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Received March 26, 2003

Five sets of 27-membered combinatorial libraries of alicyclic β-lactams were prepared *via* liquid-phase Ugi 4-center 3-component reactions (U-4C-3CR) utilizing 3 different *cis* β-amino acids, 3 different isonitriles and 5x3 sets of aldehydes. Through combinations of the building blocks of one of these libraries, all of the possible sublibraries were also generated. A few azetidinone derivatives were synthesized individually by parallel synthesis.

J. Heterocyclic Chem., **40**, 951 (2003).

In recent years, the β-amino acids have achieved importance as building blocks in the synthesis of β-lactam pharmacophores, and as segments in peptidic natural products with various biological activities [1]. Their synthesis, in either racemic or optically pure form, has therefore become an important challenge for organic chemists [2].

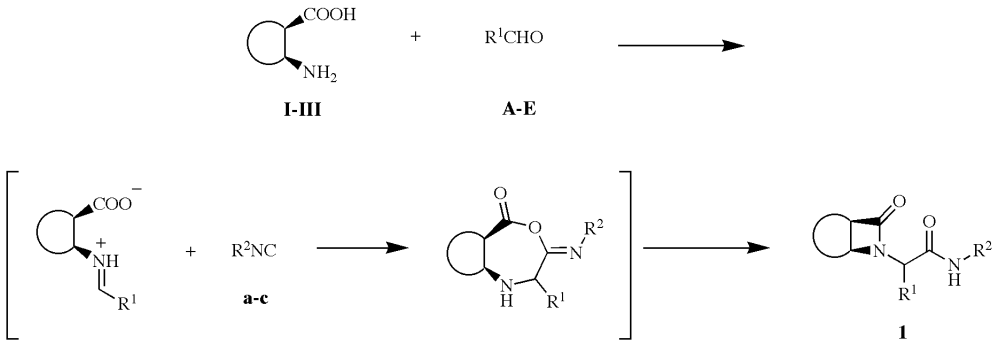
Combinatorial chemistry is a highly efficient tool in current drug discovery, yielding hundreds to thousands times more compounds with minimal time and effort as compared with classical chemistry [3]. *Via* multicomponent condensation reactions such as isocyanide-based multicomponent reactions, more complex natural product-like structures leading to libraries can be generated in fewer steps than was previously possible [4].

The traditional Ugi reaction, which combines a carboxylic acid, an amine, a carbonyl compound and an isocyanide in a one-pot condensation to yield an α-*N*-acylaminoamide, has gained considerable importance in drug discovery, *e.g.* in the synthesis of lactams [5,6], benzodiazepines [7,8], piperazines [7,9], morpholines [10], α-sulfonylamino amides [11] and other derivatives [12].

In previous work, a small (6-membered) combinatorial library of *cis* β-lactam derivatives was successfully designed in the liquid-phase *via* the Ugi 4-center 3-component reaction (U-4C-3CR) (Scheme 1) [5]. In this modified Ugi reaction, the cyclic *cis* β-amino acids as bifunctional reagents were smoothly transformed into the azetidinone derivatives in moderate to good yields. This prompted us to create a larger collection of bicyclic β-lactams which can be potential anti-inflammatory compounds or promising serine protease inhibitors [13].

In our present work we planned to create a Rubik's cube type of solution-phase libraries with 3x3x3 components in each. The diversity of the final Ugi products in one library set was increased at the aldehyde constituent, since aldehydes are available commercially in great structural variety. Figure 1 shows the selected aromatic and aliphatic aldehydes, divided into 5 sets (**A-E**). Sets **A-E** were reacted in the Ugi reaction as follows. One set of aldehydes, 3 isocyanides (cyclohexyl (**a**), *tert*-butyl (**b**), and benzyl isocyanide (**c**)) and 3 cyclic β-amino acids (*cis*-2-aminocyclohexane- (**I**) [14], *cis*-2-aminocyclopentane- (**III**) [15], and *cis*-2-aminocyclohex-4-enecarboxylic acid (**II**) [16]) were added to a vessel in equimolar amounts and the mixture was stirred in MeOH at room temperature for 3 days. The solution-phase libraries were purified by column

