Amending HIV Drugs: A Novel Small-Molecule Approach To Target Lupus Anti-DNA Antibodies

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Supporting Information

ABSTRACT: Systemic lupus erythematosus is an autoimmune disease that can affect numerous tissues and is characterized by the production of nuclear antigen-directed autoantibodies (e.g., anti-dsDNA). Using a combination of virtual and ELISA-based screens, we made the intriguing discovery that several HIV-protease inhibitors can function as decoy antigens to specifically inhibit the binding of anti-dsDNA antibodies to target antigens such as dsDNA and pentapeptide DWEYS. Computational modeling revealed that HIV-protease inhibitors comprised structural features present in DWEYS and predicted that analogues containing more flexible backbones would possess preferred binding characteristics. To address this, we reduced the internal amide backbone to improve flexibility, producing new small-molecule decoy antigens, which neutralize anti-dsDNA antibodies in vitro, in situ, and in vivo. Pharmacokinetic and SLE model studies demonstrated that peptidomimetic FISLE-412,1 a reduced HIV protease inhibitor analogue, was well-tolerated, altered serum reactivity to DWEYS, reduced glomeruli IgG deposition, preserved kidney histology, and delayed SLE onset in NZB/W F1 mice.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that disproportionately affects young women. As the disease progresses, SLE adversely impacts essentially every organ system (e.g., skin, joints, kidneys, central nervous system (CNS), hematopoietic cells). The underlying features of the disease include the development of autoantibodies, including reactivity to double stranded (ds) DNA. Anti-dsDNA antibodies are of particular importance in lupus because (i) they are diagnostic of the disease, (ii) they contribute to tissue damage in the kidney and other organs, and (iii) their titers correlate with disease activity.7 Ultimately, complexes of DNA and anti-DNA antibody are internalized through Fc receptors (FcR) or carry DNA to Toll-like receptor (TLR) 9 to activate myeloid cells and other FcR and TLR9 expressing cells. A subset of these anti-dsDNA antibodies also binds to a peptide consensus sequence (D/E/W,D/E,Y,S/G)3,4 (herein shortened to “DWEYS”), found in the N-terminal d-aspartate receptor (NMDAR) expressed on neurons,5,6 and the first component of complement (C1q).7 Both a mouse and human monoclonal antibody with this specificity, termed R4A8 and G11, respectively bind to kidney and neuronal epitopes including DNA and NMDAR.1,7,9,10 Since up to 80% of SLE patients manifest cognitive dysfunction as part of neuropsychiatric lupus and renal disease occurs in approximately one-half of all SLE patients, accounting for one-third of all lupus deaths, the deposition of pathogenic antibodies in brain and kidney is of great interest.

Current lupus therapy is aimed primarily at reducing general inflammation (synthetic glucocorticoids), targeting inflammatory mediators by elimination of autoreactive lymphocytes (cytotoxic agents) or nonspecific B-cell inhibitors (belimumab).11,12 While effective at reducing inflammation, these chronic treatments have undesirable side effects and reduce overall immunity (i.e., immunosuppression), leading to increased risk for infections and cancer.13-15 Thus, novel therapeutic approaches to lupus are greatly needed. Toward this end, we began a program to target specific autoantibody populations with small-molecule “decoy antigen” compounds, thereby rendering them unavailable to bind tissue antigen. We reasoned that this therapeutic approach should have fewer
adverse side effects and would lead to a reduction in associated lupus pathology, particularly in the kidney and brain.

