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Synthesis and diversity analysis of lead discovery piperazine-2-carboxamide libraries

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Summary

A Lead Discovery Library of piperazine-2-carboxamide derivatives was produced for general screening. This paper discloses two novel solid phase synthetic routes used to produce 15 000 single compounds via the Irori directed sorting technique. Computational methods such as reagent clustering and library profiling were used to maximize reagent diversity and optimize pharmacokinetic parameters. The results of a four center pharmacophore analysis revealed the added diversity gained by using two independent synthetic routes.

Abbreviations: BAL, backbone amide linker; HBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; DCM, dichloromethane; DIEA, diisopropylethylamine; Fmoc, fluorenylmethoxycarbonyl; Alloc, allyloxycarbonyl; DMF, dimethyl formamide; NMM, N-methyl morpholine; EDC, N,N-dimethylaminopropyl-ethylcarbodiimide hydrochloride; NMP, N-methyl pyrrolidinone; DIC, diisopropylcarbodiimide; DMAP, N,N-dimethylamino pyridine; NACC, number of hydrogen bond acceptors; NDON, number of hydrogen bond donors; MW, molecular weight; PfP, pentafluorophenol; HPLC, high pressure liquid chromatography; MS, mass spectrometry; ELS, evaporative light scattering; THF, tetrahydrofuran.

Introduction

Combinatorial library synthesis and screening is accelerating the discovery of lead compounds in the pharmaceutical industry [1]. The Irori directed sorting approach combines the benefits of parallel synthesis with the additional advantage of the speed associated with the mix and split method [2,3]. Thus discrete compound libraries in multimilligram quantities with full identification of each member of the library are synthesized with high efficiency [4]. This system has the capacity to produce single compounds in the tens of thousands range with a production time of one to two months. We have applied this method to the synthesis of piperazine-2-carboxamide derivatives and have synthesized over 15 000 discrete compounds.

The piperazine-2-carboxylic acid scaffold is a pharmacologically important [5] center core found in Angiotensin II antagonist [6] **1**, substance P antagonist [7] **2** and the aspartyl protease inhibitor Indinavir **3** [8]. Libraries built around this core scaffold are expected to be of general interest for high throughput screening.

Two novel solid phase approaches to piperazine-2-carboxamide derivatives are presented in this paper. These methods show distinct advantages over previously disclosed methods, which involved a combination of solution and solid phase chemistry [9] or encoded mix and split technology [10]. The synthetic route disclosed here is very general and allows for the production of a large number of discrete compounds in high purity.

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Experimental

General information

Chloromethyl polystyrene beads of 150–300 μM (loading 2 mmol/g) and Wang resin beads 150–300 μM (loading 1.7 mmol/g) were purchased from Polymer Laboratories. 4-Hydroxy-2,6-dimethoxybenzaldehyde was purchased from PerSeptive Biosystems. BAL resin was prepared according to the published procedure [13]. A loading of 0.8 mmol/g was determined by loading 4-bromobenzylamine to the resin and using elemental analysis. Rink amide resin was purchased from Irori^{TM}. All other reagents were purchased from standard commercial sources and used without further purification. Solvents used were EM Science of OmniSolv distilled grade unless specified otherwise. ^{1}H NMR and ^{13}C NMR spectra were recorded in 5 mm tubes on a 300 MHz Bruker ARX spectrometer in $CDCl_3$ unless otherwise stated. Mass spectra were recorded on Finnigan 4500 EI and Sciex API 3 IS spectrometers.

The libraries were constructed using the Irori^{TM} system. For the 10 000 member library, MicroKans^{TM} were filled with BAL resin by suspending the resin in an isobuoyant suspension (DMF:DCE–2:1) and dispensing the suspension with a Packard Multiprobe liquid handler. For the 5000 member library, resin bound scaffold **18** was prepared in bulk and loaded into the MicroKans^{TM} using the method described above. All reactions involving MicroKans^{TM} were performed in round bottom flasks equipped with overhead stirrers. The Autosort^{TM} 10K was used to sort the MicroKans^{TM} between combinatorial steps and cleavage of the library compounds was effected in the Accucleave^{TM} 96.

1-Alloc-4-Boc-piperazine-2-carboxylic acid **(5)**

Piperazine-2-carboxylic acid dihydrochloride (Acros) (10.0 g, 49.23 mmol) was dissolved into a 1:1 dioxane/water solvent system (320 ml). 50% Aqueous sodium hydroxide was added to bring the pH to 11. BOC-ON (15.59 g, 63.32 mmol) was dissolved into dioxane (80 ml) and added dropwise while maintaining the pH at 11 with 50% aqueous NaOH. The reaction was stirred overnight at RT. For workup, the reaction solution was extracted with diethyl ether (5 × 250 ml) and acidified to pH 2 with concentrated hydrochloric acid. The bis-protected compound was then extracted out with ethyl acetate (4 × 200 ml). The aqueous acidic solution was basified to pH 9.5 with 50% NaOH solution. This was cooled in an ice bath. Allyl chloroformate (6.1 ml, 57.10 mmol) was added portionwise by syringe while maintaining the pH at 9.5 with 50% NaOH solution. The ice bath was removed after the addition was complete and the reaction was allowed to warm to RT with stirring overnight. For workup, the basic solution was extracted with diethyl ether (4×). It was then acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate (4×). The combined ethyl acetate extracts were dried over anhydrous magnesium sulfate, filtered and concentrated. The resulting oil was placed on a high vacuum line to dry. 9.18 g (59% yield) of **(5)** as a viscous yellow oil was obtained. ^{1}H NMR δ 5.90 (m, 1H), 5.25 (m, 2H), 4.70 (m, 1H), 4.60 (m, 3H), 3.90 (m, 2H), 3.25 (m, 1H), 3.07 (dd, 1H), 2.80 (m, 1H), 1.45 (s, 9H), MS (ESI) m/z = 315 $[M+H]^{+}$.

