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Use of Parallel-Synthesis Combinatorial Libraries for Rapid Identification of Potent FKBP12 Inhibitors

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Abstract—Using simple, inexpensive equipment, we have used solution-phase parallel synthesis to rapidly prepare hundreds of sulfonamide- and urea-containing FKBP inhibitors, resulting in rapid identification of extremely potent compounds in these series.

Immunophilins are 'receptors' for immunosupressant drugs such as FK506 and cyclosporin A.[1] Immunophilins possess peptidyl-prolyl isomerase (rotamase, or PPIase) activity. Recently it has been discovered that the immunophilin FKBP12, which binds FK506, is enriched 10- to 40-fold more in the brain than in the immune tissues.[2] Immunosuppressant drugs such as FK506 have been shown to promote neuronal process extension in vitro and regrowth of damaged peripheral nerves in vivo.[2,3] It has recently been demonstrated that nonimmunosuppressive analogues of these drugs retain these neurotrophic properties.[4]

We have recently described the neurotrophic actions of simple *N*-glyoxyl prolyl and pipecolyl esters.[5,6] These small molecule FKBP12 ligands are devoid of immunosuppressive action but potently promote neurite outgrowth in neuronal cultures. Compounds such as GPI 1046 (Fig. 1) have been shown to possess neuroprotective and neuroregenerative properties in a variety of animal models of neurodegeneration, thus suggesting broad therapeutic potential for FKBP12 ligands in nervous system disorders.[6,7]

FKBP12 ligands in which the α-ketoamide (*N*-glyoxyl) moiety of FK506 and GPI 1046 has been replaced with isosteric mimetics (e.g., sulfonamides such as **1** and ureas such as **2**) have been reported in the literature,[8,9] but no extensive development of the SAR of these compounds as FKBP12 inhibitors has appeared. Our discovery that compounds such as GPI 1046 are a novel class of potent neuroregenerative agents prompted us to explore other structural classes of FKBP12 ligands.

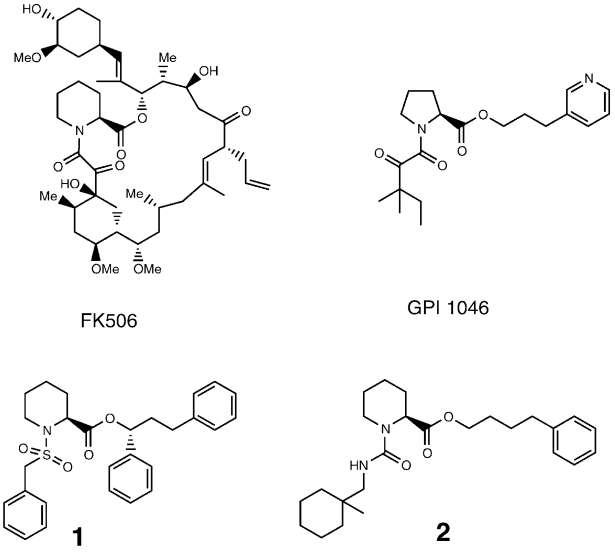

Figure 1. Ligands for FKBP12.