Scheme 1



Formation of azetidinone ring **1** from cyclic β-amino acids (**I-III**), which supply the carboxylic and amino functions in the Ugi 4-center 3-component reaction (U-4C-3CR).

chromatography and the products were collected in one pot with CH_2Cl_2:MeOH (97:3) as eluent. After evaporation of the solvent, the 27 diversely substituted derivatives of one set were submitted to electro spray ionization mass spectral (MS) analysis to determine the success of library generation. According to the MS analysis, the synthesis was effective in most cases. All expected molecular ions were detected in the mixture when aromatic aldehydes were used in the Ugi reaction. Only one set of β-lactams generated from aliphatic aldehydes (**E**) failed to provide the correct $M+H^+$.

From the sublibraries of aldehydes **2** and **3**, not all of the components were missing. These products should have been formed, because aliphatic aldehydes are more reactive than the aromatic ones in the Ugi reaction [5]. In order to prove this fact, 6 individual products **1a-f** were synthesized in liquid-phase Ugi reactions in a parallel fashion,

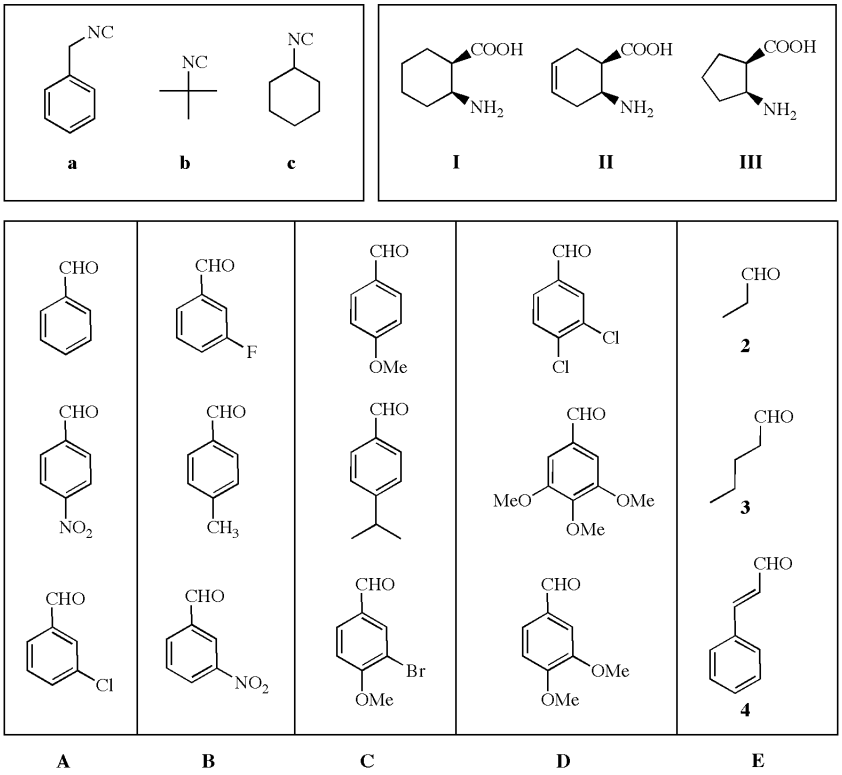


Figure 1. Building blocks of 3x3x3-membered mixture-based Ugi libraries generated from 5 sets of aldehydes (**A-E**), 3 β-amino acids (**I-III**) and 3 isocyanides (**a-c**).