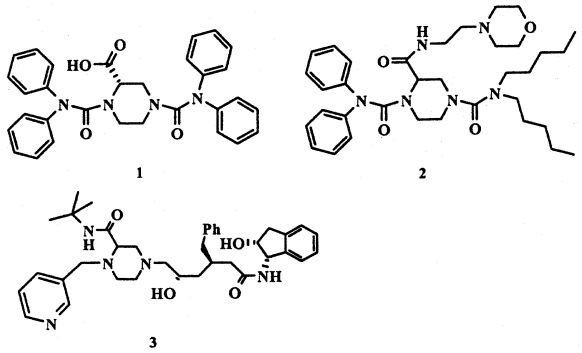


Figure 1. Examples of pharmacologically active piperazine-2-carboxamide derivatives.

*1-Alloc-4-Fmoc-piperazine-2-carboxylic acid (***6***)*

1-Alloc-4-Boc-piperazine-2-carboxylic acid (9.10 g) was dissolved into dichloromethane (100 ml) and trifluoroacetic acid (100 ml) was added. The reaction was stirred at RT for 2.5 h. It was concentrated down and the residual TFA was azeotroped off with toluene. The resulting white solid was dried on a high vacuum line. The white solid was dissolved into water (70 ml). Sodium carbonate (6.94 g, 65.50 mmol) was added slowly with stirring. Dioxane (40 ml) was added and the reaction solution was cooled in an ice bath. Fmoc-Cl (6.76 g, 26.20 mmol) was added all at once. This solution was stirred at 0 °C for 5 h. The ice bath was removed and the reaction was allowed to warm to RT with stirring overnight. For workup, the reaction was diluted with water (400 ml) and extracted with diethyl ether (4×). The aqueous solution was acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate (4×). The combined ethyl acetate extracts were dried over anhydrous magnesium

sulfate, filtered and concentrated. The resulting oil was placed on a high vacuum line to dry. 12.66 g (59% overall yield from piperazine-2-carboxylic acid) of the title compound **6** was obtained as a viscous yellow oil with a purity of 96% by HPLC analysis. ^{1}H NMR δ 7.73 (d, 2H), 7.53 (m, 2H), 7.37 (t, 2H), 7.30 (m, 2H), 5.89 (m, 1H), 5.22 (m, 2H), 4.80 (d, 1H), 4.60 (m, 3H), 4.43 (bs, 2H), 4.19 (m, 1H), 3.95 (m, 2H), 3.18 (m, 2H), 2.90 (m, 1H); ^{13}C NMR δ 177.5, 155.6, 143.7, 141.4, 132.2, 127.8, 127.2, 124.9, 120.0, 118.2, 68.0, 67.0, 66.8, 54.0, 53.6, 47.0, 44.2, 42.7, 40.9, 40.5; MS (APCI) m/z = 437 $[M+H]^+$.

Resin bound amines **(8)**

For each amine, 250 MicroKans™ (each MicroKan™ contained 12 mg of 0.8 mmol/g loaded BAL resin **7**) were placed into a 1.0 L 3-necked round bottom flask fitted with an overhead stirrer. The resin in the MicroKans™ was swelled in a 1% acetic acid in DMF solution (300 ml). The air bubbles in the MicroKans™ were removed by placing the round bottom flask under house vacuum. The amine (20.0 mmol) and sodium triacetoxyborohydride (4.24 g, 20.0 mmol) were added sequentially. The reaction was stirred at RT for 6.5 h. For workup, each reaction was individually drained and washed with DMF (1×). All of the MicroKans™ were then combined and washed with 1:1 DMF/MeOH (2×), DMF (2×), DCM (3×) and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Resin bound amides **(9)**

The 4500 MicroKans™ containing resin bound amines **8** were placed into a 12 L 3-necked round bottom flask fitted with an overhead stirrer. Dimethylformamide (4.5 L) was added to swell the resin in the MicroKans™. 1-Alloc-4-Fmoc-piperazine-2-carboxylic acid scaffold **6** (78.6 g, 180.0 mmol) was dissolved into DMF (500 ml) and added to the MicroKans™. HBTU (68.3 g, 180.0 mmol) and DIEA (62.7 ml, 360.0 mmol) were then added sequentially. The reaction was stirred at RT for 6.5 h. The solution was drained and the MicroKans™ were washed with DMF (3×), DCM (3×) and Et_2O (1×). The MicroKans™ were dried overnight with a stream of nitrogen gas.

Loading of scaffold **(6)** *to Wang resin*

Wang resin (5.0 g of 1.7 mmol/g loaded resin from Polymer Labs) was swelled in anhydrous DCM (80 ml). The piperazine scaffold **6** (7.42 g, 17.0 mmol), DIC (2.67 ml, 17.0 mmol) and DMAP (0.21 g, 1.70 mmol) were added sequentially. The reaction was then stirred overnight at RT on an orbital shaker. The reaction solution was drained and the resin was washed with DCM (2×), DMF (4×), DCM (4×), THF (4×) and Et_2O (4×). The resin was placed on a high vacuum line to dry. IR analysis was used to confirm the loading of the scaffold. This resin was then loaded into 500 MicroKans™.

