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Novel Antifungal Compounds Derived from Heterocyclic Positional Scanning Combinatorial Libraries

Sylvie E. Blondelle, Adel Nefzi, John M. Ostresh and Richard A. Houghten

Torrey Pines Institute for Molecular Studies, San Diego, CA, USA

Abstract: Synthetic combinatorial libraries composed of mixtures of tens thousands to millions of compounds have been shown to be useful in a wide range of biomedical research areas. The "libraries from libraries" concept was extended to the generation of heterocyclic synthetic combinatorial libraries from existing N-acylated dipeptide libraries. In particular, N-alkyl aminocyclic urea and thiourea SCLs were generated and assayed for their ability to inhibit *Candida albicans* growth. Potent individual anticandidal compounds were derived from these libraries.

INTRODUCTION

Fungal infection emergence

Life threatening infections caused by pathogenic fungi are becoming increasingly common, especially in those individuals with suppressed immune systems such as cancer patients and patients with AIDS (ref. 1). This is in part due to the emergence of strains resistant to currently available antibiotics. However, there are only a limited number of antifungal compounds available to counter such infections, which leads to a strong need to develop new classes of compounds having antifungal activities. In particular, *Candida albicans* and *Cryptococcus neoformans* are two of the most common opportunistic fungi responsible for infections. Candidiasis is the fungal infection most frequently associated with HIV-positive patients, while *Cr. neoformans* is the causative agent of cryptococcosis, which is the leading cause of morbidity and mortality due to fungi in patients with AIDS.

Sources for new antifungal compounds

Drug discovery has historically involved the synthesis of hundreds to thousands of individual analogs of a weakly active lead compound in an attempt to enhance the original activity, bioavailability, and/or selectivity, while at the same time decreasing its toxicity. In particular, large banks of known existing compounds, as well as of natural sources such as soil samples, marine waters, insects, and tropical plants (reviewed in ref. 2;3) have been the traditional sources for new lead antifungal compounds. The recent introduction of readily accessible synthetic combinatorial libraries (SCLs) has resulted in a fundamental shift in how drug discovery is

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carried out (ref. 4-9). This revolutionary concept enables hundreds to thousands of times more compounds to be synthesized and screened relative to traditional approaches. Early work from this laboratory has shown the broad utility of mixture-based SCLs for the *de novo* identification of potent analgesic compounds, highly active antimicrobial compounds, enzyme inhibitors, and highly specific antigenic determinants (ref. 10-13).

Development of synthetic combinatorial libraries

Due to existing optimized synthetic protocols for the preparation of peptides, the SCL concept was initially applied to the generation of millions of peptides (ref. 10;13). Recent advances in synthesis technologies and chemistries have led to the generation of collections of peptidomimetic and nonpeptide compounds (ref. 4;7;14). Such compounds are expected to be more suitable as oral drug candidates as compared to peptides. In particular, peptidomimetic and chemical SCLs were developed using an approach that involved the chemical transformation of existing peptide SCLs (this was termed the "libraries from libraries" approach (ref. 15-17)). For example, the chemical transformation of a resin-bound peptide library was carried out by permethylation of the amide nitrogens of the mixtures (ref. 15). The screening of this library led to the identification of a number of N-permethylated hexapeptides having antimicrobial activity selective to gram-positive bacteria (ref. 15).

The development of strategies for the synthesis of heterocyclic compounds on the solid phase is expanding as a greater understanding of how to successfully carry out such reactions is gained. For example, a large variety of heterocyclic compounds have been synthesized via imine formation or using multicomponent condensation (ref. 18-22). The "libraries from libraries" concept was therefore extended to the generation of heterocyclic SCLs including hydantoin **1**, thiohydantoin **2**, quinazolinone **3**, cyclic urea **4**, thiourea **5**, bicyclic guanidine **6**, and diazepines **7** SCLs (Fig. 1 - ref. 23;24). Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents. In particular, nitrogen heterocycles are important pharmacophores in drug design. For example, nitro-carbon heterocyclic structures are found in various therapeutic agents, including numerous antihistamines, as well as antiseptic, antiarrythmic, antirheumatic, antibiotic and other pharmaceutical compounds (ref. 25). We report here the generation and identification of N-alkyl aminocyclic ureas and thioureas having antifungal activities.

