atoms (the Fit Atoms command of SYBYL).

5.3.1.2. Systematic conformational search and data collection. Single bonds in each hydrophobic substituent were assigned as rotating bonds as shown in Figure 7, and data were collected at 10 degree intervals from zero to 350. Energy differences were calculated relative to the lowest energy conformer (as stability) and coordinates of both end points of an 8 Å normal passing through the centroid of the terminal phenyl ring (as location and direction) were collected for each conformer using the Molecular Spread Sheet of SYBYL.

5.3.1.3. Identifying the MFB space. Only conformers that showed an energy difference less than 10 kcal/mol were focused. The combination of three conformers representing each active type that best locate their terminal phenyl rings closely was identified. This showed the lowest RMSD value calculated from equation 1. A mirror image of each conformer was ignored. As the hydrophilic domain was optimized to be quite planar, an alternate mirror image represents an energetic equivalence in this case regarding a ligand alone. Coordinates of the three



$$RMSD = \sqrt{\frac{D1_{AB} + D1_{AC} + D1_{BC} + D2_{AB} + D2_{AC} + D2_{BC}}{6}} \quad \text{eq. 1)}$$

$D1_{AB} = (End1_A - End1_B)^2$

$End1_A - End1_B$: distance between $End1_A$ and $End1_B$

$End1_A$: $End1$ of molecule A

Figure 7. Arrows indicate rotating bonds in the systematic conformational analyses, and the Anchor-Atoms to be superimposed are emphasized by drawing bold characters for each structure, respectively. The broken line indicates an 8 Å normal passing through the centroid at the middle point. 'End 1' and 'End 2' indicate the coordinate points to be collected.

centroids and the three normals were averaged to identify the MFB space as a relative orientation based on the Anchor-Atoms.

5.3.2. Superposing the molecule 3e, 3j, and 3o representing inactive types D, E, and F over the hydrophobic pharmacophore model. Rotating bonds of the molecules **3e** and **3j** were, respectively, modeled to show the same torsion angles as the active conformer **3c** and **3k** identified above. The molecule **3o** was superimposed over the pharmacophore model based on the Anchor-Atoms using the rigid-body least-squares fit. The rotating bonds were manually rearranged to set the terminal phenyl ring close to the MFB.

5.3.3. Modeling of DKAs (L-708,906, Compound A, S-1360) comparing to the hydrophobic pharmacophore model. The 1,3-diketone region was constructed as a localized enol form. The triazol region was to be a (2*H*-[1,2,4]triazol-3-yl) form. Each of the structures was superimposed over the pharmacophore model based on the Anchor-Atoms using the rigid-body least-squares fit. The best conformation to make the terminal phenyl ring close to the MFB space was modeled by manual torsional rearrangements of the hydrophobic substituents.

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