Loading of scaffold **(6)** *on Rink Amide resin*

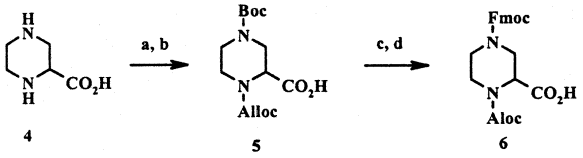
Fmoc-Rink Amide resin (11.0 g of 0.78 mmol/g loaded resin from Advanced Chemtech) was deprotected with 1:1 DMF/piperidine for 3 h. The reaction solution was drained off and the resin was washed with DMF (4×), THF (4×), Et_2O (3×) and anhydrous DMF (3×). Anhydrous DMF (120 ml) was then added to the resin. The scaffold **6** (14.98 g, 34.32 mmol), HBTU (13.0 g, 34.32 mmol) and DIEA (12.0 ml, 68.64 mmol) were added sequentially. The reaction was stirred on an orbital shaker at RT overnight. The reaction solution was drained and the resin was washed with DMF (4×), DCM (4×), THF (4×), and Et_2O (4×). The resin was placed on a high vacuum line to dry. IR analysis was used to confirm the loading of the scaffold. This resin was then loaded into 500 MicroKans™.

Removal of Fmoc protecting group from **(9)**

The 5000 MicroKans™ containing **9** were placed into a 12 L 3-necked round bottom flask fitted with an overhead stirrer. Dimethylformamide (2.5 L) and piperidine (2.5 L) were added to the MicroKans™. The reaction was stirred at RT for 3.5 h. The reaction solution was drained and the MicroKans™ were washed with DMF (3×), DCM (3×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Acylation with carboxylic acids to produce **(10)** *or* **(11)** *or* **(20)**

For each carboxylic acid, 200 MicroKans™ were placed into a 1 L 3-necked round bottom flask fitted with an overhead stirrer. The resin in the MicroKans™ was swelled in NMP (300 ml). The carboxylic acid (20.0 mmol) and EDC (3.83 g, 20.0 mmol) were added sequentially. The reaction was stirred overnight at RT. For workup, each reaction was individually drained and washed once with DMF. All of the MicroKans™ from each acid were then



Scheme 1. Synthesis of the protected scaffold. (a) Boc-ON, dioxane:water (1:1), pH = 11; (b) Allyl chloroformate, pH = 9.5; (c) 50% TFA-DCM; (d) Fmoc-Cl, Na_2CO_3, dioxane:water (1:1).

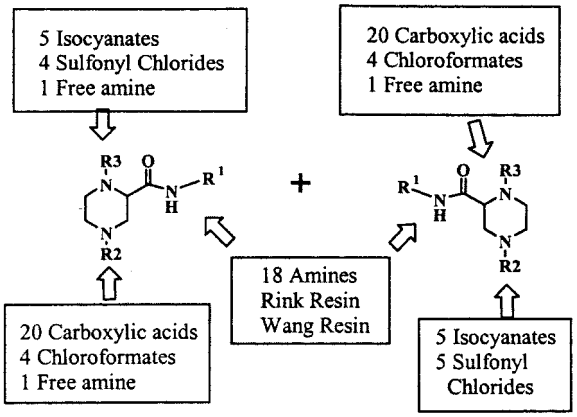


Figure 2. Composition of the 10 000 member library.

combined and washed with DMF (2×), DCM (3×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Acylation with chloroformates to produce **(10)** *or* **(11)** *or* **(20)**

For each chloroformate, 200 MicroKans™ were placed into a 1 L 3-necked round bottom flask fitted with an overhead stirrer. The resin in the MicroKans™ was swelled in anhydrous DCM (300 ml). DIEA (3.5 ml, 20.0 mmol) and the chloroformate (20.0 mmol) were then added sequentially. The reaction was stirred overnight at RT. For workup, each reaction was individually drained and washed once with DMF. All of the MicroKans™ were then combined and washed with DMF (2×), DCM (3×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Urea formation with isocyanates to produce **(10)** *or* **(11)**

For each isocyanate, 500 MicroKans™ were placed into either a 2 or 3 L 3-necked round bottom flask fitted with an overhead stirrer. The resin was swelled in anhydrous DCM (600 ml). The isocyanate (50.0 mmol) was then added neat. The reaction was stirred overnight at RT. For workup, each reaction was individually drained and washed with DMF (3×). All of the MicroKans™ were then combined and washed with DMF (2×), THF (1×), DCM (2×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Sulfonamide formation to produce **(10)** *or* **(11)** *or* **(20)**

For each sulfonyl chloride, 500 MicroKans™ were placed into either a 2 or 3 L 3-necked round bottom flask fitted with an overhead stirrer. The resin was swelled in anhydrous DCM (600 ml). NMM (5.5 ml, 50.0 mmol) and the sulfonyl chloride (50.0 mmol) were then added sequentially. The reaction was stirred overnight at RT. For workup, each reaction was individually drained and washed with DMF (3×). All of the MicroKans™ were then combined and washed with DMF (2×), THF (1×), DCM (2×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Removal of Alloc protecting group from resin **(10)** *or* **(19)**