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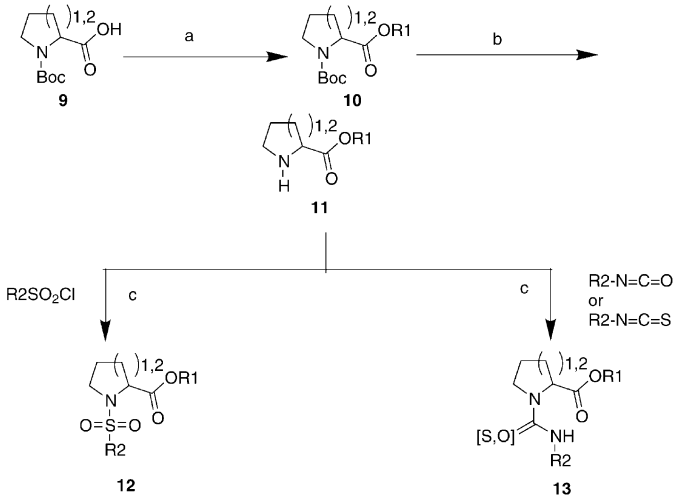
One of the approaches that we have deployed in exploring other classes of FKBP12 ligands is solution-phase, parallel synthesis. In an economical fashion, we have quickly generated several series of compounds for SAR studies and lead optimization using a Reacti-Therm stirring-heating module, an expandable multi-channel pipette, and a set up for solid-phase extraction.[10] Reacti-Therm modules have been widely used by pharmacokineticists and protein chemists. The module provides heating and stirring functionalities. When connected to a nitrogen outlet, the module can act as a blow dryer. It can also provide an inert reaction atmosphere when used in conjunction with proper septa. Vials used with the module are readily amendable to the solution transfer by the Matrix expandable multi-channel pipette.[11] In addition to allowing quick reagent addition, the multi-channel pipette facilitates product transfer from vials to 96-well plates. The cost of the module, 27 5-mL vials, caps, and septa is about $1500. With the additional cost of the expandable multi-channel pipette, which is about $1000, the total cost of the set up is no more than $3000. The low cost of the set up makes it possible for chemists to carry out parallel syntheses on the bench-top without having to invest prematurely in expensive robotics.

Compounds were synthesized as shown in Scheme 1. *N*-(*tert*-Butyloxycarbonyl) proline or *N*-(*tert*-butyloxycarbonyl)pipecolic acid were esterified with various alcohols in round bottom flasks using standard coupling conditions to provide Boc-protected proline or pipecolic acid ester. For optimization of the sulfonamide, urea, or thiourea moieties, 2 mL of 1.48 mM esters solutions in methylene chloride were pipetted to each of the nine vials in a row. Deprotection of the Boc protecting group with trifluoroacetic acid afforded proline or pipecolic acid ester trifluoroacetic salts in 2 h. Solvent was then removed by means of blow-drying. Solutions of sulfonyl chlorides, isocyanates, or thioisocyanates in methylene chloride (2 mL of 2.96 mM) were transferred to each of the three vials in the column. Triethylamine was then added. Excess sulfonyl chlorides, isocyanates, or thioisocyanates were scavenged by Tentagel-S-NH_2 resin after stirring for 16 h. The reactions were worked up by passing the crude mixture through silica solid-phase extraction columns. For optimization of the aryl alkyl moiety, esters were added to each of the three vials in a row, and sulfonyl chlorides, isocyanates, or thioisocyanates to each of the nine vials in a column. All products were checked for purity with HPLC. One-third of the reaction mixtures were checked by FABMS for the presence of products. The products (10–20 mg) were collected with an average purity of 70%. An example of a library is shown in Figure 2. Representative HPLC traces are shown in Figure 3.

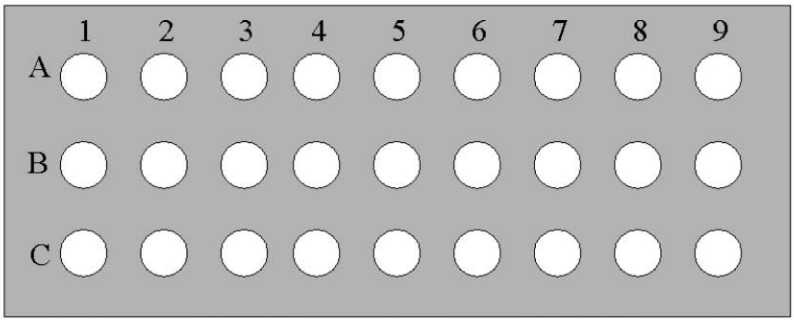
Compounds were tested for their ability to inhibit the peptidyl-prolyl isomerase (PPIase, or rotamase) activity of FKBP12 using the method of Kofron, as previously described.[12] Data are reported as apparent inhibition constants (K_i's). Although the products from the presently described parallel synthesis were used only in in vitro studies, we have reported on the activity of these compounds in in vivo models of neurodegeneration elsewhere.[13]