**RESULTS**

**HIV Protease Inhibitors and Derivatives as Anti-dsDNA Antibody Decoy Antigens.** The DWEYS peptide consensus sequence, which binds the R4A autoantibody, is present in a loop on the NMDAR. We derived a consensus peptide structure for DWEYS (Figure 1a) by overlaying the corresponding residues in an experimental structure of NMDAR2B (rat, PDB code 3JPY) and two homology models of human NMDAR2A and NMDAR2B (available in Modbase, see methods in Experimental Section). To identify compounds that might function as experimental decoy antigens, we carried out a DWEYS fingerprint similarity search against the DrugBank database (version 4.2), which comprises 5055 drug entries, including FDA approved drugs, nutraceuticals, and experimental compounds. Three fingerprint types were applied in the search, including MACCS structural keys (BIT-MACCS), 3D shape (ESshape), and pharmacophore atom triangle (piDAPH3) fingerprints to examine different aspects of the consensus structure. The three-step screening gave 100 hits out of 5055 entries from the entire database (2%). Interestingly, 8 out of 100 hits belong to the HIV protease inhibitors catalog, including saquinavir (1), nelfinavir (2), lopinavir (3), and related compounds. The molecular descriptors of the resulting 100 hits of our fingerprint search are displayed in a 3D plot for hydrogen donors, rotatable bonds, and ring numbers (Figure 1b). Our analysis suggested this subset of HIV protease inhibitors had features similar to the DWEYS peptide consensus structure. To assess whether these HIV protease inhibitors might function as anti-dsDNA antibody decoy antigens, we used a competitive ELISA to ask whether they would inhibit the binding of the R4A monoclonal antibody to DWEYS-coated plates and found several HIV protease inhibitors exhibited mild inhibition in the assay (Figure 1c).

With an eye toward elucidating even higher affinity decoy antigens, we performed computational and structural activity analyses of the top three HIV protease inhibitors (1, 2, and 3) that yielded promising results in the ELISA assay (Figure 1c). Significantly, in these modeling analyses we could draw on a rich database of HIV protease inhibitor structures, since these drugs have been cocystallized with multiple substrate proteins. We derived consensus structures for the HIV protease inhibitors, starting first with compound 1. Five distinct conformations of 1 were identified from cocrystal structures; these were modeled using MOE flexible alignment module (representative ligand conformations in PDB entries 1C6Z, 1FB7, 3PWW, 3S56, and 3UFN) and found to have good 3D structural overlap with the DWEYS consensus structural model (Figure 2a). Note that the hairpin-like conformation of 1 mimics the turn structure of the DWEYS peptide, with significant structural conservation between the backbones of the WEY sequence, and overlap of 1 hydrogen bonding and aromatic moiety of the glutamic acid (E) and tyrosine (Y) side chains (Figure 2a). However, due to the constrained amide bonds on 1, the decahydroisoquinoline (DIQ) group and the quinoline ring were not able to fit well with the peptide side chain and mimic the peptide conformation. Like compound 1, the other two HIV protease inhibitors, 2 and 3, were also found to mimic the DWEYS consensus loop structural model and have a structurally constrained internal amide backbone (Supporting Information, Figure S1). On the basis of these analyses, we reasoned that HIV protease inhibitors with reduced internal carbonyl groups would manifest increased structural flexibility and likely achieve better binding to the R4A autoantibody.

To test this prediction, we chemically reduced the backbone of the HIV protease inhibitors to increase the flexibility. Of the chemically modified HIV protease inhibitors, some were triaged due to stability issues. The newly formed compounds 5, 6, and 7, described as reduced HIV protease inhibitors (Figure 2c), were purified and characterized by mass spectrometry and nuclear magnetic resonance (NMR) spectra. Their purities...
were analyzed by HPLC and are above 95% (compound 7, >92%) (Experimental Section, 2. Chemistry, Table 1). Subsequently, we examined the structural overlay of newly formed compounds 5, 6, and 7 with the DWEYS consensus. The conformations of compounds 5, 6, 7 were constructed by modifying their parent HIV protease inhibitors’ protein database (PDB) structures and optimizing their geometry by energy minimization in the MMFF94x force field using the MOE software default parameters.25 Flexible alignments of 5, 6, and 7 to the DWEYS consensus peptide yield better fits compared to their parent HIV protease inhibitors. The reduction of the amide bonds introduced more rotatable bonds to compounds 5, 6, 7 and thus more flexibility. The resulting flexible alignment confirmed a higher degree of similarity of 5 to DWEYS as compared to the rigid compound 1, as evidenced by a better fit of the tetrahydroquinaldic moiety to the W side chain within the peptide (Figure 2b). In addition, the flexible backbone of 6 (reduced form of 2) had better hairpin mimicking conformation and facilitated side chain similarity alignment with the peptide D side chain compared to 2 (Supporting Information, Figure S1a,b). The borane reduction opened the 2-tetrahydropyrimidinone ring on 7; hence, the conformation similarity of 7 and DWEYS is lower compared to 3 (Supporting Information, Figure S1c,d). Overall, the average strain energy (U) of compounds 5, 6, and 7 decreased and the grand alignment score (S) improved (except for compound 7) in their alignment with DWEYS (Supporting Information, Table S1). Interestingly, the R4A autoantibody is known to bind dsDNA, the NMDAR receptor (containing the consensus sequence D/EWD/EYS/G),5 and the complement protein C1q (EADSV).7 It has been suggested that more cross-reactivity could be the result of increased flexibility in the