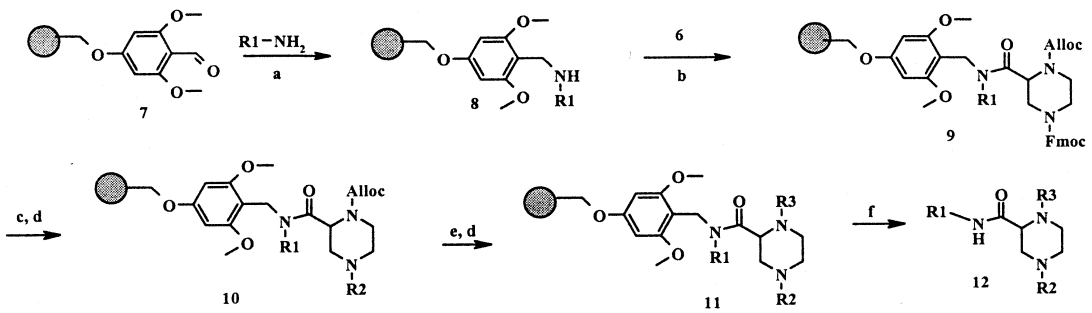
The 5000 MicroKans™ containing resin **10** or **19** were placed into a 12 L 3-necked round bottom flask fitted with an overhead stirrer. THF (2 L), DMSO (2 L), and 0.5 N HCl (1 L) were added to the MicroKans™. The reaction flask was then flushed with nitrogen. $Pd(Ph_3P)_4$ (8.06 g, 6.98 mmol) and morpholine (218 ml, 2500 mmol) were added sequentially. The reaction was stirred overnight under a flow of nitrogen gas. For workup, the reaction was drained and the MicroKans™ were washed with THF (2×), sodium diethyldithiocarbamate [0.02 M in DMF] (2×), DMF (2×), 0.5% DIEA in DCM (1×), DCM (3×), and Et_2O (1×). The MicroKans™ were then dried overnight with a stream of nitrogen gas.

Preparation of resin **(16)** *by the Yamaguchi method*

4-(Bromomethyl)phenylacetic acid (529 mg, 2.34 mmol) was poured into 5 ml of DMF, followed by pyridine (170 μl, 2.125 mmol) and 2,6-dichlorobenzoylchloride (301 μl, 2.125 mmol). The mixture was shaken for 1 h and then the Wang resin (loading 1.7 mmol/g) was added. It was shaken overnight, drained and the resin was washed with DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was dried overnight under reduced pressure.

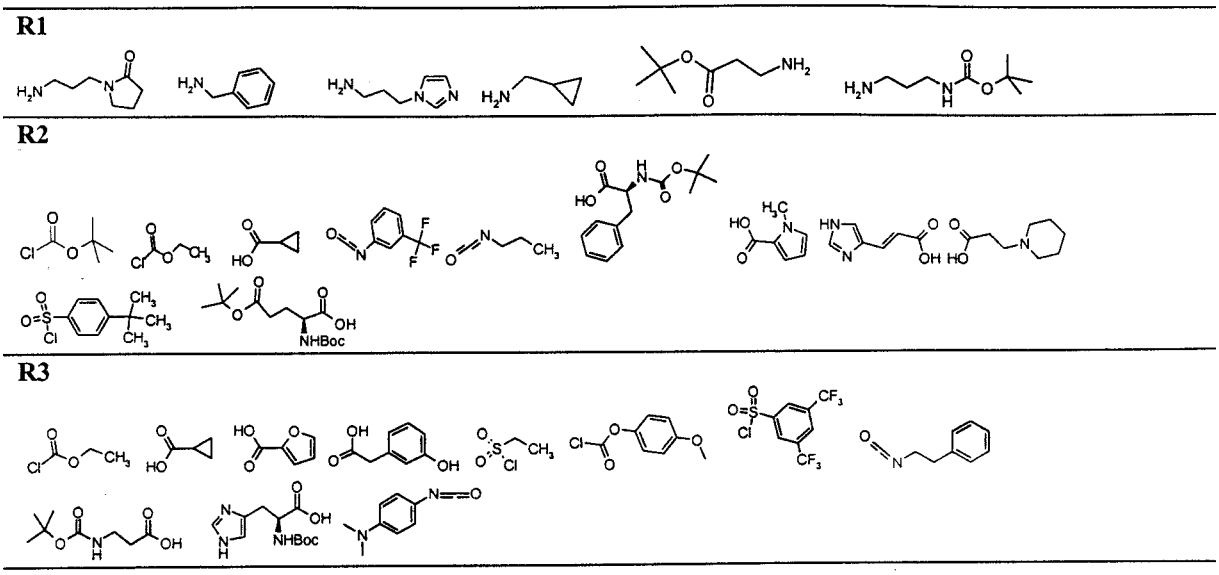
Preparation of resin **(16)** *from acid chlorides*

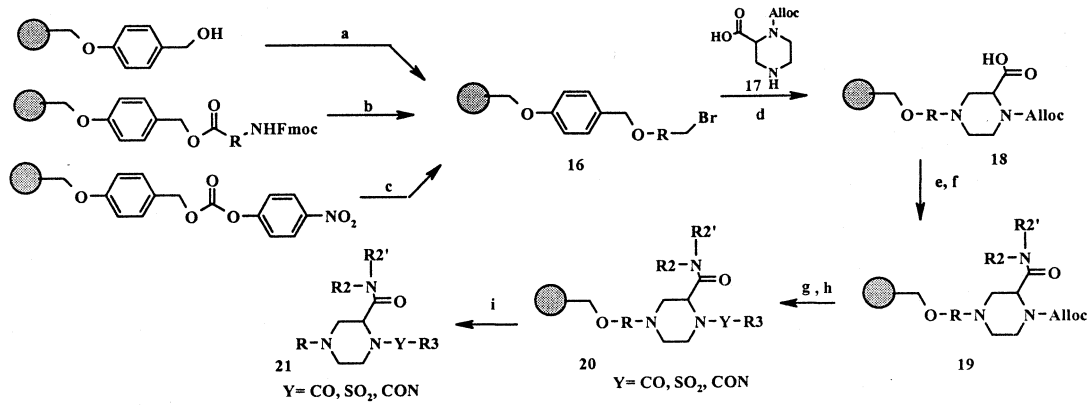
In a flask flushed with nitrogen, the acid (5.5 equiv, 2.34 mmol) and oxalyl chloride (3 ml, 6.02 mmol)



Scheme 2. 10 000 member combinatorial library: (a) $NaBH(OAc)_3$, DMF, AcOH; (b) HBTU, DIEA, DMF; (c) Piperidine:DMF (1:1); (d) RSO_2Cl, NMM, DCM or RNCO, DCM or ROCOCl, DIEA, DCM or RCOOH, EDC, NMP; (e) $Pd(PPh_3)_4$, morpholine, THF, DMSO, aqHCl; (f) 50% TFA-DCM.