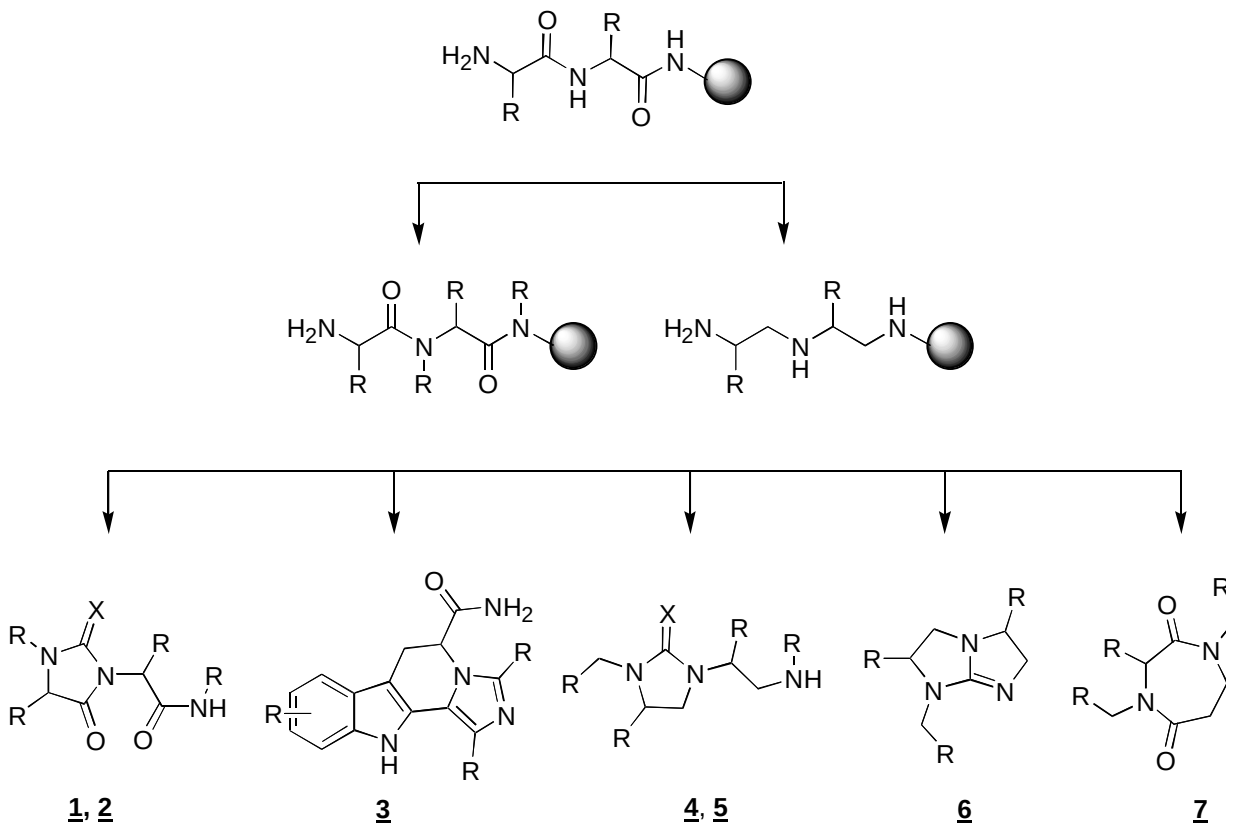

Fig. 1 Solid phase synthesis of heterocyclic compounds from modified dipeptides.