Figure 2. HIV protease inhibitors and their derivatives possess 3D structural similarities with DWEYS and inhibit SLE anti-dsDNA antibodies. Structural overlay models of DWEYS (gray) with (a) 1 (green) and (b) 5 (magenta). Hydrogens are not displayed. (c) 2D Structures of HIV protease inhibitors and reduced derivatives. (d) IC50 curves of HIV protease inhibitor derivatives as assessed in ELISA-based screens with R4A monoclonal antibody and DWEYS-coated plates.
binding regions of antibodies and/or their ligands.\textsuperscript{26–29} Taken together, these results strongly argue that reducing the structurally constrained HIV protease inhibitors’ internal backbone could produce decoy antigen therapeutics with preferred characteristics.

Previously, we have shown that compound 5, the reduced form of 1, inhibited autoantibody binding in vitro (ELISA), ex vivo (kidney glomeruli), and in vivo (hippocampal neurotoxicity).\textsuperscript{1} When assayed in parallel, compounds 5, 6 (reduced compound 3), and 7 (reduced compound 2) inhibited the ability of the anti-dsDNA monoclonal antibody R4A to bind DWEYS peptide in a dose-dependent manner (IC\textsubscript{50} values of 4.5 ± 1.6 μM, 7.27 ± 0.5 μM, and 2.08 ± 0.8 μM, respectively) (Figure 2d). Reduced compound 4 (8) also showed moderate activity with an IC\textsubscript{50} of 42.7 ± 19.7 μM. These results largely validate the computer modeling and hypothesis that introduction of a more flexible backbone into HIV protease inhibitors represents a rich genus of new scaffolds for use as potential lupus therapeutics.

Kidney Glomeruli R4A Binding Assay. We next wished to test both unmodified (parent HIV protease inhibitors) and chemically reduced derivatives 5, 6, 7 in a more physiologically relevant setting, namely, the isolated kidney glomerulus. In this assay we incubated the R4A antibody with mouse glomeruli in the presence of compound and assessed binding with fluorescently conjugated secondary antibodies. In agreement with previous work,\textsuperscript{1} R4A bound to glomeruli (Figure 3). In marked contrast, R4A pretreated with either 1 or 2 showed diminished binding to glomerular antigens (Figure 3a). Qualitatively similar results were observed when R4A was pretreated with 5, 6, or 7 (Figure 3b); each of these chemically reduced HIV protease inhibitor derivatives decreased the binding of R4A to kidney tissue. Since renal disease occurs in approximately one-half of all SLE patients and accounts for one-third of all lupus deaths, a compound that decreases the deposition of pathogenic antibodies in the kidney is a potentially valuable therapeutic.

