Ketones as Building Blocks for Dynamic Combinatorial Libraries: Highly Active Neuraminidase Inhibitors Generated via Selection Pressure of the Biological Target

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Received October 1, 2002

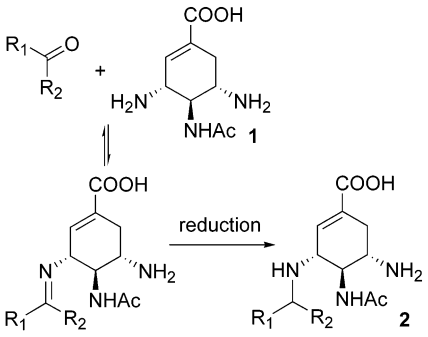
Abstract: New and potent inhibitors of neuraminidase, a key enzyme in the influenza virus activity, have been discovered in dynamic combinatorial libraries based on ketones and amines as building blocks. Selective synthesis of a number of inhibitors among multiple theoretically possible combinations of building blocks is driven by the presence of the target enzyme.

Dynamic combinatorial chemistry (DCC) is a new approach to integration of combinatorial synthesis and screening based on the shift of chemical equilibrium in a mixture of interconverting components driven by a molecular target.[1−8] This process results in the preferred formation of one or a few mixture components, which form strongest noncovalent complexes with the target. DCC has been implemented in a variety of chemical systems and applied to different targets, including proteins,[9−13] peptides,[14−16] nucleic acids,[17,18] and inorganic[19,20] and organic[21−23] ions.

Successful application of DCC to discovery of new ligands for biological targets relies on synergy of several important factors, such as use of reversible reactions compatible with aqueous media, availability of large functional diversity of library building blocks, and robust methods of mixture analysis. Earlier studies showed that reversible exchange of imines formed from aldehyde and amine building blocks can be influenced by biological targets promoting increased formation of strongly binding imines. We report here the first use of ketones as building blocks of dynamic libraries. The structures of library components selected and amplified by the target reveal building block combinations that can be used to generate highly active inhibitors with properties at least matching those of commercial drugs.

We used neuraminidase (NA), a key enzyme involved in the influenza virus propagation, as the DCC target. Diamine **1**, which is structurally similar to some known NA inhibitors,[24] was used as the scaffold (common building block) for the dynamic libraries.[25] Equilibration of the scaffold with a mixture of ketones (see Supporting Information for the complete list) was expected to produce a set of imines (Scheme 1), the distribution of which would be altered by the addition of neuraminidase.[26] The imines were then reduced to the secondary amines of general structure **2**, the composition of which was analyzed by LC/MS.[27]

Scheme 1



Following the previously developed strategy, we used the set of conditions for in vitro virtual screening, i.e., where little or no components of the reduced library could be detected in the absence of the target.[13] The library analysis is thus greatly simplified, and one can clearly see appearance of hits induced by the target. The results of a typical DCC experiment are shown in Figure 1.

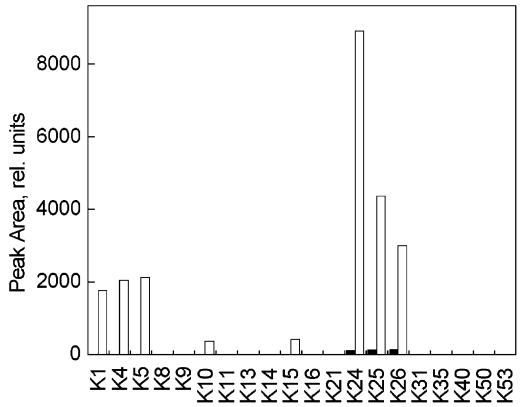


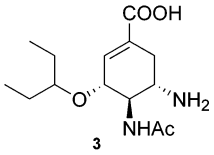
Figure 1. Relative amounts of amines **2** produced in the DCC experiments with **1** and 20 ketones in the absence (black bars) and in the presence (white bars) of 43 μM NA.

Clearly, addition of the enzyme target results in dramatic amplification of selected amine peaks. The amplification factors estimated in Table 1 can actually be even higher, because the concentrations of many amines cannot be measured in the absence of the target. The effect of the target is probably due to its thermodynamic control of the imine composition, rather than kinetic effect, as the latter would require enzymatic catalysis of the rate-limiting reduction process. Since the imines are very unstable in aqueous solutions and cannot be directly used as ligands and inhibitors, it is very important to learn whether structurally similar amines **2** will maintain binding properties of the imines. We have synthesized (see Supporting Information) and tested in kinetic enzyme assays several amines that have been selected by the target, as well as one that has not been selected. The K_i values (Table 1) show that the binding properties of the individual amines correlate well with their amplification in the DCC experiment.

