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DISCOVERY OF ANTIRHINOVIRAL LEADS BY SCREENING A COMBINATORIAL LIBRARY OF UREAS PREPARED USING COVALENT SCAVENGERS

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Abstract. Solution phase parallel synthesis of equimolar mixtures of ureas was accomplished using a solid-supported "covalent scavenger" (aminomethylpolystyrene) to remove isocyanate impurities. Screening of these purified mixtures for antirhinoviral activity in a whole cell assay and subsequent deconvolution of hit mixtures afforded novel antirhinoviral agents with low cytotoxicities.

The human rhinoviruses are members of the picornavirus family, and are the primary cause of the common cold.[1] As part of our program to discover novel antirhinoviral agents,[2,3] we elected to employ combinatorial chemistry techniques for lead generation.[4,5] Combinatorial mixture synthesis and screening has proven to be a viable tool for the identification of novel leads against a variety of biological targets. Although peptide mixtures prepared using solid phase synthesis served as the springboard for early research in the field, more recently investigators have explored the synthesis and screening of mixtures of non-oligomeric "small molecules".[5] For example, both Smith and Parlow have described the utility of amide mixtures prepared by a variety of techniques for lead generation.[6,7] We recently reported on the use of solid-supported nucleophiles and electrophiles ("covalent scavengers") for the facile parallel synthesis of amine derivatives in high purity.[8] In this Letter, we disclose the application of this methodology to the discovery of novel antirhinoviral agents with low cytotoxicity.

As shown in Scheme 1 and Figure 1, a library of 4,000 ureas was prepared as 400 ten compound mixtures. For a given reaction vessel, 1.25 equiv of an isocyanate was added to a limiting amount of an equimolar mixture of 10 amines[9] in $CHCl_3$ at room temperature (Scheme 1). After approximately 3 h, 1.00 equiv of aminomethylpolystyrene (0.8 mequiv/g) was added, and agitation by shaking was performed for 3 h. The mixture was then filtered and evaporated to dryness to afford an equimolar mixture of 10 ureas for assay. In a typical run, a 10x10 grid of reaction vessels was set up to produce 1000 products (Figure 1).

Scheme 1

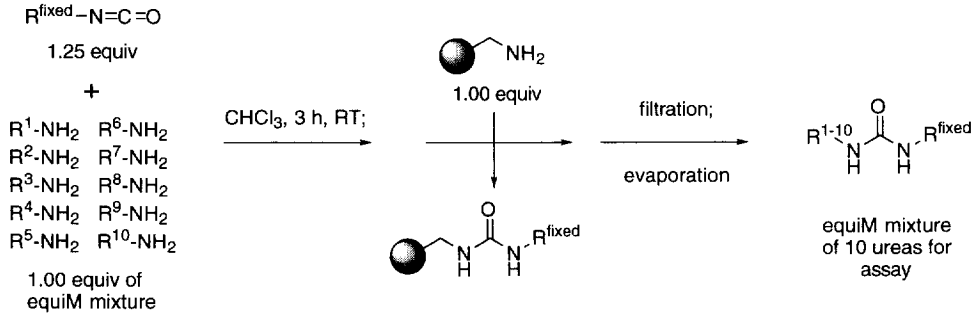
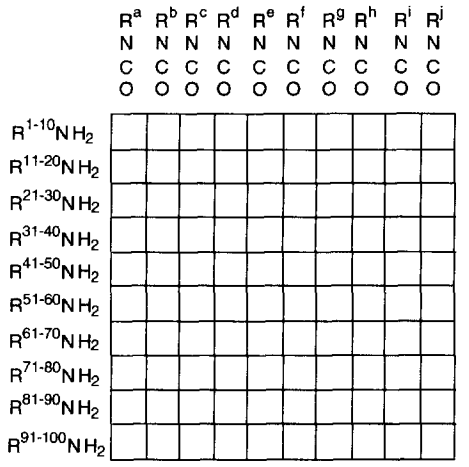
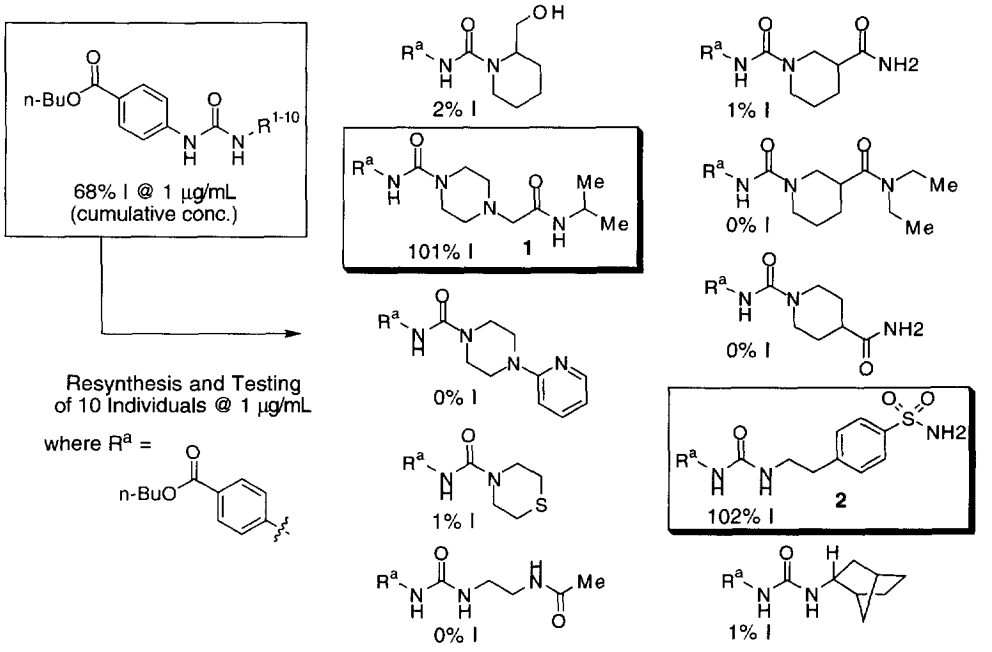