Neurotoxicity Model. It is known that R4A or NMDAR-binding antibodies can elicit excitotoxic death of neurons in the mouse hippocampus and that this, in turn, contributes to cognitive impairment and memory loss.\textsuperscript{30–33} Accordingly, we next assayed whether the reduced HIV protease inhibitors would block the neurotoxicity of R4A in vivo. In this assay, R4A antibodies were stereotactically injected into the dorsal hippocampus of a living mouse in the presence or absence of compound or vehicle. One day later, the animal was sacrificed, and apoptosis was revealed by TUNEL staining of the brain at the injection site. As expected,\textsuperscript{1,4,5,30,33} R4A alone caused neuronal apoptosis as indicated by positive TUNEL staining (Figure 4, left). In contrast, pretreatment with 5 and 6 abrogated this neurotoxic effect, while 7 did not, possibly due to lower solubility (Figure 4).

Taken together, these glomerular ex vivo and brain in vivo experiments demonstrate that parent (unmodified) and chemically reduced HIV protease inhibitors can function as decoy antigens to protect the kidney and brain from the pathogenic effects of anti-dsDNA antibodies.

Preliminary Pharmacokinetic Studies. Having made significant investments in other small molecule therapeutic programs only to reach an impasse due to toxicology and/or pharmacokinetic (PK) issues, we next sought to use these criteria to assist in deciding which drug candidate to test in more rigorous in vivo disease models. All of the top three leads advanced through pilot toxicology studies, with no obvious behavioral changes or tissue abnormalities following a week of daily dosing. We next determined the pharmacokinetics of each compound after either intravenous (iv) or oral (gavage) administration at 1 mg/kg and 10 mg/kg, respectively (Supporting Information, Figure S2). In both cases, 5 exhibited the best PK profile and was well tolerated at the administered...
dose levels. On the basis of these criteria, we began testing 5 in the NZB/W F1 lupus mouse model.

**NZB/W F1 Lupus Model: Efficacy Testing.** NZB/W F1 mice spontaneously develop lupus-like phenotypes, including elevated serum anti-dsDNA autoantibodies and immune-mediated glomerulonephritis that leads to kidney failure as the animals age. Like the human situation, disease in this strain is strongly biased toward females. In our study, we began dosing female NZB/W F1 mice with either vehicle or compound 5 (5 mg kg\(^{-1}\) day\(^{-1}\) in two daily i.p. injections) at 22 weeks of age, prior to onset of severe proteinuria. Body weights, blood, and urine were taken weekly. The blood was used to assess anti-dsDNA reactivity and urine used to assess proteinuria. Humane end points included ≥3+ proteinuria or 20% body weight, or prostration. Terminal blood and kidney tissues were taken for subsequent analysis.

Body weights throughout the study were similar between the vehicle and 5-treated groups (Figure 5a), and no 5-treated animals had to be removed due to weight loss or prostration, suggesting that the decoy antigen was well tolerated. 5-treated mice showed delayed onset of disease (26–36 weeks) and a trend toward increased disease-free survival (Figure 5c), although it did not reach statistical significance (perhaps owing to small cohorts). Strikingly, the kidneys from 5-treated animals had significantly less staining for mouse IgG (autoantibody deposition) in the glomeruli and exhibited preserved histology compared to vehicle-treated mice (Figure 6). As displayed in representative sections from mice at 60 weeks, lymphocyte aggregates (thin arrows), tubule dilation and casts (★), and glomerulonephropathy (thick arrows) are prominent in vehicle-treated mice while minimal in 5-treated mice (Figure 6b). Conglomerate blinded analyses of kidney histopathology scores (60 weeks) resulted in significantly lower standard combined pathology scores in 5-treated mice (Figure 5d). This combined pathology score encompassed four criteria (glomerular sclerosis, glomerular cellularity, crescents, and interstitial inflammation). Of these criteria, the 5-treated animals showed improvement in all, suggesting that their deaths may not be due to impaired renal function. Finally, sera from 5-treated animals manifest higher anti-DWEYS reactivity relative to sera from vehicle-treated mice (Figure 5b), presumably due to reduced IgG deposition in target organs of 5-treated mice. Taken together, these results demonstrate that 5 represents a novel class of potential therapeutics that results in a trend toward increased disease-free survival and preserved kidney histology in the NZB/W F1 mouse model of SLE. The hypothesized decoy antigen mechanism of action was supported by the reduction of immune complex deposition and downstream pathology in kidneys from 5-treated mice compared to vehicle-treated mice.