In order to find the reason why several compounds were absent from the above-mentioned main library, we created 12 sublibraries, combining the components of aldehyde sets **C** and **E** with the building blocks of β-amino acids **I-III** and isocyanides **a-c**. Three sublibraries of β-amino acids with 6x1x3 components in each, 3 sublibraries of isocyanides with 6x3x1 components in each, and 6 sublibraries of aldehydes with 1x3x3 components in each, were synthesized similarly to the positional scanning deconvolution approach [18]. Each of the 3 positional sublibraries contains exactly the same diversity of β-lactams. The mixture-based sublibraries were purified by column chromatography and the products were collected in one pot with CH_2Cl_2:MeOH (97:3) as eluent. Some Ugi products derived from propionaldehyde and valeraldehyde were found by MS analysis to be missing from certain sublibraries (**a**, **b**, **c**, **III**, **2** and **3**).

starting from propionaldehyde and valeraldehyde (Figure 2). These reactions were carried out in MeOH, with stirring of the mixture at room temperature for 24 h. The compounds were successfully purified by column chromatography using a less polar solvent mixture of CH_2Cl_2:MeOH, 99:1 as eluent than in the cases of the libraries resulting in the β-lactam derivatives **1a-f** in moderate to good yields. It should be noted that in all cases the U-4C-3CR leads to the formation of a new stereogenic center at position C2 of the acetamido group in the final products. This means that each the components consist of 2 diastereomers.

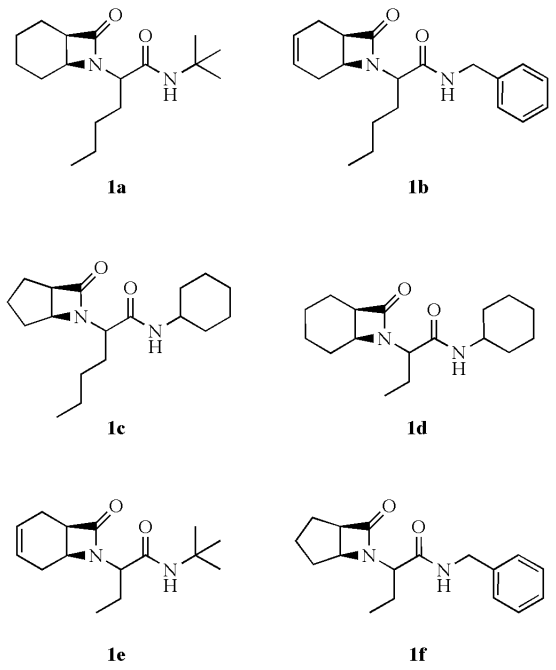


Figure 2. Products synthesized in parallel liquid-phase Ugi reactions.

Considering the polarity of the aliphatic side-chain derived from the aldehydes, we assumed that those library products were eluted off with the unreacted isocyanides during the purification process. Hence, the reactions of 1x3x3-membered sublibrary of valeraldehyde (**3**) with β-amino acids (**I-III**) and isocyanides (**a-c**) were repeated. In column chromatography the products were eluted with a less polar solvent mixture of CH_2Cl_2:MeOH, 99:1. The accurate masses of the purified library components were measured by MS, with detection of their protonated molecular ions ($M+H^+$). This confirmed our expectation that all of the components were present in the mixture.

The combination of an MCR (multicomponent reaction) with a subsequent secondary reaction is a very powerful concept. Therefore, acid-catalysed solvolysis of the azetidinone ring in the case of one 27-membered library generated from aldehyde set **D** was also carried out in the presence of water or EtOH, resulting in the corresponding carboxylic acid or the ethyl ester derivatives, respectively (Scheme 2). After evaporation of the solvents, its MS analysis gave the correct molecular masses.