We began by synthesizing libraries in which the sulfonamide, urea, and thiourea substituents were varied. Adamantyl, cyclohexyl, and benzyl groups were found to be particularly effective substituents in the urea, thiourea, and sulfonamide series, respectively (**19**, **21**, and **48**, Figs. 4 and 5). These moieties were held constant in subsequent libraries while the ester side chains were varied. Potent compounds in each series, such as compounds **42**, **43**, and **56**, were rapidly generated by this method.

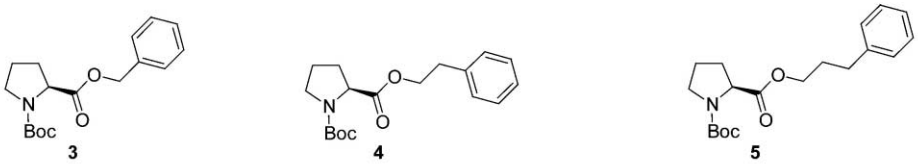
Structure–activity trends were observed in both the sulfonamide and urea series. Examples of the trends observed are shown in Tables 1 and 2. In general, monosubstitution of the ring of the arylalkyl ester chain with lipophilic substituents enhanced activity in the sulfonamides. Among the 4-halophenyl propyl side chains, 4-flourophenyl propyl was generally better than the



Scheme 1. Reagent and conditions: (a) R1OH, DCC, DMAP, CH_2Cl_2, rt; (b) trifluoroacetic acid; (c) 2 equiv R2SO_2Cl or R2NCO or R2NCS, Et_3N, CH_2Cl_2.



Esters added to rows A, B, and C were:



Sulfonyl chlorides, isocyanate, and thioisocyanate added to columns 6–8 for the above reactions were:

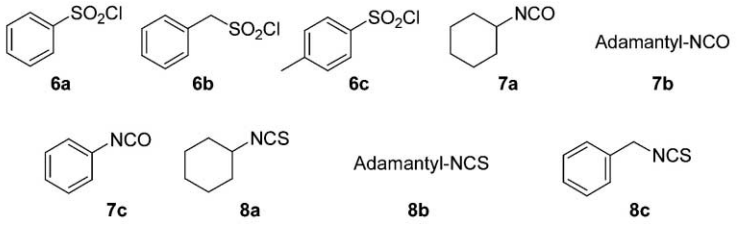


Figure 2. An example of the library.