**DISCUSSION**

In this manuscript we report a fingerprint similarity search of existing compounds/drugs (either FDA approved or experimental) with structural features common to the cross-reactive antigen peptide DWEYS\(^*\) that resulted in 100 hits, 8 of which belonged to the HIV protease inhibitor family. To further refine our "decoy antigens", we employed computational modeling to...
investigate aspects of the HIV protease inhibitor compounds which could be modified to improve the three-dimensional fit to DWEYS. We discovered that reducing the structurally
constrained HIV protease inhibitors’ internal backbone could improve their flexibility and enhance the fitting profile to DWEYS. Using an ELISA-based screen, we confirmed that many HIV protease inhibitors, including 1, 2, and 3, dose-dependently inhibited the binding of anti-dsDNA antibodies to target antigens. Next, we chemically reduced these HIV protease inhibitors and identified several more potent derivatives that could neutralize anti-dsDNA antibodies in vitro, in situ, and in vivo. Among these, one small molecule (S) previously described was well-tolerated in vivo and delayed SLE disease progression in NZB/W F1 mice. At necropsy, S-treated animals displayed significantly less staining for mouse IgG (autoantibody deposition) in the glomeruli and preserved kidney histology compared to vehicle-treated mice.

Using a small molecule compound (MW under <700 Da) to target an autoimmune antibody is novel, and the strategy of targeting an anti-DNA antibody with a larger drug complex has only been tested once before to our knowledge. Abetimus (Abetimus sodium, LJF 394, Rentol, Riquent) is a tetrameric set of oligodeoxyribonucleotides attached to a proprietary nonimmunogenic PEG carrier (approximately 54 000 Da) that was tested in several clinical trials. It was found to lower anti-DNA Ab levels and reduce anti-DNA B-cell clones in animal studies, the latter causing it to be termed a “tolerogen”. Initial human trials were promising and advanced to the phase III level (LJP394-90-09 and LJP394-90-14). The last phase III trial was halted after it was determined that significance differences were not going to be achieved between placebo and drug-treated groups (results of this study have not been published to our knowledge). Poststudy analyses of the published trials led to the conclusions that there were significantly fewer renal flares, and time to renal flare in the drug-treated group was half that of placebo when a responding subset of patients (patients with high affinity DNA binding antibodies) were compared with the placebo group.34 There has been criticism of patient inclusion criteria, end points measured, and dosage of drug used in the study that was halted.35 Furthermore, the oligonucleotides present in the abetimus conjugate were not tested for TLR9 binding. It is possible that the immune complexes formed by anti-DNA antibody and abetimus were, in fact, proinflammatory, thereby exacerbating lupus symptoms. In contrast, compound 5 is monovalent and will not form immune complexes and is a mimotope of a peptide that does not bind TLR9 (unpublished data). Our data suggest that blocking anti-dsDNA antibody from binding tissue or oligonucleotides that constitute TLR9 ligands may still be a valid therapeutic approach in lupus but requires carefully planned testing. As our kidney evidence in a lupus model suggests, the use of small molecule agent 5, rather than a large drug-conjugate, to neutralize autoactive antibodies may allow for less tissue binding and generate fewer side effects than the drug-complex used in the aforementioned study.

In summary, our investigation has led us to the discovery that clinically approved HIV drugs may be themselves repurposed, or modified to be made more flexible, to target autoantibodies prevalent in SLE. These compounds appear to function as decay antibodies, reducing IgG deposition and associated pathology in the kidney, although not neutralizing all contributors to early death in SLE. Our approach of increasing flexibility of structurally relevant drugs/compounds to increase binding affinity to pathogenic autoantibodies could serve as a valuable tool in structure–activity analyses going forward. The fact that our starting compounds have a proven safety profile in HIV patients who, like SLE patients, may be immune-compromised is a promising back-drop for the reductively modified offspring we have termed “reduced HIV protease inhibitors”. We are continuing tests of our lead compounds for safety parameters and oral dosing using mouse models of lupus.

