Nanomolar Inhibitors for Two Distinct Biological Target Families from a Single Synthetic Sequence: A Next Step in Combinatorial Library Design?**

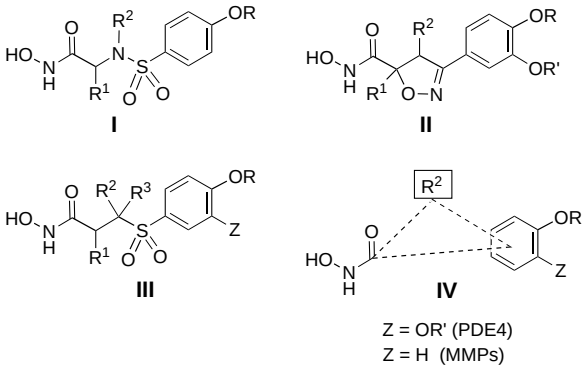
Christopher J. Burns,* Robert D. Groneberg, Joseph M. Salvino, Gerard McGeehan, Stephen M. Condon, Robert Morris, Matthew Morrissette, Rose Mathew, Shelley Darnbrough, Kent Neuenschwander, Anthony Scotese, Stevan W. Djuric, John Ullrich, and Richard Labaudiniere

The application of combinatorial chemistry and parallel synthesis tools to drug discovery has significantly increased the chemist's ability to prepare large sets or libraries of pharmacologically relevant compounds.[1] The design of small molecule libraries has traditionally been directed at either general lead generation[2] or more focused lead optimization libraries.[1] Recent empirical evidence suggests that certain minimum pharmacophore patterns[3] present within small-molecule lead structures can result in recognition by more than one member of a specified target family of interest (e.g. G-protein-coupled receptors).[4] Consequently a third class of combinatorial library has emerged: the target family directed library.[5] A key element in the design of such libraries is the inclusion of specific pharmacophore patterns or pharmacophoric points (e.g. hydroxamic acids or thiols for metalloenzymes) considered to be "privileged" for recognition of a target class of interest. Target family directed libraries offer the possibility of rapidly furnishing new lead structures for novel biomolecular targets as they arise within each family from molecular biology and genome research.[6]

Ideally, a target family directed library should incorporate the appropriate privileged pharmacophore pattern(s), and should also introduce sufficient diversity to ensure a range of pharmacological, pharmacokinetic, and physicochemical properties for the library members. Screening of a target family library may thereby not only provide lead structures for biological targets within a specific family, but initial structure – activity relationships (SAR) and selectivity data as well. It is therefore important to identify combinatorial-based synthetic approaches that allow rapid, efficient entry into such compound libraries.

Here we report a specific embodiment of the combinatorial chemistry paradigm that may have more general implications: the generation of multiple libraries—from a single synthetic sequence—that have been constructed to recognize members of two unrelated biological target families. Central to this approach was our identification of a novel structural template (**III**) that, with only a small but important change in functional decoration, could independently display the necessary pharmacophore patterns for inhibition of members of either of two different biomolecular target families (matrix metalloproteinases (MMPs) or phosphodiesterases (PDEs)). This recognition has permitted the pursuit of a straightforward synthetic route that efficiently generates a variety of combinatorially diversified α,β-unsaturated ester intermediates ($\mathbf{I}_{m-n}$, see Scheme 1). These intermediates can then be directed—depending upon the choice of the pharmacophoric characteristics chosen for the chemical input used in the divergent step—toward two structurally related but functionally distinct β-sulfonyl hydroxamic acid libraries.

Inhibition of the MMP collagenase-1 (MMP-1, EC3.4.24.7), the 72-kDa gelatinase (MMP-2, EC3.4.24.24), and the enzyme stromelysin-1 (MMP-3, EC3.4.24.17), and inhibition of the cAMP-specific phosphodiesterases (e.g. PDE4) has formed the basis for considerable research into therapies for oncology, asthma, and rheumatoid arthritis.[7] Within both of these target families, small-molecule inhibitors have been identified and are presently in clinical development.[7] Although there is little structural similarity between the natural substrates of these two distinct enzyme families (a peptide sequence for the MMPs and a cyclic nucleotide monophosphate for the PDEs), we have noted an apparent convergence of pharmacophore patterns (**IV**) for two independent series of hydroxamate-based MMP and PDE4 inhibitors (**I**[8] and **II**,[9] respectively). This convergence was consistent with the readily accessible structural template **III** designed in our lab. Contingent upon aryl substituent Z, structure type **III** can



display key pharmacophoric elements for recognition of either the MMP or the PDE target families, and therefore was used as the basis for construction of our first multiple target family directed libraries.