GENERATION OF N-ALKYL AMINOCYCLIC UREA AND THIOUREA SCLS

Acylated dipeptide SCLs have been used as starting materials for the synthesis of cyclic urea and thiourea SCLs as described earlier for individual control compounds (ref. 24). In brief, two key methods developed in our laboratory were used for the generation of such compounds. The first method that allowed the straightforward generation of dipeptidomimetic SCLs, used in this case as precursors, was the tritylation of resin-bound amino acids followed by a selective N-alkylation (ref. 26). Thus, N-alkylation was performed on the amide-linked resin-bound N-tritylated amino acid using lithium t-butoxide in tetrahydrofuran, followed by the addition of the alkylating agent (methyl iodide or benzyl bromide) in dimethyl sulfoxide. Following removal of the trityl protecting group with 2% trifluoroacetic acid in dichloromethane, the second amino acid was added using traditional peptide chemistry. The resulting dipeptide was acylated with one of a wide range of available carboxylic acids to obtain the acylated dipeptide. The second key step in the synthetic process of cyclic ureas was the reduction of the amide groups of the resin-bound acylated dipeptidomimetics using diborane in tetrahydrofuran at 65°C to generate a tertiary and two secondary amines (ref. 17). Cyclization to obtain the five-member cyclic ureas and cyclic thioureas was then carried out using carbonyldiimidazole and thiocarbonyldiimidazole in anhydrous dichloromethane (ref. 24). The desired products were obtained in excellent yield and purity (average 80% by HPLC) following cleavage from the resin with anhydrous hydrogen

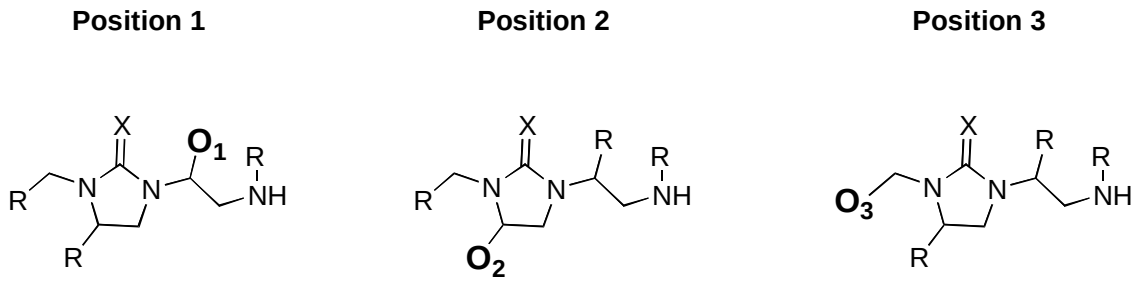

Fig. 2 Representation of the N-alkyl aminocyclic urea and thiourea positional scanning SCLs. O is defined with a single building block, R is a mixture of building block, and X=S,O.

fluoride. The cyclization step has also been successfully carried out using triphosgene or thiophosgene. The strength of this approach is that well-characterized peptide libraries can be prepared with the confidence that the yields and deconvolution approaches can also be used for the resulting heterocyclic library.

Four SCLs (N-methyl aminocyclic urea and thiourea, and N-benzyl aminocyclic urea and thiourea) were prepared in a positional scanning (PS) format, which involves the generation and subsequent screening of separate, single defined position SCLs to individually identify the most important functionalities at each position of diversity within a library (ref. 27). As shown in Fig. 2, a complete PS-SCL consists of three sublibraries, each of which has a single defined functionality at one position and a mixture of functionalities at each of the other two positions. Each of the four SCLs (ureas and thioureas) contains 118,400 compounds (40x37x80). The pooling of each sublibrary, which contain the same 118,400 compounds, varied based on the functionality at the defined position of that sublibrary. The structure of individual compounds can be determined from such a screening since each compound is present in only one mixture of each sublibrary.

IDENTIFICATION OF NOVEL ANTICANDIDAL COMPOUNDS

Each mixture of the four PS-SCLs described above was initially screened at 250µg/ml against 1-5x10^5 CFU/ml blastoconidial *C. albicans* as described earlier (ref. 28). In order to differentiate the most active mixtures from each library, the IC_{50} values were then determined for those mixtures that exhibited more than 50% inhibition at 250µg/ml. As illustrated in Fig. 3 and Table 1, different profiles in activity were observed between the four SCLs, which indicate sequence specificity for the active compounds present in the SCLs. Thus, the most active mixtures from each library were defined with building blocks that had significantly different chemical characters. For example, a 4-dimethylaminobenzoic acid group at position R_3 was an important building block only for the activity of N-alkyl aminocyclic urea mixtures, while the cyclohexanebutyric acid group was more important for the activity of N-alkyl aminocyclic thioureas (Table 1).