## Experimental Section

1. General. Unless otherwise indicated chemicals and reagents were purchased from Sigma-Aldrich. D-WEYES was acquired from Keck Biotechnology Resource Laboratory (New Haven, CT) and was greater than 99% pure as indicated by HPLC and MS (Supporting Information, page S9). Saquinavir, nelfinavir, and other HIV protease inhibitors were obtained from the pharmacy of North Shore University Hospital (Manhasset, NY), extracted from capsules, purified by flash chromatography, and confirmed by mass spectrometry (MS). Animals were housed under standard conditions and were assigned to groups by random selection upon arrival. All procedures were in accordance with the IACUC of The Feinstein Institute. Excel or Prism was used for graphing and statistical analyses. Results are reported as mean values with standard deviations unless otherwise noted. Student’s t tests were used to distinguish significance with the criteria of p < 0.05 and are indicated in figure by an asterisk (∗) symbol.

2. Chemistry. Chemicals were obtained from commercial suppliers and used without further purification unless otherwise indicated. All 1H NMR spectra were recorded on a Bruker Avance DRX 500 and 600 MHz. Chemical shifts are relative to the deuterated solvent peak and are in ppm. The coupling constants (J) are measured in Hz. The 1H signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Low- and high-resolution mass spectrometry was carried out at the LCOQ DECA XP MS. HPLC was performed using Waters system combining a 600 PUMP and a 996 PAD UV detector. The analytical column was a Phenomenex Luna C18 100 Å 250 mm x 46 mm.

### General Procedure for the Reduction of Selected HIV Protease Inhibitors (HIVPIs).

To the solution of 100 mg of HIVPI in 20 mL of dry THF was added 20 mL of 1 M BH3 in THF. The solution was stirred at 65 °C under N2 for 1 h. The crude residue was dried under vacuum pump to yield white solid, which was dissolved in 1 mL of MeOH and diluted with 10 mL of piperidine. The solution was stirred at 65 °C under N2 for another 1–2 days. The solvent was concentrated, and the residue was dissolved in methanol, then using ether to precipitate the crude product which was purified by HPLC for the animal studies and characterizations. Information regarding the analytical data of the analogues is provided in Table 1 and Supporting Information, sections I and II.