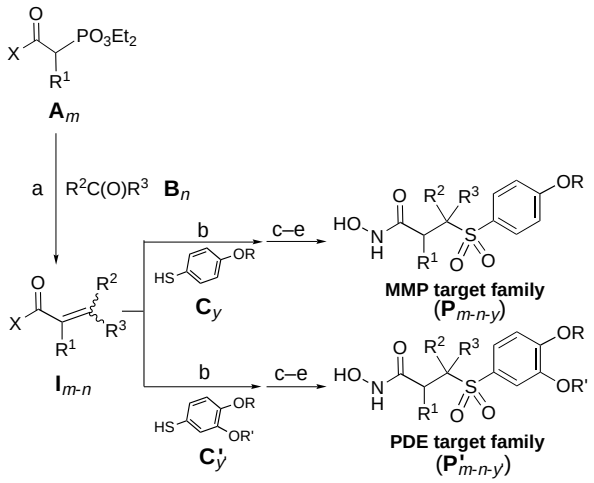
Synthetic routes were designed to allow the construction and subsequent elaboration of the diversified intermediate esters ($\mathbf{I}_{m-n}$) in both solution and the solid phase (Scheme 1).[10] The solution-phase synthesis (method A) served to explore the requisite chemical transformations, to perform the reactions on millimole scale, and to incorporate functional groups deemed incompatible with the solid-phase route. The solid-phase methodology (method B) rapidly provided micromole quantities of material for in vitro screening.[11]

[*] Dr. C. J. Burns, Dr. R. D. Groneberg, Dr. J. M. Salvino, Dr. G. McGeehan, Dr. S. M. Condon, R. Morris, M. Morrissette, R. Mathew, S. Darnbrough, Dr. K. Neuenschwander, A. Scotese, Dr. S. W. Djuric, Dr. J. Ullrich, Dr. R. Labaudiniere
Rhône-Poulenc Rorer
500 Arcola Rd., Collegeville, PA 19426 (USA)
Fax: (+1) 610-454-3311
E-mail: christopher.burns@rp-rorer.com

[**] We thank Dr. R. Harrison, Dr. J. Souness, Dr. J. Uhl, and J. Kline for the pharmacological data.

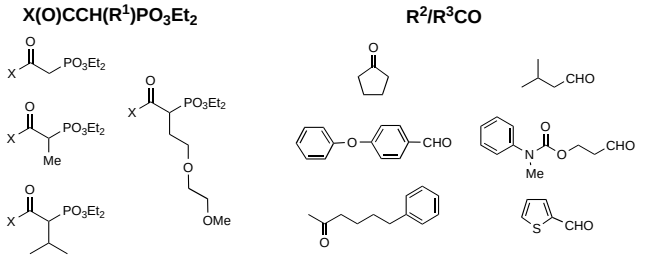
Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

  1433-7851/98/3720-2848 $ 17.50+.50/0



Scheme 1. Single synthetic route to multiple target family directed libraries. Method A (solution-phase synthesis): a) NaH (1 equiv), THF, $-40\,^{\circ}C \rightarrow RT$, then $R^2C(O)R^3$ (1 equiv); b) *n*BuLi (0.1 equiv), ArSH (1.5 equiv), THF, $0\,^{\circ}C \rightarrow RT$; c) TFA/$CH_2Cl_2$, 1:5 (v/v); d) $(COCl)_2$ (2 equiv), CH_2Cl_2, DMF, then TMS-ONH_2 (5 equiv), CH_2Cl_2; e) oxone (1.5 equiv), MeOH/water, 1/1 (v/v), $0\,^{\circ}C \rightarrow RT$. Method B (solid-phase synthesis): a) KHMDS or LiHMDS (4 equiv), THF, $0\,^{\circ}C \rightarrow RT$, 0.5 h, filter, then $R^2C(O)R^3$ (5 equiv), THF/cyclohexane, 72 h; b) *n*BuLi (4 equiv), ArSH (20 equiv), THF, 100 h; c) *m*-CPBA (13 equiv), 1,4-dioxane, 16 h; d) TFA/CH_2Cl_2 1/1 (v/v), 2 h; e) EDCI (4 equiv), hydroxylamine-based Wang resin, 20 h, then TFA/CH_2Cl_2, 1/1 (v/v), 1.5 h. TFA = trifluoroacetic acid; TMS = trimethylsilyl; HMDS = bis(trimethylsilyl)amide; X = O*t*Bu, Wang resin.