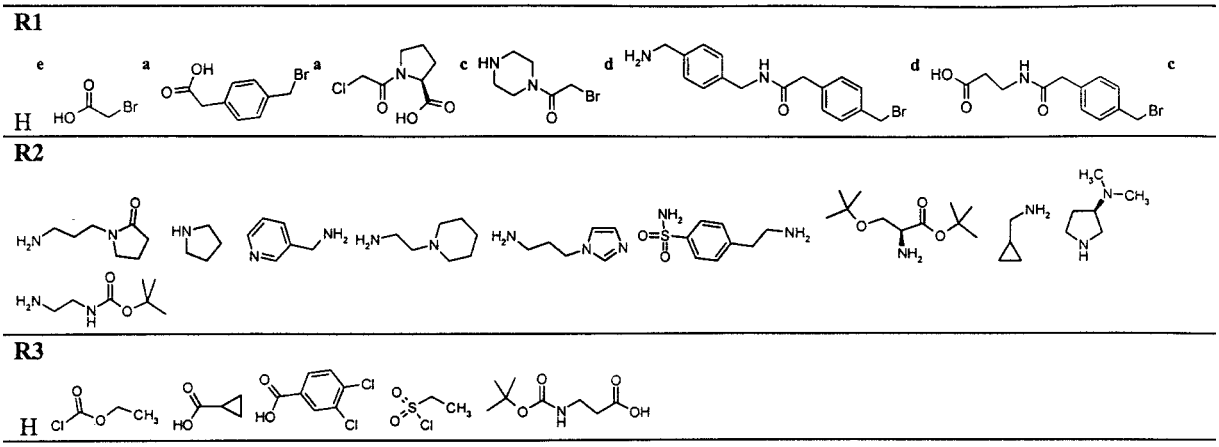
Table 1. Representative sample of the reagents used in the 10 000 member combinatorial library



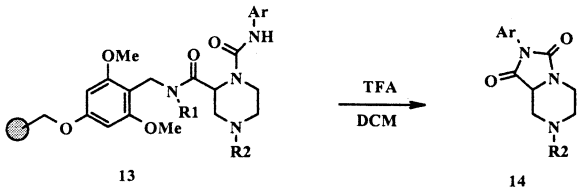


Scheme 3. 5000 member library. (a) Br-R-COOH, dichlorobenzoyl chloride, pyridine, DCM or Br-R-COCl; (b) (i) piperidine, DMF; (ii) Br-R-COOH, DIC, HOBt, DMF; (c) (i) symmetrical diamine, DMF; (ii) Br-R-COOH, DIC, HOBt; (d) KI, DMF, 80C; (e) DIC, PfP, DMF; (f) R2-NH-R2′, DMF; (g) $Pd(PPh_3)_4$, morpholine, THF, DMSO, aq HCl; (h) RSO_2Cl, NMM, DCM or ROCOCl, DIEA, DCM or RCOOH, EDC, NMP; (i) 50% TFA-DCM.

Table 2. Representative sample of the reagents used in the 5000 member 4-alkyl-piperazine-2-carboxamide combinatorial library



(a): Loaded with the Yamaguchi method. (b) Loaded by conversion to the acid chloride. (c) Fmoc-amino acid loaded with DIC/DMAP, deprotected and reacted with bromo-acid. (d) Symmetric diamine loaded on the nitrophenyl carbonate derivative of Wang resin and subsequently reacted with bromo-acid. (e) Piperazine carboxamide scaffold reacted with the nitrophenyl carbonate derivative of Wang resin.



Scheme 4. Cyclization of aromatic ureas to hydantoins.

were mixed in DCM (10 ml). One drop of DMF was added. The mixture was stirred for 1 h. The solvent and oxalyl chloride were then removed by evaporation. The acid chloride was dissolved in 9 ml of a mixture of DCM and pyridine (9:1). Wang resin (loading 1.7 mmol/g) was added and the mixture was shaken overnight, drained and the resin was washed with DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was dried overnight under reduced pressure.

Preparation of resin **(16)** *from amino acids*

Wang resin (150 mg, 0.255 mmol) was suspended in DCM (2 ml). The Fmoc-amino acid (1.25 mmol) was added, followed by DIC (200 μl, 1.25 mmol) and DMAP (6.4 mg, 0.052 mmol). The mixture was shaken overnight, drained and the resin was washed with DCM (2×), DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was then suspended in a mixture of piperidine and DMF (1:1). The mixture was shaken overnight, drained and the resin was washed with DCM (2×), DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin (100 mg, 0.17 mmol) was suspended in DCM (15 ml). The bromo-acid (2.55 mmol) was added, followed by DIC (400 μl, 2.55 mmol). The mixture was shaken overnight, drained and the resin was washed with DCM (2×), DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was dried overnight under reduced pressure.