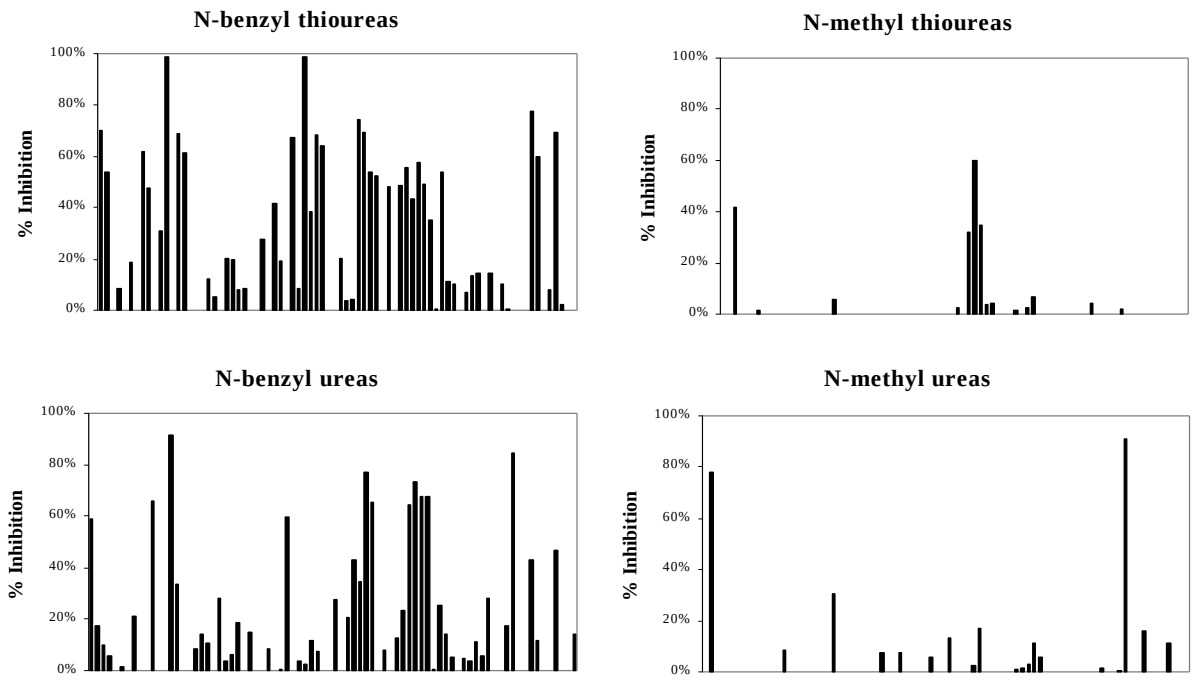

Fig. 3 Screening at 250µg/ml of the four N-alkyl aminocyclic urea and thiourea positional scanning, SCLs. Each graph represents the activity against *C. albicans* of the corresponding sublibrary defined at position 3.

TABLE 1. Comparison between the % inhibition[a] against *C. albicans* of mixtures defined with the same building block

Defined building block	Thiourea		Urea	
	N-benzyl	N-methyl	N-benzyl	N-methyl
Position 1:				
L-lysine	92	0	0	65
L-norleucine	23	84	80	100
Position 2:				
L-methionine	89	37	69	86
D-histidine	0	95	82	51
Position 3:				
p-methoxyphenylacetic acid	100	0	0	0
cyclohexanecarboxylic acid	74	60	43	0
p-dimethylaminobenzoic acid	0	0	84	91

Following the screening of the four SCLs, four sets of individual compounds were prepared. Each set corresponded to all possible combinations of the building blocks defining the most active mixtures at each position of the given SCL. These individual compounds were generated in order to confirm the screening data and determine their relative activities. Thus, a total of eight N-benzyl aminocyclic thioureas, twelve N-methyl aminocyclic thioureas, twelve N-benzyl aminocyclic ureas, and six N-methyl aminocyclic ureas were synthesized and assayed in a manner similar to the SCLs. Greater activities were found for the N-benzylated compounds relative to the N-methylated compounds (MIC values of the most active compounds varied from 8 to 64μg/ml, and 64 to 125μg/ml, respectively), with greater activities found for the thioureas versus ureas. These results show the ability to draw structure-activity relationship conclusions from the screening of any related SCLs. Two representative active compounds are shown in Fig. 4A and B. These N-benzyl aminocyclic ureas were derived from L-asparagine and D-cyclohexylalanine at position 1, L-norleucine at position 2, and cyclohexylacetic acid and p-dimethylaminobenzoic acid at position 3, respectively. Their respective MIC values against *C. albicans* were 20-30μg/ml and 30-60μg/ml.