The five-step synthesis permits independent variation at three regions of template **III** (R^1, R^2/R^3, and SAr(OR)(Z)). Diversification at R^1 and R^2/R^3 is achieved early in the synthetic route by Wadsworth–Horner–Emmons olefination of selected aldehydes and ketones. Selection of the substituents at these positions was based on several parameters including size, shape, and hydrophobicity/hydrophilicity (Scheme 2). The key chemical transformation that directs

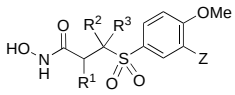


Scheme 2. Sampling of inputs used in the construction of diversified intermediates $\mathbf{I}_{m-n}$.

the olefinic ester intermediates $\mathbf{I}_{m-n}$ away from one target family and toward another (i.e., the divergent step) is the conjugate addition of aromatic thiols that are appropriately functionalized to complete the desired pharmacophore patterns.

As exemplified in Table 1, the choice of the appropriate arylthiolate ($\mathbf{C}_y$ or $\mathbf{C}'_{y'}$) in the divergent step of Scheme 1 defines the final products as either metalloproteinase or phosphodiesterase inhibitors. Use of *p*-alkoxythiophenols

Table 1. Enzyme inhibitory activity for examples from multiple target family directed libraries.[a]



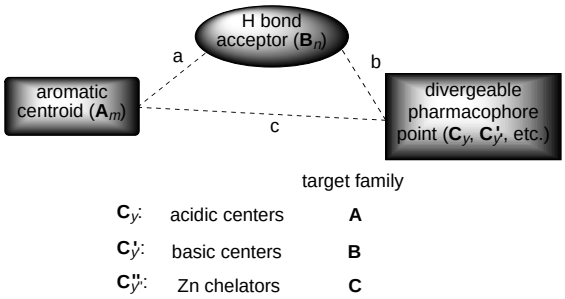
Compd[d]	K_i [μM][b]			IC_{50} [μM][c]
	MMP-1	MMP-2	MMP-3	PDE4
1	0.2	0.01	0.05	> 1
2	7	0.02	9	> 1
3	> 10	> 10	> 10	0.001

[a] K_i and IC_{50} are mean values from two or more determinations of purified compounds (see the supporting information). [b] Ref. [12]. [c] Ref. [13]. [d] Compound **1** (RPR 121683): $R^1 = H$; $R^2/R^3 = (-CH_2CH_2CH_2CH_2-)$; Z = H; **2**: (RPR 123303): $R^1 = H$; R^2 = 4-phenylbenzyl; $R^3 = Z = H$; **3** (RPR 123145): $R^1 = H$; $R^2 = Ph(CH_2)_4$; $R^3 = H$; Z = OMe.

such as 4-methoxythiophenol afforded potent inhibitors of MMP-1, MMP-2, and MMP-3.[14a] Selectivity among the MMPs varied according to the substituents R^1 and R^2/R^3 incorporated during the initial diversification steps. In fact, both broad-spectrum (**1**) and selective (**2**) MMP inhibitors were revealed during the screening process, thus demonstrating the versatility of this combinatorial approach. Alternatively, addition of discrete 3,4-dialkoxyphenyl thiolates to $\mathbf{I}_{m-n}$ elicited a switch in target family, and provided inhibitors of PDE4 effective at nanomolar concentrations while suppressing activity against the MMPs (e.g. **3**).[14b]

The application of combinatorial chemistry to drug discovery can result in structurally similar inhibitors for multiple targets (including members of multiple target families) as a natural consequence of general library screening.[15] It is the lure of adding front-end design elements to expedite such events that holds particular appeal. The advantages of combinatorial chemistry "directed" toward multiple target classes by a single synthetic sequence are the potential for higher hit rates during screening of the resulting libraries against biochemical targets within the targeted families, and the diminished need to develop two different routes (to two different libraries) to accomplish the same end.

We speculate that such a method of focusing combinatorial design could have applications beyond the example cited here, and could represent a logical evolutionary step from libraries directed at targets within a single target family.[5] Computational analysis of empirical structure–activity data has begun to permit the explicit identification of key pharmacophore points and complete pharmacophore patterns that are common (or similar yet distinct) to more than one target family (for a simplified hypothetical example, see Scheme 3). The identification of these privileged pharmacophore patterns can be used to drive the design of structure types and synthetic routes that are amenable to combinatorial or parallel diversification. Optimally, the synthetic design would incorporate a divergent step that can direct the library members toward one pharmacophore pattern and away from another. Such a divergent step can impart a high level of selectivity for one target family over another despite the partial pharmacophore pattern commonalities that they share (as was observed in the MMP/PDE example presented here).