Preparation of resin **(16)** *from symmetrical diamines*

Nitrophenol carbonate resin (100 mg, 0.16 mmol) was suspended in 2 ml of DMF. The diamine (1.6 mmol) was added and the mixture was shaken overnight at room temperature. The mixture was drained and the resin was washed with DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was suspended in DCM (10 ml). The bromo-acid (1.7 mmol) was added, followed by DIC (267 μl, 1.7 mmol). The mixture was shaken overnight, drained and the resin was washed with DCM (2×), DMF (3×), THF (3×), DCM (3×), and ether (2×). The resin was dried overnight under reduced pressure.

Alkylation with piperazine-2-carboxamide scaffold to produce **(18)**

The resin **16** (4.6 g, 7.82 mmol) was suspended in DMF (15 ml). 1-Alloc-2-carboxy-piperidine trifluoroacetate salt (2.71 g, 23.4 mmol) was added followed by potassium iodide (458 mg, 23.4 mmol) and DIEA (2.89 ml, 45 mmol). The reaction mixture was heated overnight at 80 °C, then drained and the resin was

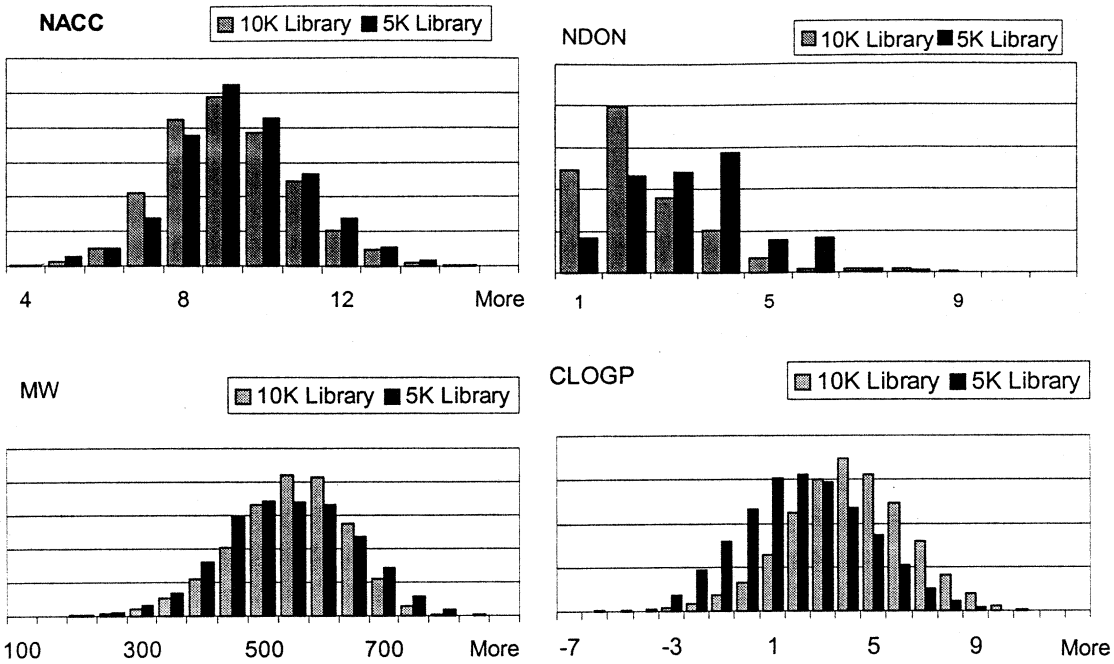


Figure 3. Libraries assessed against the Lipinski rule of 5: Histograms of the distribution of (1) number of hydrogen bond acceptors, (2) number of hydrogen bond donors, (3) molecular weight, (4) C-LogP.

washed with DMF (3×), THF (3×), DCM (3×), and ether (1×).

Amide bond formation to produce **(19)**
For each amine, 402 MicroKans™ (each MicroKan™ contained 6 mg of 1.7 mmol/g loaded resin **18**) were placed into a 1 L 3-necked round bottom flask fitted with an overhead stirrer. The resin in the MicroKans™ was swelled in DMF (300 mL). Diisopropylcarbodiimide (4.79 ml) and pentafluorophenol (5.63 g) were added and the resulting mixture was stirred at RT for 2 h. Each reaction was individually drained and washed with DMF (2×). The MicroKans™ in each round bottom flask were again suspended in DMF (300 ml) and the corresponding amine (20.4 mmol) was added to each vessel. In the case of hydrochloride salts, DIEA (10 equiv) was added. This reaction was stirred at room temperature overnight. Each reaction was individually drained. All of the MicroKans™ were then combined and washed with DMF (3×), THF (3×), DCM (3×), and ether (1×). The MicroKans™ were dried overnight with a stream of nitrogen.

Cleavage
The three different resins used in these libraries were separated. The MicroKans™ containing BAL resin were cleaved with 50% TFA in DCM for 1 h. The MicroKans™ containing Wang resin were cleaved with 30% TFA in DCM for 1 h. The kans containing Rink amide resin were cleaved with 10% TFA in DCM for 1 h. All of the above cleavage solutions contained a small amount of water. 99+% spectrophotometric grade TFA was used. The cleavage plates were then concentrated down in a Savant from 25 to 43 °C.

Results

The piperazine-2-carboxylic acid scaffold **4** is well suited for a combinatorial approach as it is a small, constrained structure with three functional groups (one carboxylic acid and two amines) which may be conveniently substituted by solid phase chemistry. Orthogonal protection of the two amino groups can easily be carried out on a large scale by solution phase chemistry (Scheme 1).