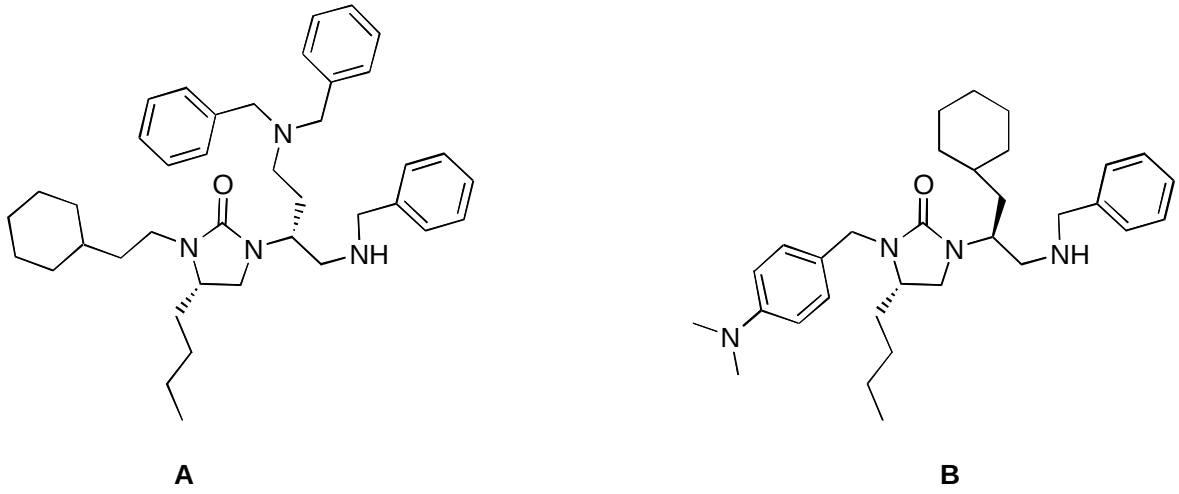

Fig. 4 Structure of two representative antifungal N-benzyl aminocyclic ureas.

IMPORTANCE OF SCLS IN BIOMEDICAL RESEARCH

One of the fundamental objectives of organic and medicinal chemistry is the design, synthesis, and production of molecules having value as human therapeutic agents. The successes of organic and medicinal chemistry have fundamentally changed human therapeutics over the past 30 to 40 years by the steady design, preparation, and improvement of compounds that affect virtually all human medical conditions. As illustrated by the studies described above, the

broadly applicable concepts and tools encompassed by combinatorial chemistry accelerate advances within synthetic organic and medicinal chemical research and discovery. It can be expected from the current successes that the ability to synthesize and densely search the molecular space of therapeutically important receptors will permit not only highly active analogs of existing pharmacophores to be identified, but through the use of combinatorial variance of synthetic reaction conditions allow the identification of entirely new synthetic approaches to novel pharmacophores. Combinatorial chemistry and synthetic approaches can then be expected to rapidly lead to more active, more specific, safer and less expensive therapeutics.

Finally, mixture-based SCL approaches as described may be considered an "optimized approach" of the identification of novel pharmacophores from natural sources. Indeed, both SCLs and natural product extracts are composed of mixtures of compounds that are directly screened without intensive initial purification. In both cases, a large number of active individual compounds were identified from such mixtures. In contrast, SCL approaches offer a number of advantages over natural product extracts: i) each individual compound is present at a close to equimolar concentration in an SCL mixture; ii) the chemical structures as well as the synthetic pathway of each compound within an SCL mixture are known; and iii) as shown in the reported studies, structure-activity relationship information can be derived from screening a given SCL.

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