Communications to the Editor

Drug Leads from Combinatorial Phosphodiester Libraries

Peter W. Davis,* Timothy A. Vickers, Laura Wilson-Lingardo, Jacqueline R. Wyatt, Charles J. Guinosso, Yogesh S. Sanghvi, Elizabeth A. DeBaets, Oscar L. Acevedo, P. Dan Cook, and David J. Ecker

Isis Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, California 92008

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The current interest in the creation of large, searchable libraries of organic compounds has captured the imagination of organic chemists and the drug discovery community. Efforts in numerous laboratories focused on the introduction of chemical diversity into oligomeric motifs have been recently reviewed,[1-3] and pharmacologically interesting compounds have been identified from libraries of widely different composition, including oligonucleotide,[4] peptide,[5] and peptoid building blocks.[6,7] This activity prompted our efforts to use a collection of modified and natural nucleosides and other simple monomers for the generation of combinatorial phosphodiester libraries. These monomers include nucleoside 2′-*O*-alkylpurines, pyrimidines, and benzotriazoles, plus simple carbazole, imidazole, and amine building blocks[8] (Figure 1).

This report describes the deconvolution of one oligomer library to select unique inhibitors of two inflammatory mediators, phospholipase A_2 (PLA_2) and leukotriene B_4 (LTB_4). The hydrolysis of phospholipids by the enzyme PLA_2 is the rate-limiting step in the release of pro-inflammatory mediators, and type II PLA_2 is implicated in the pathogenesis of a number of human inflammatory diseases.[9,10] LTB_4 mediates the inflammatory response through interaction with specific cell-surface receptors[11] and is also associated with inflammatory disease.[12,13]

An iterative method of synthesis and screening, SURF,[14] was used to select unique inhibitors of these targets from a phosphodiester-based library. Twelve subsets of pentamers were synthesized,[15] each subset containing 1728 compounds (Table 1, round 1). The sequence of each subset was defined by fixing the leftmost position (X) with a unique monomer followed by three "randomized" positions (N) composed of an equimolar mixture of all 12 monomers.[16,17] At the right end, there was a defined position (T) corresponding to the thymidine-derivatized CPG used for solid phase synthesis.[18]

The 12 subsets of the oligomer library were screened in a number of assay systems including the two inflammatory targets described above. These included both cell-based and biochemical assays for inhibitors of a number of pharmacologically relevant targets. Subsets displayed activity warranting deconvolution only against the two inflammatory targets. In the LTB_4 and PLA_2 assays, only two subsets had significant activity at the highest concentration tested. In other assay systems, no activity was observed at a concentration of the subset below 50 μM (data not shown).

The library subsets were screened for inhibition of phospholipid hydrolysis by human type II PLA_2[19] in an assay using *Escherichia coli* labeled with [^{3}H]oleic acid as substrate.[20,21] The IC_{50} values (concentration at which enzymatic activity is 50% relative to the control) of subsets in each round of the deconvolution are given in Table 1. In the first two rounds of the deconvolution, the subsets fixed with the monomer noC were clearly most active. However, as the deconvolution progressed, increasing numbers of subsets displayed activity. In the fourth and final round, unique compounds were screened, and although several had activity below 10 μM, the subset with T in the fixed position had the best activity with an IC_{50} of 2 μM.

Several compounds related to the selected inhibitor, noC-noC-dG-T-T (Figure 2a), were synthesized to determine whether the order of the monomers and the length of the oligomer were important (Table 2). Two compounds with the same monomer composition but different sequence did not have activity at 25 μM. Truncation of the sequence from the 3′-end also led to loss of activity. A "homo-oligomer" of noC-noC-noC-noC-T was 3-fold less active than the selected compound. These observations suggest that both length and order are important for activity.