In the course of this study, 135 *cis*-β-lactam derivatives were synthesized efficiently in 5 sets of mixture-based libraries *via* the U-4C-3CR by reacting *cis* β-amino acids, aldehydes and isocyanides. The 5 main libraries were submitted to biological screenings, *e.g.* to antiproliferative, elastase inhibition and plasma membrane glycoprotein inhibition assays. Two of the main libraries (generated from aldehyde sets **C** and **E**) exhibited promising inhibitory effects on proliferation. Since the positional scanning deconvolution approach is a convenient technique with which to determine the biologically most active components of the libraries, the sublibraries were also submitted to biological screening. In contrast to our expectations, they did not exert antiproliferation effect. In conclusion, the pronounced effects in the main libraries are presumed to be consequences of super-additive effects of the 27 compounds.

EXPERIMENTAL

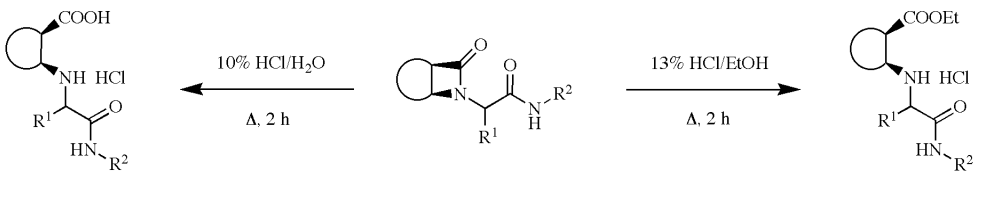
^1H nmr spectra were recorded at 400 MHz and ^{13}C nmr spectra at 100 MHz, in deuteriochloroform at ambient temperature on a Bruker AM 400 spectrometer. Chemical shifts are given in δ (ppm) relative to tetramethylsilane (deuteriochloroform) as internal standards. Elemental analyses were performed with a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer.

The molecular masses of compounds were determined by electro spray ionization, using a Bruker (Bruker Daltonics, Billerica, USA) BioAPEX 47e Fourier transform ion cyclotron resonance mass spectrometer equipped with a 4.7 Tesla, 160-mm bore superconducting magnet, an Infinity cell and an electro spray ion source (Analytica of Brandford Inc., Brandford, CT, USA). The solutions of library sample mixtures were continuously introduced into the interface sprayer through a glass microlitre syringe at a flow rate of 40 μl/h under atmospheric pressure. The measurements were performed in a 50:50:1 mixture of MeOH:water:AcOH, using the broad-band mode with a resolution of ≥ 25000. Under these conditions, only protonated molecular ions were observed.

General Procedure for the Preparation of 27-Membered Libraries.

Three different types of aldehydes (5 mmol of each) and 3 cyclic β-amino acids: *cis*-2-aminocyclohexane- (**I**) (0.71 g,

Scheme 2



5 mmol), *cis*-2-aminocyclopentane- (**III**) (0.64 g, 5 mmol), and *cis*-2-aminocyclohex-4-enecarboxylic acid (**II**) (0.70 g, 5 mmol) were dissolved in MeOH (20 ml), and the solution was kept at room temperature for 1 h. Cyclohexyl (**a**) (0.54 g, 5 mmol), *tert*-butyl (**b**) (0.41 g, 5 mmol), and benzyl isocyanide (**c**) (0.58 g, 5 mmol) were then dissolved in a small amount of MeOH and added to the previous solution. The resulting mixture was stirred at room temperature for 3 days at room temperature. The reaction was followed by TLC on silica gel plates (CH_2Cl_2:MeOH, 97:3). When the aldehyde spot disappeared, the solvent was evaporated and the residue was purified by column chromatography on silica gel. The unreacted isonitrile was eluted with CH_2Cl_2:MeOH (97:3), and all of the compounds were collected in one vessel.

Hydrolysis of Library Generated from Aldehyde Set D.

(**5**). A 0.2 g sample of the library generated from aldehyde set **D** was dissolved in 10% aqueous HCl (10 ml) and refluxed for 2 h. When the reaction was completed, the solvent was evaporated off and the residue was crystallized from Et_2O.