Two distinct libraries were synthesized and combined to produce 15 000 discrete compounds. The first library was prepared according to the chemistry outlined in Scheme 2. Thus a primary amine was attached to the dimethoxy-benzaldehyde linker **7** (BAL) [12,13] via reductive amination. The secondary amines were acylated with the orthogonally protected

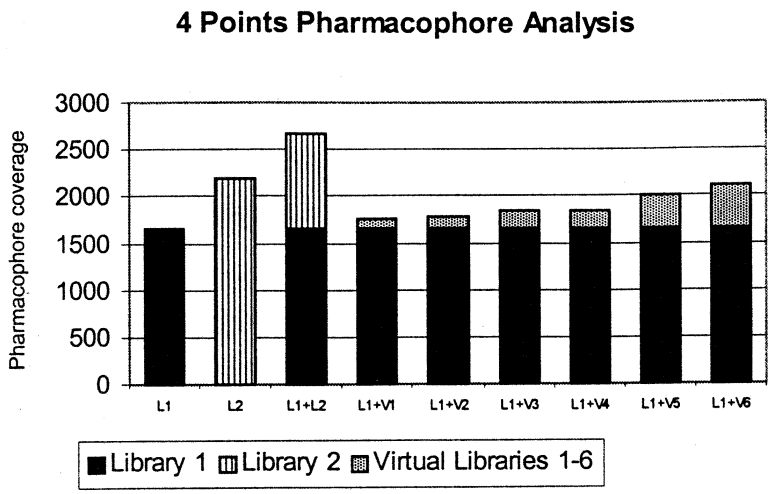


Figure 4. Four center pharmacophore analysis: Diversity coverage of the two combinatorial libraries, their combination, and the combination of the 10 000 member library with 6 virtual libraries built using the same chemistry.

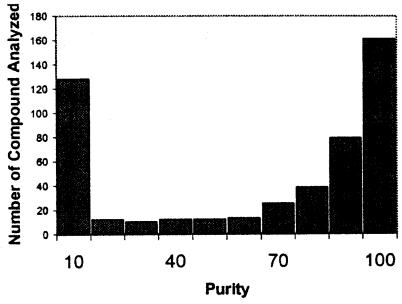
piperazine-2-carboxylic acid scaffold **6** in the presence of HBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate) and diisopropylethylamine (DIEA) to yield **9**, in which both amines can be selectively deprotected and functionalized. The fluorenylmethoxycarbonyl (Fmoc) group was removed with piperidine and the 4-amine was reacted with sulfonyl chlorides, isocyanates, chloroformates and carboxylic acids. The allyloxycarbonyl (Alloc) group was subsequently removed with $Pd(PPh_3)_4$ and the 1-amine was functionalized similarly to afford sulfonamides, ureas, carbamates and amides. This chemistry was used to produce a 10 000 member library. Two combinatorial matrixes of 5000 compounds each were defined, the first containing amides and carbamates at R2, and sulfonamides and ureas at R3. The second matrix contained amides and carbamates at R3, and sulfonamides and ureas at R2 (Figure 2). See Table 1 for a representative sample of the reagents used.

The second library, built around the piperazine-2-carboxylic acid scaffold, was designed to fill some of the diversity gaps left by the first library. In the first library, all of the compounds contained the 2-secondary amide which was the point of attachment to the resin. Also, none of the compounds contained a basic tertiary amine in the piperazine ring.

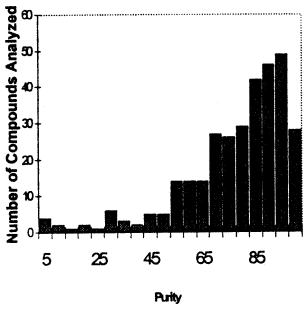
The synthetic scheme for the second library is outlined in Scheme 3. The scaffold **17** was synthesized on a large scale in solution. Bifunctional reagents containing a handle (carboxylic acid or amine) and a halide were loaded onto Wang resin. Bromo- or chloro-carboxylic acids were reacted with Wang resin using either the Yamaguchi method (2,6-dichlorobenzoyl chloride) [14] or by conversion to the acid chloride. Alternatively, Fmoc-amino acids were loaded onto Wang resin with diisopropylcarbodiimide (DIC)–dimethylaminopyridine (DMAP). The Fmoc group was removed and the free amine was then acylated with bromo- or chloro-carboxylic acids. Symmetric diamines were also loaded onto the nitrophenol carbonate derivative of Wang resin and acylated with bromo- or chloro-carboxylic acids. The bromides or chlorides were converted in situ to the corresponding iodide and then reacted with the unprotected amine of **17**. Site isolation on the resin ensured clean monoalkylation. The carboxylic acid was then converted to the pentafluorophenyl ester and reacted with amines in a second combinatorial step. After the removal of the Alloc group, the third combinatorial step was completed. This consisted of acylation, sulfonylation and carbamate formation, which were performed under the same conditions as in the first library. Table 2 lists a representative sample of the reagents used in the 5100 member library. A full $17\times25\times10$ matrix was constructed for this library.

Discussion

The size of the libraries which can be generated has been increasing steadily, however, chemists are still limited to a tiny portion of the chemical space which can be covered by functionalization of a scaffold. In



a



b

Purity Range	% Library
76-100%	53%
51-75%	12%
26-50%	6%
0-25%	29%

Purity Range	% of Library
76-100%	62%
51-75%	28%
26-50%	5%
0-25%	5%

Figure 5. Purity distribution for (a) the 10 000 member library and (b) the 5000 member library.

the case of lead generation libraries, the objective is to maximize the coverage of this chemical space with a limited number of compounds, while keeping the physical properties of the compounds in the typical range of orally available compounds [15]. For this purpose, clustering tools were used to guide the choice of reagents and both libraries were profiled against the Lipinski rule of five [16]. Also a four point pharmacophore analysis was used to quantify the additional coverage of the chemical space gained by applying two different chemistries to functionalize the piperazine-2-carboxamide scaffold.