Scheme 3. Similarities and differences for the hypothetical pharmacophore pattern of three distinct target families, which have been superposed to aid in the design of multiple target family libraries. Design of the corresponding library structure types and synthetic route would optimally include diversification of the conserved pharmacophore points $\mathbf{A}_m$ and $\mathbf{B}_n$ (various aromatic rings and H-bond acceptors separated by a distance of *a* in this example) followed by divergence of the resulting combinations (upon incorporation of the input bearing the distinguishing pharmacophore points $\mathbf{C}_y$, $\mathbf{C}_{y'}$, or $\mathbf{C}_{y''}$) toward product libraries with different final pharmacophore patterns, therein biased to recognize different biological target families.

In summary, we have exemplified a method by which the convergence of template design and combinatorial principles can be exploited to maximize the impact of a single synthetic route. The approach allowed for the efficient creation of two functionally distinct target family directed small-molecule libraries from this route. In applying this methodology, we have rapidly discovered novel, potent, and selective inhibitors within two therapeutically important biomolecular target families: the matrix metalloproteinases and the phosphodiesterases. We propose that the incorporation of multiple target family directed front-end design elements into combinatorial library design could ultimately expedite the pharmaceutical lead discovery process.

Received: May 11, 1998 [Z 11843 IE]
German version: *Angew. Chem.* **1998**, *110*, 3044 – 3047

Keywords: combinatorial chemistry · drug design · enzyme inhibitors · metalloenzymes · phosphodiesterases

[1] For recent reviews, see a) F. Balkenhohl, C. von dem Bussche-Hünnefeld, A. Lansky, C. Zechel, *Angew. Chem.* **1996**, *108*, 2436 – 2487; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288 – 2337; b) E. M. Gordon, M. A. Gallop, D. V. Patel, *Acc. Chem. Res.* **1996**, *29*, 144 – 154; c) D. V. Patel, E. M. Gordon, *Drug Discovery Today* **1996**, *1*, 134 – 144.

[2] General compound libraries for lead generation are often constructed around maximized pharmacophoric diversity and novelty. In some cases, a bias is incorporated toward discrete molecular recognition motifs (e.g. β-turn motifs) and/or inclusion of substructural elements with historically attractive pharmacokinetic (or other "druglike") characteristics. Such libraries are not normally directed at a particular target or class of targets. For a recent overview, see R. A. Fecik, K. E. Frank, E. J. Gentry, S. R. Menon, L. A. Mitscher, H. Telikepalli, *Med. Res. Rev.* **1998**, *18*, 149 – 185.

[3] P. Gund, *Annu. Rep. Med. Chem.* **1979**, *14*, 299 – 308.

[4] See, for example: T. W. Schwartz, U. Gether, H. T. Schambye, S. A. Hjorth, *Curr. Pharm. Design* **1995**, *1*, 325 – 342.

[5] For an introduction, see J. C. Hogan, *Nature* **1996**, *384 (Suppl.)*, 17 – 19. Examples of libraries implicitly or explicitly constructed to interact with multiple members of a particular target family: 1) Seven-transmembrane G-protein-coupled receptors: a) R. N. Zuckermann, E. J. Martin, D. C. Spellmeyer, G. B. Stauber, K. R. Shoemaker, J. M. Kerr, G. M. Figliozzi, D. A. Goff, M. A. Siani, R. J. Simon, S. C. Banville, E. G. Brown, L. Wang, L. S. Richter, W. H. Moos, *J. Med. Chem.* **1994**, *37*, 2678 – 2685; b) R. Hirschmann, W. Yao, M. A. Cascieri, C. D. Strader, L. Maechler, M. A. Cichy-Knight, J. Hynes Jr., R. D. van Rijn, P. A. Sprengeler, A. B. Smith III, *J. Med. Chem.* **1996**, *39*, 2441 – 2448, and references therein; 2) aspartyl proteases: E. K. Kick, J. A. Ellman, *J. Med. Chem.* **1995**, *38*, 1427 – 1430; 3) kinases: a) J. K. Chen, S. L. Schreiber, *Angew. Chem.* **1995**, *107*, 1041 – 1058; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 953 – 969; b) T. C. Norman, N. S. Gray, J. T. Koh, P. G. Schultz, *J. Am. Chem. Soc.* **1996**, *118*, 7430 – 7431; 4) matrix metalloproteinases: D. A. Campbell, J. C. Bernak, *J. Am. Chem. Soc.* **1994**, *116*, 6039 – 6040.

[6] L. J. Beeley, D. M. Duckworth, *Drug Discovery Today* **1996**, *1*, 474 – 480.

