Communications to the Editor

Non Nucleic Acid Inhibitors of Protein·DNA Interactions Identified through Combinatorial Chemistry†

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The autoimmune disease systemic lupus erythematosus (SLE) afflicts at least 500 000 Americans, significantly shortening their life expectancy.[1] Women are affected in 90% of cases and African Americans are several times more likely to develop the disease than Caucasians.[2] Antibodies that bind single- and double-stranded DNA (ssDNA and dsDNA, respectively) are present in the serum of lupus patients.[3] A subset of these antibodies are also pathogenic: they mediate a complement-dependent inflammatory response in kidney tissue (glomerulonephritis) often resulting in renal damage.[4] Nonspecific immunosupressive and cytotoxic agents can curtail glomerulonephritis in some patients; yet, in others they are ineffective and side effects often force discontinuation of treatment.[5] A clear need exists for specific, broadly effective, and better tolerated therapeutics.

Recognition of DNA adherent to the glomerular basement membrane can anchor pathogenic anti-DNA to glomeruli, the sites of injury within kidney tissue.[6] Hence, blocking this binding should be an effective (and as of yet unexplored) way to combat anti-DNA-mediated glomerulonephritis.[7] As a first step in testing this hypothesis, we describe non nucleic acid inhibitors that block DNA recognition by an anti-DNA monoclonal antibody (mAb). These compounds were identified by library screening and represent the first reported use of a combinatorial small-molecule library to identify inhibitors of a protein without precise knowledge either of the natural ligand that is recognized *in vivo* or of other known inhibitors.[8,9]

For these experiments, library screening was conducted using 11F8 which is a DNA-binding mAb isolated from a lupus-prone mouse.[11] 11F8 binds ssDNA exclusively and shows a base preference for thymine.[11,12] Several lines of evidence suggest that stacking of thymine bases between aromatic amino acids in the binding site of 11F8 is important for stabilizing 11F8·ssDNA complexes.[11] Because specific "hydrophobic stacking"[13] may be important for binding, we postulated that aromatic compounds could compete with thymine for binding to 11F8. On the basis of this rationale, we elected to screen a 1,4-benzodiazepine library.[14]

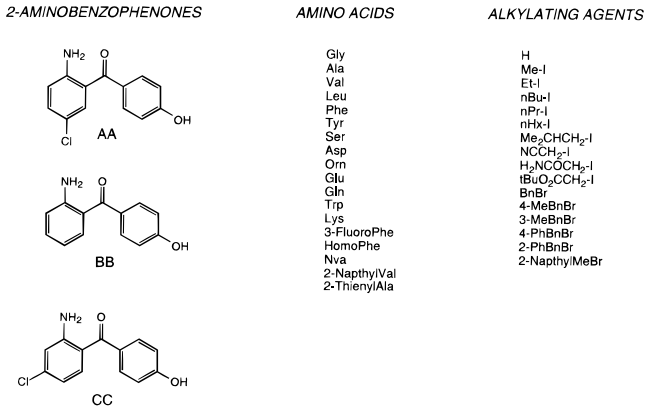

Figure 1. Library components.

Our library was constructed from three different 2-aminobenzophenones, 35 amino acids, and 16 alkylating agents (Figure 1)[15] using previously reported methods.[16] Enantiomeric amino acids were pooled, and the synthesis was performed using the Chiron Mimotopes multipin method to provide 1680 different 1,4-benzodiazepines within individual wells of 96-well microtiter plates.[17] Racemic benzodiazepines were screened by competition ELISA for the ability to inhibit 11F8 from binding to ssDNA.[10] Approximately 30% of the molecules inhibit to some extent at 20 μM, and several possess IC_{50} values $\leq$20 μM, comparing favorably to those of other first generation inhibitors.[18] Most of the compounds that inhibit binding incorporate 2-aminobenzophenone AA, indicating that this particular aminobenzophenone may make important contacts with the antibody. This conclusion is also supported by the observation that for a given R_1 and R_2, the compounds

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(7) Anionic dyes have been used to inhibit serum samples containing anti-DNA, see: Ben-Chetrit, E.; Eliat, D.; Ben-Sasson, S. A. *Immunology* **1988**, *65*, 479−485.