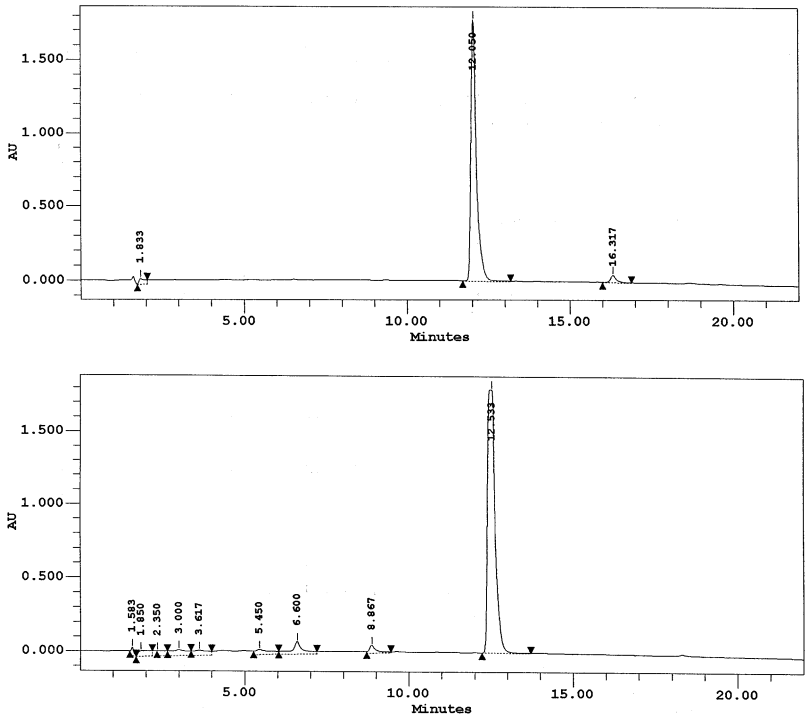


Figure 3. HPLC traces of two compounds from the parallel synthesis.