Deconvolution of the library in the LTB_4 assay led to the identification of the inhibitor egCB-egCB-BT1-egCB-T (Table 3). The LTB_4 assay measured the effect of subsets of the combinatorial library on the binding of a radiolabeled LTB_4 to its receptor on a membrane preparation from guinea pig spleen.[22] Unlabeled LTB_4 was used as a control in the assay. LTB_4 binds to its receptor with a K_d value in the 1 nM range.[23] The activity of the selected inhibitor was sensitive to both length and sequence; however, minor chemical alterations had little effect on activity (Table 4). Truncation of the oligomer led to loss of activity. Oligomers containing a (*R*)-*N*-carbazolylpropane-2,3-diol monomer exhibited activities similar to those containing the (*S*)-isomer used in deconvolution. Substitution of phosphorothioate for phosphodiester linkages in some of these shorter compounds did not alter activity.

The inhibitors described above were selected from a library of compounds which would not *a priori* have been predicted to contain them. Despite the fact that the library was not biased in any way, the selected compounds have inhibitory activities that are comparable to leads identified from natural product screening.[24,25] Manolide, a well-known inhibitor of PLA_2, has an IC_{50} of approximately 3 μM in our assay (data not shown). Inhibitors of LTB_4 isolated from sponge also have IC_{50}'s in the 1–10 μM range.[26] The inhibitors selected from the combinatorial library are specific for the particular targets. Inhibition required a specific subset of functional groups per round, and different inhibitors were selected in each assay. The PLA_2 inhibitor was not active in the LTB_4 assay (Figure 3); however, the LTB_4

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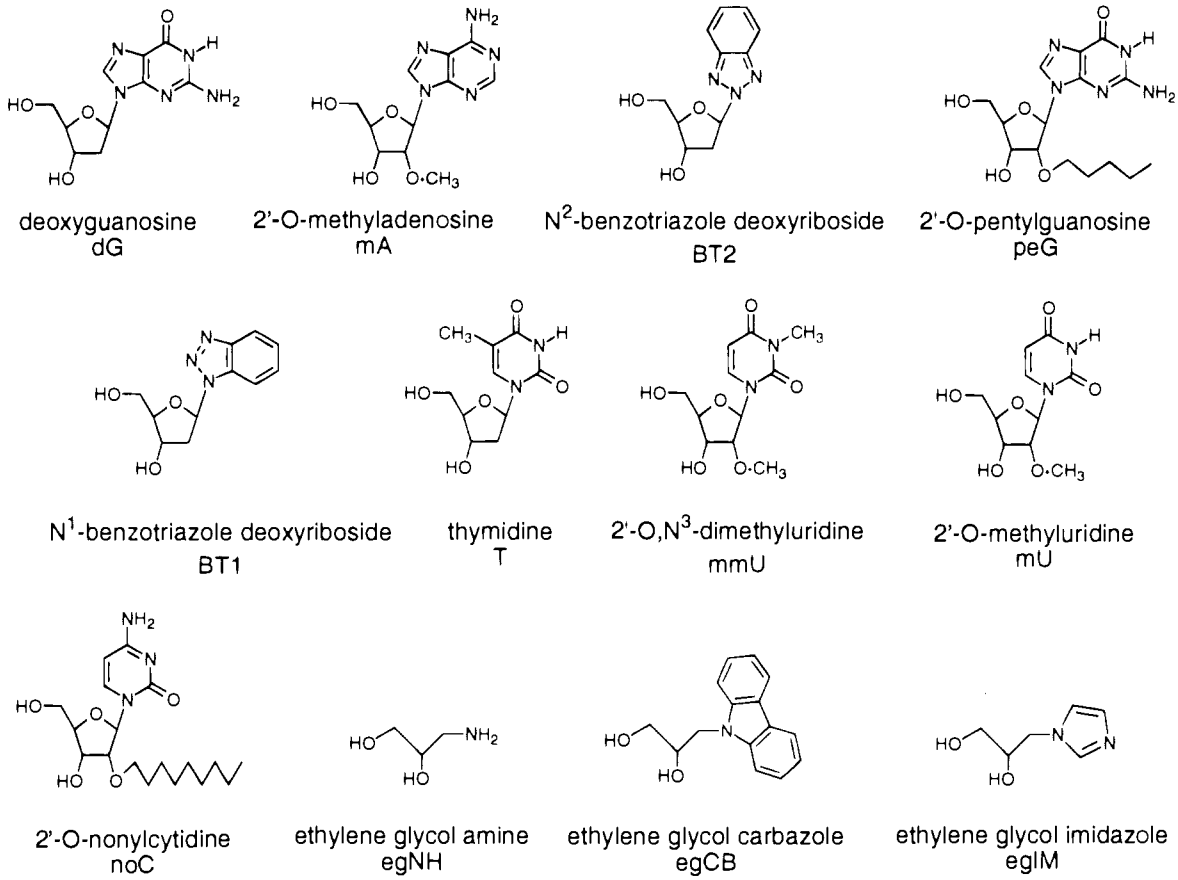