(8) The exact DNA antigen(s) recognized *in vivo* are not known.

(9) The best inhibitors obtained from screening a library of pentapeptides composed of the common 20 L-amino acids by competition ELISA[10] have IC_{50} values > 50 μM. Glick, G. D. Unpublished observations.

(10) See Supporting Information for further details.

(11) Swanson, P. C.; Ackroyd, C.; Glick, G. D. *Biochemistry* **1996**, *35*, 1624−1633.

(12) 11F8 does not cross-react with dsDNA, other nucleic acids, ribonucleoproteins, or structurally related antigens such as phospholipids.[11]

(13) By stacking we refer to intercalation of a DNA base in-between two aromatic amino acids on the protein. For an example of this type of stacking, see: Swanson, P. C.; Cooper, B. C.; Glick, G. D. *J. Immunol.* **1994**, *152*, 2601−2612.

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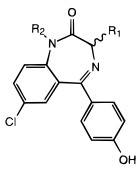

R_2	R_1	Entry	% Inhibition
CH_3-	2-napthylAla-	1	60
$C_6H_5CH_2$-	$HO_2CCH_2CH_2$-	2	50
$CH_3(CH_2)_3CH_2$-	$HOCH_2$-	3	40

Figure 2. Structures and inhibitory potency of selected 1,4-benzodiazepines determined by competition ELISA in the initial library screen (measured at 20 μM inhibitor). In this assay,[10] 20 μM d(pT)$_6$ completely blocks binding. The data presented represent the average of at least two separate determininations and are within ±15%. The inhibition data for **1** and **2** were confirmed using purified and fully characterized compounds prepared on solid support.

that incorporate aminobenzophenone AA inhibit to a greater extent than those that incorporate aminobenzophenones BB or CC.

The best inhibitors generally could be placed into two broad classes: one group possesses R_1 = hydrogen bond donor−acceptor and R_2 = an aromatic or nonpolar group and the other has R_1 = aromatic and R_2 = H or Me (Figure 2). To help elucidate the structural features that are important for binding, we prepared several derivatives of **1**, one of the most potent inhibitors. Removing either the aromatic chloride or the phenolic OH, replacing the napthylalanine with phenylalanine, or methylating the phenolic oxygen affords molecules with IC_{50} values >50 μM, as determined by competition gel shift measurements.[19] However, replacing the *N*-methyl group with hydrogen does not affect inhibition. Furthermore, it was observed that only the *R*-isomer of **1** inhibits binding.[20] When this isomer is titrated into a solution of 11F8, the intrinsic protein fluorescence produced upon excitation at 283 nm is quenched by 30%, similar to that obtained when titrating with oligo(dT).[21] By contrast, the *R*-isomer of **1** quenches to a much lesser extent. Collectively, these data suggest that only the *R*-isomer binds specifically to 11F8 and that binding is not due to nonspecific (hydrophobic) interactions. Furthermore, that **1** quenches the protein fluorescence suggests that, upon binding, this benzodiazepine may interact with the unique tryptophan in the combining site of 11F8.[22]

In summary, we have demonstrated the use of combinatorial chemistry to identify inhibitors of an anti-DNA mAb.[22] It is not clear how potent anti-DNA antagonists must be *in vitro* to show an effect *in vivo*. Natural mechanisms exist to clear immune complexes, and in lupus, these pathways become saturated, ultimately leading to renal damage and failure.[23] Therefore, a weak inhibitor *in vitro* may produce a large effect *in vivo* even if it does not completely block all antibody·DNA interactions. Testing of **1** in lupus-prone mice is currently underway. Finally, because murine and human anti-DNA possess similar binding-site structures, the compounds described here may likely be valuable leads for inhibiting the anti-DNA produced in patients afflicted with SLE.[24]

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Supporting Information Available: Details on the synthesis of the inhibitors along with descriptions of the ELISA and gel shift assays (5 pages). See any current masthead page for ordering and Internet access instructions.

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(19) These experiments were conducted using purified and fully characterized inhibitors.[10]

(20) As expected, the *R*-isomer is roughly 2-fold more potent than racemic **3** in the inhibition assays.

(21) The maximum quenching determined using oligo(dT) as the ligand is ≤40%.

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