For both libraries, a diverse range of reagents were tested at the three combinatorial positions to determine the scope and limitation of these reactions. Lists of commercially available amines, sulfonyl chlorides, carboxylic acids and isocyanates were subjected to Ward's clustering using Daylight 2D fingerprints [17]; cluster centroids having 75% or greater similarity to the original set were discarded with the remaining centroids (or similar replacement, based on cost, availability and other factors) used to form the candidate lists. Reagents for the library were then chosen from these lists to maximize diversity within compatibility of the chemistry.

The profile of each library was tested against the Lipinski rule of 5 [16] to assess the potential absorption or permeability properties of the compounds produced. The rule of 5 is an empirical observation from a review of the current drugs on the market which may predict if a compound will exhibit poor absorption and/or permeability if it has a number of hydrogen bond acceptors (NACC)>10, a number of hydrogen bond donors (NDON)>5, a molecular weight (MW)>500, a logP>5. Figure 3 shows for each library a set of histograms describing the distribution of (1) number of hydrogen bond acceptors, (2) number of hydrogen bond donors, (3) molecular weight, (4) ClogP. For all the rules other than MW, 80% of each library is below the threshold suggested by Lipinski. For molecular weight, 80% of each library is below 650. Overall, for library 1, nearly 60% of the library passed at least three criteria, and most of the failures were due to combinations of MW and NACC or MW and C-LogP. For library 2, 70% of the library passed at least three criteria and most of the failures were also for combinations of MW and NACC or MW and C-LogP.

The additional coverage of the chemical space gained by applying two different chemistries to the same scaffold was measured by the four-point pharmacophore method using ChemDiverse/Chem-X software [18] as customized and extended at Rhone-Poulenc Rorer [19]. A four-point pharmacophore is defined as a combination of four pharmacophore center types (points) and the six distances that separate them. Four-point pharmacophore analysis is well suited to compare compounds within the same structural class as it quantifies the distribution of pharmacophores around a scaffold. Seven center types, assigned

using a molecule's atom types, are available and were defined as: H-bond donor, H-bond acceptor, H-bond donor and acceptor, aromatic ring, hydrophobe, acid, and base. Distances between centers in a molecule are measured exactly but stored using a binning scheme; here a nonlinear scheme that uses 10 bins (8 for distances between 2–19.5 Å and one each for higher and lower values) was used. All pharmacophores within a molecule are stored in a pharmacophore key, which is a bit string where each bit represents a specific combination of center types and distances. Each molecule was subjected to a conformational analysis using either random or systematic sampling based on molecular flexibility, and all bits in the pharmacophore key corresponding to observed pharmacophores in all acceptable conformers were set. Logical operations (e.g. AND, NOT) were then performed on the keys to generate a library key representing all pharmacophores shown by all molecules in the library. This was then used to compare libraries.

Figure 4 illustrates the additional pharmacophore diversity obtained by changing the point of attachment of the scaffold and the chemistry used to functionalize it. Six 5000 member virtual libraries were constructed with the chemistry depicted in Scheme 2, using either the same reagents with different attachment points to the scaffold (Virtual Library 1 and 2), random reagents chosen within a set where the molecular weight was kept below 200 (Virtual Library 3 and 4) and random reagents chosen without molecular weight cutoff (Virtual Library 5 and 6). The additional diversity gained from those libraries compared to the first 10 000 member library was measured using the four-point pharmacophore approach and compared to the additional diversity obtained with the 5000 member library depicted in Scheme 3. Although smaller, the 5000 member library depicted in Scheme 3 actually covered more diversity space than the first 10 000 member library. In addition, the combination of the two libraries covered significantly more diversity space than the combination of the 10 000 member library with any of the six virtual libraries.

Both libraries were constructed as a combination of a small number of reagents, therefore analysis of a small random portion of the library was expected to be representative of the overall purity. The quality of both libraries was assessed by calculating the percentage crude yield on 20 random samples and analyzing 5% of the compounds by HPLC (High Pressure Liquid Chromatography) with a combination of Mass Spectrometry (MS) and Evaporative Light Scattering (ELS) detection. The MS detector was used to confirm identity of the compounds and purity was based on ELS detection. For the 10 000 member library the average yield was 62% and for the 5000 member library 67%. For both libraries, all of the samples analyzed had a yield above 45%. Figure 5 shows the purity distribution for the two libraries. In both cases, the majority of the compounds were present in high purity. For the 10 000 member library, a significant portion of the library was affected by an unexpected side reaction. Compounds containing aromatic ureas at the R3 position cyclized to form hydantoins in high purity (Scheme 4) [20]. In the 5000 member library, 95% of the expected compounds were detected and none of the reagents used were found to produce any significant side reactions.

Conclusions

Two novel general solid phase routes to piperazine-2-carboxamide derivatives were developed and the directed sorting method was used to produce 15 000 discrete compounds. This illustrates that it is now possible to generate large combinatorial libraries of discrete compounds using multistep solid phase synthesis. Maximum diversity in the series was obtained by (1) devising two different routes to functionalize the piperazine-2-carboxylic acid scaffold to maximize pharmacophore display and (2) choosing reagents to maximize diversity within 'drug like' physicochemical properties. These compounds are currently being screened against a wide variety of targets and results will be published in the future.

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