[7] Matrix metalloproteinases: R. P. Beckett, M. Whittaker, *Exp. Opin. Ther. Patents* **1998**, *8*, 259 – 282. Phosphodiesterases: J. A. Karlsson, D. Aldous, *Exp. Opin. Ther. Patents* **1997**, *7*, 989 – 1003.

[8] L. J. MacPherson, D. T. Parker (Ciba-Geigy), EP-B 606046 A1, **1994**; recent detailed report: L. J. MacPherson, E K. Bayburt, M. P. Capparelli, B. J. Carroll, R. Goldstein, M. R. Justice, L. Zhu, S. Hu, R. A. Melton, L. Fryer, R. L. Goldberg, J. R. Doughty, S. Spirito, V. Blancuzzi, D. Wilson, E. M. O'Byrne, V. Ganu, D. T. Parker, *J. Med. Chem.* **1997**, *40*, 2525 – 2532.

[9] E. F. Kleinman (Pfizer), WO-A 95/14681, **1995;** recent detailed report: E. F. Kleinman, E. Campbell, L. A. Giordano, V. L. Cohan, T. H. Jenkinson, J. B. Cheng, J. T. Shirley, E. R. Pettipher, E. D. Salter, T. A. Hibbs, F. M. DiCapua, J. Bordner, *J. Med. Chem.* **1998**, *41*, 266 – 270.

[10] Quality and purity for all library members was tested by HPLC/MS, with further characterization (NMR spectroscopy, elemental analysis) of all final products from method A. Note that when ketones are used as $\mathbf{B}_n$ in method A, steps b and c are reversed, with the thiol addition typically performed in pyridine with heating. The final steps d and e remain the same. In examples where R^1 and either R^2 or R^3 are not hydrogen, both the solution and solid-phase methods give principally the *E* olefin (for $\mathbf{I}_{m-n}$) and stereospecific *anti*-thiol addition ("erythro" product) consistent with a recent study in solution by Naito and co-workers: O. Miyata, T. Shinada, I. Ninomiya, T. Naito, T. Date, K. Okamura, S. Inagaki, *J. Org. Chem.* **1991**, *56*, 6556 – 6564. Levels of the "*threo*" epimer were typically below 15%, but did reach as high as 40 – 50% in method B when R^1 was large (e.g. CH_2CH_2SPh).

[11] For our first libraries, almost 300 compounds (more than 200 from method B) were prepared and screened as singles using these routes (see ref. [14]). In general, yields from method A were greater than 30% overall, with an average yield of more than 80% for each step. For method B, overall yields were typically 5 – 25% (see the supporting information).

[12] Inhibitory activity on recombinant human MMP-1, MMP-2, and MMP-3 (biogenesis) is measured according to the methods of C. G. Knight, F. Willenbrock, G. Murphy, *FEBS Lett.* **1992**, *296*, 263 – 266.

[13] Inhibitory activity against guinea pig macrophage homogenate PDE4 was performed according to a two-step radioisotopic method: W. J. Thompson, W. L. Terasaki, P. M. Epstein, S. J. Strada, *Adv. Cyclic Nucl. Res.* **1979**, *10*, 69 – 92.

[14] a) In total, 73% of compounds prepared with the MMP pharmacophore pattern (Scheme 1, $\mathbf{P}_{m-n-y}$) gave greater than 90% inhibition when screened at a concentration of 10 μM for at least one MMP, which typically resulted in K_i values of 1 μM or less; b) 33% of compounds prepared with the PDE motif ($\mathbf{P}^-_{m-n-y}$) afforded $IC_{50} < 100$ nM in the PDE4 assay.

[15] Consider, for example, compounds based on the 1,4-benzodiazepine (BZD) scaffold, which are rigid β-turn peptide mimics with known biopharmaceutical pedigree. Such features, combined with the plausibility of introducing three to four points of diversity onto the scaffold during its construction, have made BZD an appealing substructure for combinatorial library synthesis (for reviews, see S. H. DeWitt, A. W. Czarnik, *Acc. Chem. Res.* **1996**, *29*, 114 – 122 and J. A. Ellman, *Acc. Chem. Res.* **1996**, *29*, 132 – 143) and a prolific source of hits and leads against targets ranging across a number of biological target classes (CCK-A, FTase, gpIIb/IIIa, etc.). In such cases, the desired end—hits and leads against multiple target families using a single synthetic route (or at least similar synthetic routes)—is ultimately achieved without the need for explicit pharmacophore pattern comparison across the target types to be screened.

  1433-7851/98/3720-2850 $ 17.50+.50/0