Figure 1. Monomers used in synthesis of oligomer library.

Table 1. Selection of a Type II PLA_2 Inhibitor from the 12-Monomer Combinatorial Library

			IC_{50}[d] for X =											
R[a]	sequence[b]	Q[c]	dG	T	mA	mU	BT1	BT2	egNH	noC	mmU	peG	egCB	egIM
1	XNNNT	1728	>100	>100	>100	>100	>100	>100	>100	30	>100	60	>100	>100
2	(noC)XNNT	144	>100	>100	>100	>100	35	>100	>100	20	>100	35	>100	>100
3	$(noC)_2XNT$	12	10	>50	>50	>50	>50	>50	>50	20	45	15	>50	>50
4	$(noC)_2(dG)(X)T$	1	5	2	6	5	>10	>10	>10	8	>10	5	>10	>10

[a] Round of synthesis and screening. [b] X represents a monomer from Figure 1 fixed at position shown; N represents an equimolar incorporation of 12 monomers. [c] Number of compounds per subset. [d] IC_{50} values are in μM/subset. Underlined value indicates a most active subset.

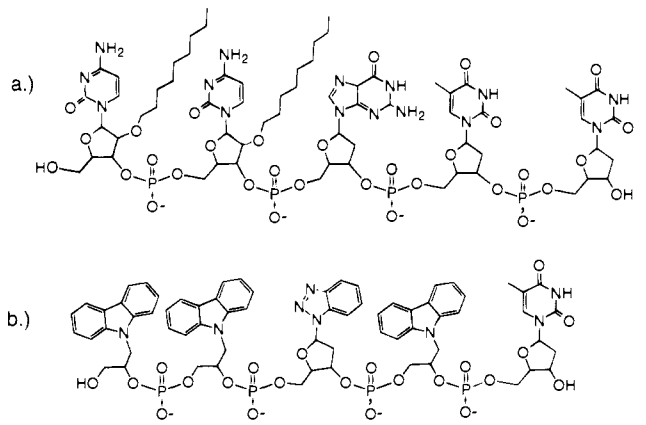

Figure 2. Structures of a selected (a) PLA_2 inhibitor and (b) LTB_4 inhibitor.

inhibitor did show modest activity in the PLA_2 assay with an IC_{50} approximately 3-fold higher than the selected oligomer (data not shown).

The compounds selected may be the most active in the library against these particular targets, but other compounds also contributed to the IC_{50}'s of the round 1 subsets. If only a single compound were responsible for inhibitory activity, one would expect a 12-fold improvement in each round of deconvolution. The lower than expected round-to-round improvement is due to the inhibitory effect of a number of compounds in the library and has been observed for other combinatorial library deconvolutions.[3,27]

Table 2. Comparison of PLA_2 Binding Inhibition by Related Compounds

oligomer	binding constant (μM)	oligomer	binding constant (μM)
$(dG)(noC)_2(T)_2$	>25	$(noC)_4(T)$	7
$(noC)(dG)(noC)(T)_2$	>25	$(noC)_2(dG)(T)$	5
$(noC)_2(dG)$	15	$(noC)_2(dG)(T)_2$[a]	2

[a] Selected inhibitor.