(**6**). A 0.2 g sample of the library generated from aldehyde set **D** was dissolved in 13% HCl/absolute EtOH (10 ml) and refluxed for 2 h. The solvent was evaporated and the residue was crystallized from Et_2O.

General Procedure for the Preparation of Sublibraries.

The aldehydes and the β-amino acids were dissolved in MeOH, and the solution was allowed to stand at room temperature for 1 h. The isonitriles were dissolved in a small amount of MeOH and added to the mixture of aldehydes and isonitriles. The solution was stirred for 3 days at room temperature. The reaction was followed by TLC on silica gel plates (CH_2Cl_2:MeOH, 97:3). When the aldehyde spot disappeared, the solvent was evaporated and the residue was purified by column chromatography on silica gel. The unreacted isonitrile was eluted with CH_2Cl_2:MeOH (97:3). All of the compounds were collected in one vessel.

General Procedure for Individual U-4C-3CR (**1a-f**).

The β-amino acid (1.2 mmol, 1.2 equiv) and aldehyde (1.0 mmol, 1.0 equiv) were dissolved in 5 ml of MeOH. The solution was allowed to stand at room temperature for 30 min. The isonitrile (1.0 mmol, 1.0 equiv) was added and the resulting mixture was stirred at room temperature for 1 day. When the reaction was indicated by TLC on silica gel plates (CH_2Cl_2:MeOH, 99:1) to be complete, the solvent was evaporated and the residue was purified by column chromatography on silica gel, the product being eluted with 1% MeOH in CH_2Cl_2.

N-*tert*-Butyl 2-Butyl-2-*cis*-(8-oxo-7-azabicyclo[4.2.0]oct-7-yl)-acetamide (**1a**).

Diastereomeric ratio of purified product: 4:1, yield: 56%; R_f on silica gel plates: 0.59 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.90 (t, J = 7.0, 3H, CH_2CH_3) 1.34 (s, 9H, 3xCH_3), 1.37-2.0 (m, 14H, 7xCH_2) 3.19 (m, 1H, CH_2CHCO), 3.85 (m, 1H, CHN), 3.92 (t, J = 7.6, 1H, NCHCO), 6.45 (s, 1H, NH); minor diastereomer: δ 0.90 (t, J = 7.0, 3H, CH_2CH_3) 1.34 (s, 9H, 3xCH_3), 1.37-2.0 (m, 14H, 7xCH_2) 3.19 (m, 1H, CH_2CHCO), 3.61 (t, J = 7.8, 1H, NCHCO), 3.85 (m, 1H, CH_2CHN), 7.00 (s, 1H, NH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 14.47, 17.83, 19.56, 20.43, 22.90, 24.29, 28.59, 29.06, 29.16, 30.24, 46.66, 51.17, 51.89, 57.70, 61.35, 169.80, 172.31; minor diastereomer: δ 14.67, 17.16, 19.07, 20.23, 22.84, 23.84, 29.19, 29.90, 31.59, 46.73, 51.82, 53.18, 170.57, 172.14; ms (electro spray ionization): *m*/*z* 295.18 ($M+H^+$, 100).

Anal. Calcd. for $C_{17}H_{30}N_2O_2$: C 69.35, H 10.27, N 9.51 Found: C 69.52, H 10.04, N 9.05.

N-Benzyl 2-Butyl-2-*cis*-(8-oxo-7-azabicyclo[4.2.0]oct-3-en-7-yl)acetamide (**1b**).