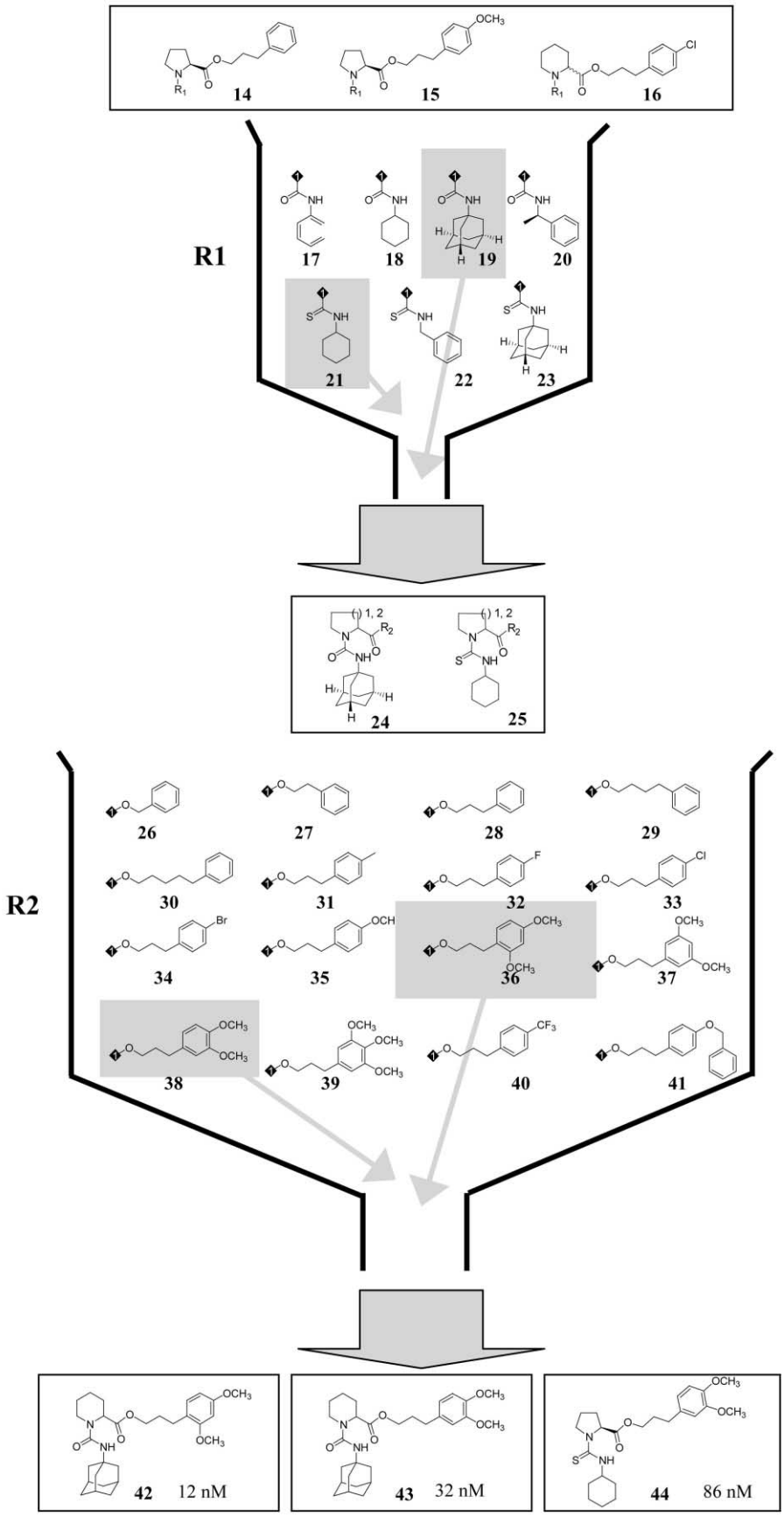


Figure 4. Procedure showing the lead optimization in the urea and thiourea series.

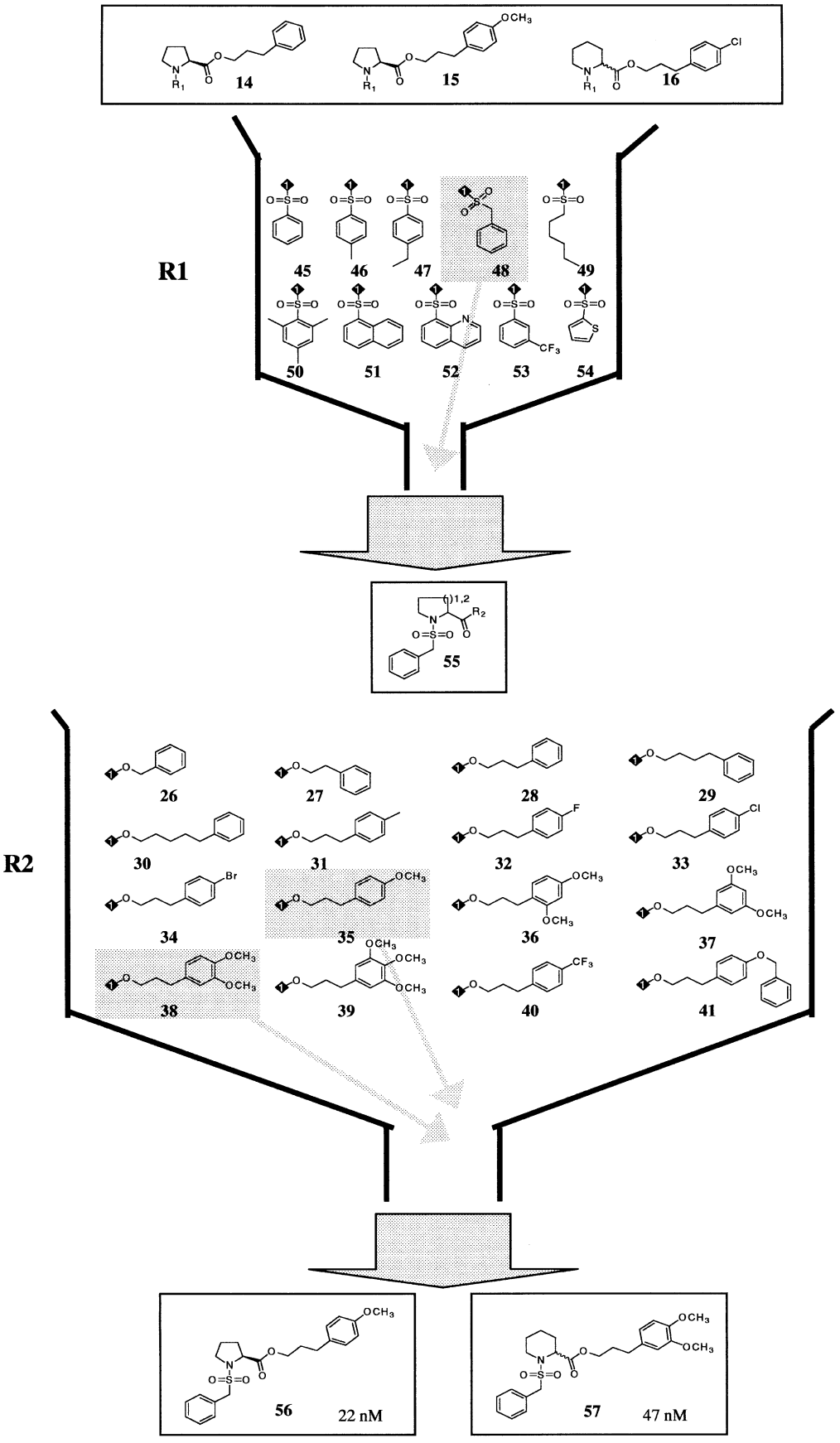
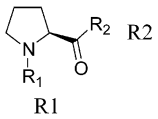