In summary, unique inhibitors of two inflammatory targets, PLA_2 and LTB_4, were selected from a phosphodiester-linked combinatorial library. The library was composed of approximately 20 000 oligomers synthesized from 12 biologically occurring and synthetic monomers and deconvoluted using an iterative synthesis and screening approach. The inhibitors have activity

Table 3. Selection of an LTB_4 Inhibitor from the 12-Monomer Combinatorial Library

			IC_{50}[a] for X =											
R[a]	sequence[a]	Q[a]	dG	T	mA	mU	BT1	BT2	egNH	egIM	mmU	noC	egCB	peG
1	XNNNT	1728	>50	>50	>50	>50	>50	>50	>50	>50	>50	48	40	>50
2	(egCB)XNNT	144	>10	>10	>10	>10	9	>10	>10	>10	>10	>10	6	>10
3	$(egCB)_2$XNT	12	1.9	>5	>5	>5	1.7	1.9	>5	>5	>5	>5	2.2	>5
4	$(egCB)_2$(BT1)XT	1	0.92	>1	>1	>1	0.76	>1	>1	0.90	0.80	0.88	0.68	0.71

[a] See the legend to Table 1.

Table 4. Comparison of LTB_4 Binding Inhibition by Related Compounds

	relative IC_{50}[a]		
oligomer	P=O[b]	P=O[c]	P=S[d]
carbazole	>25		
(gCB)(T)	>25		>25
$(egCB)_2$(T)	11.9		
$(egCB)_2$(dG)	6.8		
(egCB)(BT1)(egCB)	6.4		
(egCB)(BT1)	5.9	6.4	
(BT1)(egCB)	5.2		7.3
$(egCB)_2$(BT1)	3.0	2.8	5.0
$(egCB)_2$(BT1)(egCB)T[e]	1.0	0.9	

[a] IC_{50}'s are normalized to a value of 1 for the selected inhibitor. [b] Phosphodiester oligomers were made with (*S*)-*N*-carbazolylpropane-2,3-diol monomer. [c] Oligomers made with (*R*)-*N*-carbazolylpropane-2,3-diol monomer. [d] Activity of phosphorothioate analog. [e] Selected inhibitor.

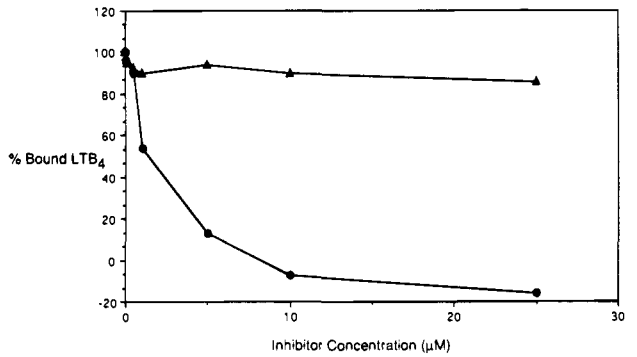

Figure 3. Relative potency of selected PLA_2 inhibitor and LTB_4 inhibitor in the LTB_4 receptor binding assay. The LTB_4 inhibitor (●) effectively competes with labeled LTB_4 for binding to its receptor, while the PLA_2 inhibitor (▲) does not.

in the micromolar range. The combinatorial approach allows screening of a large number of molecules in an efficient fashion. By comparing activities of subsets through the deconvolution, information is obtained about the relative importance of functional groups, which can be instructive in the design of new libraries or analogues of the selected inhibitor. It seems likely that further combinations of functionality and linkage chemistries[28] will yield novel inhibitors against a greater variety of disease targets.

Supporting Information Available: Synthesis of BT1, BT2, and noC phosphoramidites (5 pages). Ordering information is given on any current masthead page.

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