To verify that the amplification effect was due to interactions with the neuraminidase active site, rather than other nonspecific effects of the protein, two sets of

10.1021/jm025589m CCC: $25.00
Published on Web 01/03/2003

Table 1. Amplification Factors for DCC Hits and K_i of Their Individually Synthesized Analogs

Compound[a]	Amplification factor[b]	K_i, nM
1	N/A	31 300
2a $R_1 = R_2 = Et$	> 30	85
2b $R_1 = Et$; $R_2 = Pr$	> 90	92
2c $R_1 = Me$; $R_2 = (CH_2)_2(4\text{-OH-Ph})$	84	700
2d $R_1 = Me$; $R_2 = (CH_2)_3NEt_2$	below detection limit	54 000
 **3**	N/A	1.3

[a] Compounds **2a**, **2b**, **2c**, and **2d** were formed from ketones K1, K4, K24, and K14, respectively. [b] Amplification factors for **2a** and **2b** are given as their signal-to-noise ratios in the LC/MS profiles.

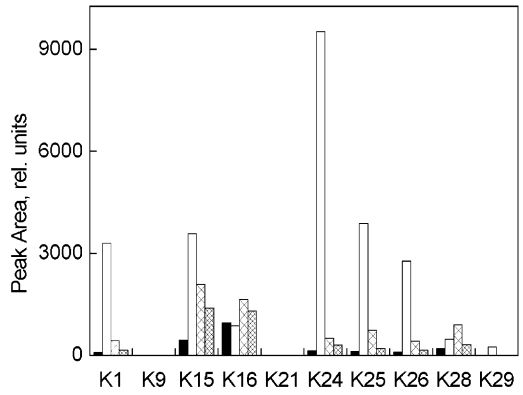


Figure 2. Relative amounts of amines **2** produced in the DCC experiments with **1** and 10 ketones in the absence of NA (black bars), in the presence of 43 μM NA (white bars), in the presence of 40 μM BSA (sparse pattern), and in the presence of 43 μM NA and 100 μM Zanamivir (dense pattern).

control experiments have been performed (Figure 2). In the first set, bovine serum albumin (BSA) was used instead of neuraminidase. In most cases the effect of BSA was small or negligible, with the notable exception of ketone K15, where significant amplification by BSA was observed. In the second set, the DCC experiment with NA was performed in the presence of a potent NA active site inhibitor zanamivir.[28] The presence of zanamivir inhibited amplification in all cases, except for K15. Apparently, the NA effect in the latter case is caused by some nonspecific interactions with the protein surface. The two controls thus serve as convenient means to eliminate false positives from the DCC hits.

DCC experiments and the initial activity assays were performed with the extracellular domain of NA. To verify that the compounds discovered by DCC were potent inhibitors of native enzyme forms, amines **2** were tested with various human pathogenic NA viral forms. The results summarized in the Supporting Information show that the compounds identified by DCC are overall even more potent inhibitors of the native NA forms with the activity in the low nanomolar range.

In conclusion, we have shown that ketones can be efficiently used as building blocks for dynamic combinatorial libraries leading to rapid identification of high affinity protein ligands. It should be emphasized that the key result of any DCC experiment is the information about the most effective structural combinations of building blocks. Because selection pressure of the target applies to the imines, rather than amines **2**, various structural analogues of the imines, such as ethers, can be synthesized and tested as stable ligands. For example, compound **3**, a commercial drug,[24] can be considered as a DCC hit resulting from ketone K1 and corresponding to amine **2a**, which has thus been rediscovered through in vitro virtual screening.[29] Compound **3** improves binding affinity of the original scaffold **1** by the factor of 2×10^4 for the NA extracellular domain and by more than 2×10^5 for the native enzyme! Syntheses or ether analogues of other DCC hits are currently in progress.

Acknowledgment. This work was financed in part by the grant BEO 0312391 from BMBF, Germany. We are grateful to Prof. J.-M. Lehn (Strasbourg, France) for helpful discussions and to Prof. H.-D. Klenk (Marburg, Germany) for samples of neuraminidase cDNA.

Supporting Information Available: Complete list of ketones and K_i values; synthesis and analytical data of compounds **2a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Scaffold **1** was synthesized as described previously (ref 13).
(26) (a) The amounts of ketimines at equilibria were below detection limit of NMR. (b) No reaction products with the less reactive second amino group could be detected (cf. ref 13).
(27) Scaffold **1** (100 μM) and a mixture of ketones (25 mM each) was incubated with neuraminidase (43 μM) in the presence of tetrabutylammonium cyanoborohydride (8 mM) for 12 h. The resulting reaction mixture was analyzed by LC/MS using ESI−MRM.
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(29) In the case of neuraminidase, the ether forms of the DCC hits have higher inhibitory activity than the amino forms, probably, because they lack positive charge in neutral aqueous solutions and are therefore structurally closer to the imino forms selected by the target.

JM025589M