Diastereomeric ratio of purified product: 2:1, yield: 57%; R_f on silica gel plates: 0.5 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.89 (m, 3H, CH_3) 1.25-1.36 (m, 4H, 2xCH_2), 1.70-2.10 (m, 4H, 2xCH_2) 2.36-2.60 (m, 2H, CH_2), 3.29 (m, 1H, CH_2CHCO), 3.98 (m, 1H, CH_2CHN), 4.05 (m, 1H, NCHCO), 4.35 (m, 2H, $NHCH_2C$), 5.38 (m, 1H, CHCH), 5.72 (m, 1H, CHCH), 7.09 (m, 1H, NH), 7.25 (m, 5H, 5xCH); minor diastereomer: δ 0.89 (m, 3H, CH_3) 1.25-1.36 (m, 4H, 2xCH_2), 1.70-2.10 (m, 4H, 2xCH_2) 2.36-2.60 (m, 2H, CH_2), 3.29 (m, 1H, CH_2CHCO), 3.82 (m, 1H, NCHCO), 3.98 (m, 1H, CH_2CHN), 4.35 (m, 2H, $NHCH_2C$), 5.52 (m, 1H, CHCH), 5.72 (m, 1H, CHCH), 7.25 (m, 5H, 5xCH), 7.47 (m, 1H, NH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 14.36, 21.52, 22.67, 25.43, 28.48, 29.06, 43.95, 46.86, 51.13, 53.18, 56.47, 59.25, 124.90, 126.84, 127.83, 128.20, 129.01, 138.45, 170.44, 170.89; minor diastereomer: δ 21.63, 22.66, 25.75, 31.42, 43.78, 124.98, 126.81, 127.74, 128.09, 128.95, 138.60, 170.82; ms (electro spray ionization): *m*/*z* 327.22 ($M+H^+$, 100).

Anal. Calcd. for $C_{20}H_{26}N_2O_2$: C 73.59, H 8.03, N 8.58. Found: C 73.62, H 8.39, N 8.27.

N-Cyclohexyl 2-Butyl-2-*cis*-(7-oxo-6-azabicyclo[3.2.0]hept-6-yl)acetamide (**1c**).

Diastereomeric ratio of purified product: 11:1, yield: 52%; R_f on silica gel plates: 0.6 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.90 (m, 3H, CH_3) 1.17-2.09 (m, 22H, 11xCH_2), 3.44 (m, 1H, CH_2CHCO), 3.71 (m, 1H, NHCH) 3.92 (1H, m, NCHCO) 4.08 (t, J = 4.2, 1H, CHN), 6.49 (d, J = 7.7, 1H, NH); minor diastereomer: δ 0.90 (m, 3H, CH_3) 1.17-2.09 (m, 22H, 11xCH_2), 3.44 (m, 1H, CH_2CHCO), 3.71 (m, 1H, NHCH) 3.92 (m, 1H, NCHCO) 4.08 (t, J = 4.2, 1H, CHN), 6.96 (d, J = 6.8, 1H, NH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 14.51, 22.93, 23.59, 25.29, 25.33, 25.64, 26.08, 28.55, 28.77, 28.98, 33.32, 33.46, 48.93, 54.66, 57.17, 58.49, 169.60, 170.93; minor diastereomer: δ 22.86, 23.37, 25.47, 29.18, 31.62, 48.71, 54.45, 60.19, 60.85; ms (electro spray ionization): *m*/*z* 307.20 ($M+H^+$, 100).

Anal. Calcd. for $C_{18}H_{30}N_2O_2$: C 70.55, H 9.87, N 9.14. Found: C 70.19, H 9.52, N 8.85.

N-Cyclohexyl 2-Ethyl-2-*cis*-(8-oxo-7-azabicyclo[4.2.0]oct-7-yl)acetamide (**1d**).