**Compound Characterization NMR Data.** 2-[(3-Amino-1-{(1-benzyl-3-[3-(tert-butylamino)octahydro-isoquinolin-2-yl]-2-hydroxy-propylamino}-methyl)-propylamino]-methyl]-2,3,4-tetrahydroquinolin-3-ol (S). P.SILE-412 (S) was published in 2011.1 3-[(3-{3-(tert-Butylaminomethyl)octahydro-isoquinolin-2-yl}-2-hydroxy-1-phenylsulfonyl-methyl)propylamino]methyl-2-methylphenol (R). White foam; MS (ESI) m/z 540.4 [M + H]+; 1H NMR (500 MHz, CDCl3, δ 1.08 (s, 9H, C(CH3)3), 1.14–1.21 (m, 3H), 1.31–1.37 (m, 2H), 1.48–1.51 (m, 3H), 1.61–1.86 (m, 4H), 2.22 (s, 3H), 2.24 (m, 1H), 2.32 (dd, J = 3.4, 11.65 Hz, 1H), 2.46 (dd, J = 6.4, 13.7 Hz, 1H), 2.66 (dd, J = 3.9, 12 Hz, 1H), 2.73–2.78 (m, 2H), 2.87–2.90 (m, 1H), 3.04 (dd, J = 5.7, 13.7 Hz, 1H), 3.18 (dd, J = 7.2, 13.2 Hz, 1H), 3.56 (dd, J = 4.3, 13.2 Hz, 1H), 3.73 (dd, J = 12.3, 29.9 Hz, 2H), 3.87 (dd, J = 5.75, 11.5 Hz, 1H), 6.74 (dd, J = 7.95, 13.85 Hz, 2H), 6.91 (t, J = 7.75 Hz, 1H), 7.16–7.19 (m, 1H), 7.27–7.31 (m, 2H), 7.42–7.44 (m, 2H). 13C NMR (125 MHz, CDCl3) δ 11.51, 21.48, 26.65, 27.35, 29.02, 32.12,32.23, 35.07, 35.56, 37.76, 46.96, 54.04.
Colorless oil; MS [ESI] m/z 589.33 [M + H]+; 1H NMR (500 MHz, (pyridine- d5), δ 0.82 (d, 3H, J = 6.9 Hz), 0.87 (d, 3H, J = 6.9 Hz), 1.93 (m, 1H), 2.03–2.09 (m, 3H), 2.24 (s, 3H), 2.49 (s, 3H), 2.62–2.69 (m, 1H), 2.75–2.77 (m, 2H), 2.95–3.08 (m, 3H), 3.16 (t, 1H, J = 12 Hz), 3.28–3.32 (m, 3H), 3.35–3.45 (m, 3H), 3.62–3.64 (m, 1H), 3.73–3.77 (m, 1H), 3.84 (m, 1H), 4.04 (m, 1H), 4.13–4.17 (m, 1H, 4.26–4.31 (m, 1H), 4.36–4.39 (m, 1H), 6.93–7.01 (m, 2H), 7.16–7.29 (m, 9H), 7.4 (m, 2H). 13C NMR (125 MHz, (pyridine-d5), δ 16.83, 17.17, 26.72, 28.8, 33.40, 35.17, 35.50, 38.93, 41.5, 44.18, 44.74, 46.98, 48.11, 56.95, 61.33, 65.22, 68.99, 70.04, 125.24, 127.77, 127.32, 129.68, 129.76, 129.80, 130.15, 130.29, 131.53, 137.85, 138.33, 156.22.

3. DWEYS Peptide Structure Modeling. Four experimental three-dimensional (3D) structures of rat NR2B were identified in the Protein Data Bank (PDB codes 3JPW, 3JPY, 3QEL, 3QEM). All four structures were aligned and their sequences were aligned onto the superimposed default parameters. Proteins were removed and their three-dimensional (3D) structures bound to HIV protease were identified in the superimposed ligands by visual inspection. The five conformations of saquinavir were used as a starting point conformational database for flexible alignment of 1 onto the DWEYS structural model kept rigid using the MOE small molecule flexible alignment module (MMFF94x force field used for potentials). Resulting overlays of 1 to DWEYS were triaged and prioritized using the 3D similarity score generated by the similarity function of this module. The overlays of other HIV protease inhibitors and derivative compounds to DWEYS were generated using a similar methodology: A database of reduced derivative conformations was generated by structural modification of their parent HIV protease inhibitors initial conformations followed by geometry optimization of the resulting structure by energy minimization in the MMFF94x force field, leading to the nearest stable conformer.

7. Glomerular Binding with R4A Antibody. Murine glomeruli were isolated, adhered to glass slides, and the glomerular binding assay was carried out essentially as described.4 Monoclonal antibody R4A (mouse) was applied at 20 μg/mL for 1 h at room temperature and visualized with anti-mouse IgG antibody conjugated to FITC (Jackson ImmunoResearch Laboratories). Slides were mounted using VECTASHIELD. For inhibition of antibody binding, R4A was preincubated with test compound (50 μM) for 1 h at 37 °C before incubation with glomeruli, as was done for ELISA inhibition assays. Slides were washed with PBS and mounted with coverslips using VECTASHIELD mounting medium (catalog no. H-1000, Vector Laboratories). Images were acquired at room temperature on an upright microscope (Axioplan 2; Zeiss) with a Zeiss Plan-Neofluar 40× oil lens using the OpenLab, version 4.04, software (Improvision). For each experimental condition, images were acquired with a Hamamatsu ORCA-ER digital camera (model no. C4742-80) at the same exposure, imported into Adobe Photoshop CS3, and then adjusted for contrast and brightness identically in all frames in each treatment group. Images were enlarged 2× for the figures.