Figure 1



The urea mixtures were tested for their activity against human rhinovirus-14 (HRV-14) in cell culture.[10] Ten compound mixtures with significant antirhinoviral activity and low to moderate cytotoxicity (XTT assay) were then resynthesized as single compounds and reassayed.[11] A representative "deconvolution" experiment is shown in Scheme 2. A mixture of ten ureas produced using 4-n-butoxycarbonylphenylisocyanate provided

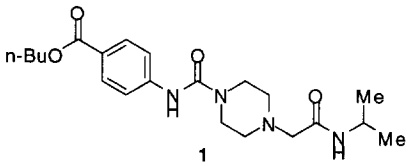
Scheme 2



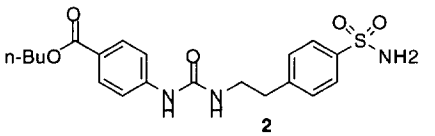
68% inhibition of HRV-14 at a cumulative concentration of 1 μg/mL. The ten ureas represented in this hit mixture were resynthesized individually using an experimental protocol analogous to that outlined in Scheme 1, and the ten products were then assayed discretely for their antirhinoviral activity.[12] Ureas **1** and **2** proved to be quite potent antivirals when tested at 1 μg/ml, whereas the remaining eight ureas were essentially devoid of activity.

Ureas **1** and **2** were resynthesized using standard synthetic procedures and were purified to homogeneity by recrystallization or column chromatography.[12-14] Comparative biological data for the original combinatorial samples and resynthesized products are depicted in Table 1. Within experimental error, the original and resynthesized samples afforded identical activities and cytotoxicities.[15]

Table 1



1



2

Product	Method of Preparation	IC50 (μM)	TC50 (μM)
1	Combinatorial	1.8 (n=1)	>40
1	Standard Chem.	2.4±0.40 (n=2)	>40
2	Combinatorial	0.69 (n=1)	34
2	Standard Chem.	0.81±0.24 (n=2)	>40

assays: RV-14 CPE/XTT whole cell

In summary, equimolar mixtures of ureas have been synthesized using covalent scavenging techniques and assayed for their ability to inhibit human rhinovirus in a whole cell assay. Validated low to subμM leads with low cytotoxicities have been identified by subsequent deconvolution of hit mixtures. Further results from the synthesis and screening of these and related mixtures against other biological targets will be reported in due course.

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References and Notes

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9. Although primary amines are depicted in Scheme 1, Figure 1, and Scheme 2 for simplicity, both primary and secondary amines were employed for urea synthesis.
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11. A liability associated with examining mixtures of compounds in whole cell assays is the enhanced probability of cellular toxicity. For this reason, resynthesis criteria for initial mixtures were relaxed to include hits with both low and moderate cytotoxicities in the XTT assay.
12. All new compounds provided satisfactory spectral data (^{1}H NMR, MS) and were homogeneous by TLC and/or HPLC.
13. Compound **1**: ^{1}H NMR (300 MHz) δ 1.05 (t, 3H), 1.22 (d, 2H), 1.43-1.80 (m, 4H), 2.68 (m, 4H), 3.12 (m, 2H), 3.62 (m, 4H), 4.18 (m, 4H), 4.37 (t, 2H), 6.77 (br s, 1H), 6.91 (br s, 1H), 7.47 (d, 2H), 8.02 (d, 2H); MS m/z 404 (MH^+ 100).
14. Compound **2**: ^{1}H NMR (300 MHz) δ 0.90 (t, 3H), 1.39 (m, 2H), 1.66 (m, 2H), 2.82 (m, 2H), 3.41 (m, 2H), 4.21 (m, 2H), 7.27 (d, J = 8.6 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 7.98, 1H), 7.84 (d, J = 8.6 Hz, 1H); MS m/z 420 (MH^+ 100).
15. At present, we are uncertain as to the exact mode of action of these inhibitors. They do not inhibit rhinovirus 3C protease (Ref. 3 and 10), and their spectrum of activities against other picornaviruses is narrower than the standard control, Enviroxime (Ref. 2). Interestingly, these inhibitors bear some resemblance to previously reported capsid binding inhibitors: Diana, G. D.; Nitz, T. J.; Mallamo, J. P.; Tresurywala, A. *Antiviral Chem. Chemother.* **1993**, *4*, 1.

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