Diastereomeric ratio of purified product: 3:1, yield: 42%; R_f on silica gel plates: 0.54 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.96 (m, 3H, CH_3) 1.10-2.10 (m, 18H, 9xCH_2), 3.20 (m, 1H, CH_2CHCO), 3.74 (m, 1H, NHCH), 3.86 (m, 2H, NCHCO, CHN), 6.54 (d, J = 7.2, 1H, NH); minor diastereomer: δ 0.96 (m, 3H, CH_3) 1.10-2.10 (m, 18H, 9xCH_2), 3.20 (m, 1H, CH_2CHCO), 3.62 (t, J = 7.8, 1H, NCHCO), 3.74 (m, 1H, NHCH), 3.86 (m, 1H, CHN,) 7.16 (d, J = 7.5, 1H, NH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 11.59, 17.85, 19.60, 20.41, 22.63, 24.25, 25.28, 26.08, 33.35, 33.42, 33.50, 46.69, 48.86, 51.26, 59.12, 169.69, 172.45; minor diastereomer: δ 17.24, 19.16, 20.23, 23.90, 25.39, 30.27,

46.63, 46.74, 48.69, 53.33, 62.27, 170.37, 172.36; ms (electro spray ionization): *m/z* 293.18 ($M+H^+$, 100).

Anal. Calcd. for $C_{17}H_{28}N_2O_2$: C 69.83, H 9.65, N 9.58. Found: C 70.16, H 9.24, N 9.79.

N-tert-Butyl 2-Ethyl-2-*cis*-(8-oxo-7-azabicyclo[4.2.0]oct-3-en-7-yl)acet-amide (**1e**).

Diastereomeric ratio of purified product: 2:1, yield: 45%; R_f on silica gel plates: 0.58 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.92-0.98 (m, 3H, CH_2CH_3) 1.32 (s, 9H, 3xCH_3), 1.92-2.01 (m, 2H, CH_2CH_3) 2.13 (m, 2H, CH_2CHCO), 2.50 (m, 2H, CH_2CHN), 3.36 (t, J = 6.1, 1H, CH_2CHCO), 3.84 (m, 1H, NCHCO) 4.02 (m, 1H, CH_2CHN), 5.70 (m, 1H, CHCH), 5.85 (m, 1H, CHCH), 6.39 (s, 1H, NH); minor diastereomer: δ 0.92-0.98 (m, 3H, CH_2CH_3) 1.32 (s, 9H, 3xCH_3), 1.71 (m, 2H, CH_2CH_3) 2.13 (m, 2H, CH_2CHCO), 2.50 (m, 2H, CH_2CHN), 3.36 (t, J = 6.1, 1H, CH_2CHCO), 3.50 (t, J = 7.6, 1H, NCHCO) 4.02 (m, 1H, CH_2CHN), 5.70 (m, 1H, CHCH), 5.85 (m, 1H, CHCH), 6.67 (s, 1H, NH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 11.54, 21.59, 22.00, 25.57, 29.08, 46.83, 51.00, 51.79, 53.26, 58.94, 62.28, 125.37, 126.58, 169.43, 170.88; minor diastereomer: δ 11.62, 21.76, 25.20, 25.76, 46.88, 51.69, 126.79, 170.06, 170.29; ms (electro spray ionization): *m/z* 265.11 ($M+H^+$, 100).

Anal. Calcd. for $C_{15}H_{24}N_2O_2$: C 68.15, H 9.15, N 10.60. Found: C 68.47, H 9.32, N 10.31.

N-Benzyl 2-Ethyl-2-*cis*-(7-oxo-6-azabicyclo[3.2.0]hept-6-yl)acetamide (**1f**).