8. Neurotoxicity Model. All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, the National Institutes of Health Guide for the Use and Care of Laboratory Animals, and the Research Animal Resource Center Users Guide. Male C57BL/6 mice between 30 and 34 g were anesthetized with 2.5% isoflurane (ip), placed in a stereotactic frame, and injected stereotaxically (2 μL) into the hippocampus, as described.45 Forty-eight hours later, mice were euthanized, brains fixed, removed, and hippocampal sections (40 μm) were cryoprotected and subjected to TACS-Tdt apoptosis detection (R&D Systems, no. TA4625) with methylene green counterstain as described.36 Sections were visualized with an upright microscope (Axio-Imager; Zeiss), and images were captured digitally (12-bit camera, 25 MHz, 1388 × 1040 resolution), processed (AxioVision 4.7, Zeiss), imported to Photoshop CS4 as 8-bit TIFF files, and scale-adjusted equally for all frames.
9. Pharmacokinetic and Lupus Disease-Free Survival Studies. Pharmacokinetic analyses of intravenous and oral dosing of reduced HIV protease inhibitor compounds dissolved in PBS were performed on male CD-1 mice of 6–10 weeks of age (n = 3/group) at time point. Whole blood was protein precipitated with acetonitrile (50:50 v/v), and supernatant was analyzed for compound recovery by HPLC/MS (Supporting Information, Figure S2). For SLE disease-free survival, female NZB/W F1 mice were used at 22 weeks of age, prior to onset of severe proteinuria. Prior to the initial dose, proteinuria scores and body weights were measured and groups were assigned such that each group had an equivalent mean proteinuria score and body weight (n = 10 mice/group). Prestudy body weight measurements were performed once weekly, and proteinuria measurements were performed once every 2 weeks beginning at 22 weeks of age.

Animals received dose administrations of test or vehicle twice daily by intraperitoneal (ip) injection beginning on day 1 (at 22 weeks of age). Doses were administered at 5.0 mL kg\(^{-1}\) day\(^{-1}\) (2.5 (mL/kg)/injection) per animal. Urine was collected before the initial dose administration on day −1 and once every 2 weeks thereafter by either spontaneous urination or gentle pelvic compression during manual restraint of the animal. Interim blood samples were collected by either tail nick or facial vein puncture and terminal blood samples by cardiac puncture.

Whole blood samples were collected into serum separator tubes inverted gently several times and allowed to clot for at least 30 min at room temperature. Samples were then centrifuged at 9300 g for 10 min at room temperature, serum was extracted by pipet and stored at −70 °C. Euthanasia (isoflurane, to effect, in conjunction with exsanguination [terminal blood collection], followed by thoracotomy) was performed in accordance with accepted American Veterinary Medical Association (AVMA) guidelines at 46 weeks of age or presentation of humane disease-specific exit criteria which were as follows: if a mouse exhibited a body weight loss of ≥20%, it was removed from study for euthanasia; if a mouse exhibited a body weight loss of ≥20% and weight loss rate exceeded 5% per day, it was removed from study; if a mouse exhibited ≥3+ proteinuria on two consecutive weeks, it was removed from study for euthanasia.

10. Kidney Histology Studies in NZB/W F1 Mice. Kidney sections from each animal were analyzed as previously described. The sections were evaluated by one investigator (JMC) in a blinded fashion, without knowledge of the mouse treatment. The severity of disease was scored semiquantitatively on a scale of 0 to 3+ for each of the following features: glomerular sclerosis, glomerular hypercellularity (including glomerular cell proliferation, leukocyte exudation), glomerular crescents, interstitial nephritis (infiltration of mononuclear cells), and vasculitis (perivascular) inflammatory infiltrates.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00694.

NMR data, HPLC profiles, flexible alignment scores and models, DWEYS peptide purity data, and pharmacokinetic studies in CD-1 mice (PDF)

Molecular formula strings and some data (CSV)

PDB information (PDB)
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