Diastereomeric ratio of purified product: 3:1, yield: 65%; R_f on silica gel plates: 0.53 (toluene-MeOH, 4:1). ^{1}H nmr (deuteriochloroform): major diastereomer: δ 0.91-0.95 (m, 3H, CH_3) 1.10-2.10 (m, 8H, 4xCH_2), 3.37 (m, 1H, CH_2CHCO), 3.91 (t, 1H, J = 7.6, NCHCO) 4.05 (t, J = 4.1, 1H, CH_2CHN), 4.40 (d, J = 6.0, 2H, NHCH_2C), 7.26 (m, 5H, 5xCH); minor diastereomer: δ 0.91-0.95 (m, 3H, CH_3) 1.10-2.10 (m, 8H, 4xCH_2), 3.37 (m, 1H, CH_2CHCO), 3.82 (t, J = 7.7, 1H, NCHCO) 4.10 (t, J = 4.1, 1H, CH_2CHN), 4.44 (d, J = 6.0, 2H, NHCH_2C), 7.26 (m, 5H, 5xCH); ^{13}C nmr (deuteriochloroform): major diastereomer: δ 11.39, 22.63, 23.28, 25.44, 28,41, 43.97, 54.60, 58.41, 58.51, 127.99, 128.26, 129.07, 138.59, 170.37, 170.85; minor diastereomer: δ 11.48, 23.21, 24.98, 25.20, 29.15, 43.82, 54.41, 59.95, 60.84, 127.85, 128.11, 129.19, 170.37, 170.85; ms (electro spray ionization): *m/z* 287.12 ($M+H^+$, 100).

Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C 71.30, H 7.74, N 9.78. Found: C 71.77, H 7.48, N 9.43.

Cell Culture.

HeLa cells (human cervix carcinoma) were obtained from the American Type Culture Collection (ATCC), while the multidrug resistant subline (MCF-7/ADR (breast adenocarcinoma, human) was a gift of Dr. J. Carmichael (University of Nottingham, UK) and were originally obtained from the laboratory of Dr. K. Cowan (NCI, Bethesda, MD, USA). The cells were cultured at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air, in Minimal Essential Medium (Gibco BRL, Paisley, UK) with Earle's salts containing L-glutamine supplemented with non-essential amino acids, penicillin G, streptomycin and amphotericin B.

Antiproliferative Assay.

Cells were seeded onto 96-well tissue culture plates at 5000 cells/well. After an overnight incubation, libraries were added and the cells incubated for another 3 days at 37 °C. Cell proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Sigma, St. Loius, MO) reduction assay. Thus, MTT was dissolved in PBS (0.01 *M*, pH 7.4) and added to the cells (20 µl/well). After an incubation at 37 °C for 4 hours, the amount of the formazan formed was extracted with 100 µl of DMSO and assayed by measuring the absorption at 550 nm using a microplate reader. Results were expressed as the percentage of cell proliferation with respect to control (*i.e.*, cells without treatment).

Elastase Inhibition Assay.

The elastase-inhibiting capacity of the libraries was determined by EnzChek™ Elastase Assay kit (Molecular Probes, Leiden, The Netherlands) using 96-well microtiter plates according to the instructions of the manufacturer. The final concentrations of pig pancreas elastase (enzyme) and bovine DQ elastase (substrate) was 0.1 U/ml and 25 µg/ml, respectively. N-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (10 µM) was used as a positive control. The enzymatic reaction was followed for one hour by detecting the fluorescence at 485/530 nm (excitation/emission) using a Biotek FL600 microplate fluorescence reader. Results were expressed as the percentage of enzyme inhibition with respect to control (*i.e.*, no inhibitor added).

Plasma Membrane Glycoprotein Inhibition Assay.

MCF7/ADR cells were seeded in 96-well microplates (40000/well) and preincubated overnight. Afterwards, the libraries were added together with rhodamine 6G (0.3 µM) (Sigma, St. Louis, MO) and incubated for 2 h. Reserpine (20 µM) (Sigma, St. Louis, MO) was used as a positive control. After washing three times with ice-cold buffer, the cells were treated with 100 µl of phenol-free trypsine (Gibco BRL, Paisley, UK) and 100 µl of 4 % sodium dodecyl sulfate (SDS) solution. Fluorescence was measured at 530/590 nm, and the results were expressed as the ratio of fluorescence of treated versus control (treated with rhodamine only) cells.

Acknowledgement.

The authors acknowledge financial support from OTKA (Grant TS 04888).

REFERENCES AND NOTES

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