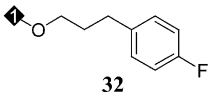
Figure 5. Procedure showing the lead optimization in the sulfonamide series.

4-chlorophenyl propyl, which was better than the 4-bromophenyl propyl (Tables 1a and 2a). Best activities were generally found with compounds with alkyl chains length of 3–4 carbons (Table 1b). The presence of electron donating groups such as methoxy was favorable for rotamase inhibition activity, and the 3,4- and 2,4-dimethoxy substitution patterns were particularly active in the sulfonamide and urea series, respectively

Table 1. SAR examples in proline urea/thiourea series

(a)





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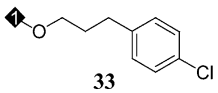

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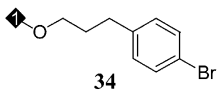

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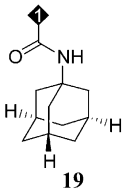
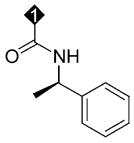
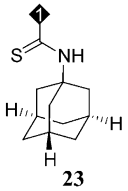
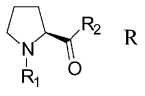
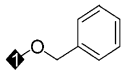
 **19**	**58** 1200 nM	**59** 2300 nM	**60** 3100 nM
 **20**	**61** 4400 nM	**62** 6000 nM	**63** > 10,000 nM
 **23**	**64** 850 nM	**65** 1500 nM	**66** 6300 nM

(b)





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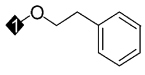
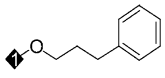


27



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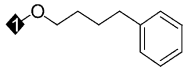

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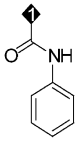
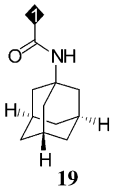
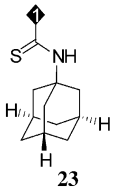
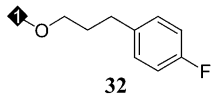
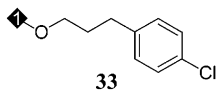
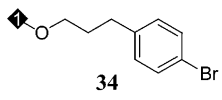
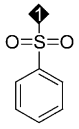
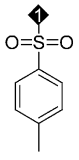
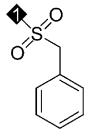
 **17**	**67** 24,000 nM	**68** 1300 nM	**69** 820nM	
 **19**	**70** 2100 nM	**71** 1300 nM	**72** 1000 nM	**73** 470 nM
 **23**	**74** 18,000 nM	**75** 47,000 nM	**76** 5200 nM	**77** 7200 nM

(e.g., **57** and **42**). Benzylsulfonyl, adamantylcarbamoyl, and cyclohexylthiocarbamoyl groups are found to be the most efficacious *N*-substitutions in both proline and pipecolic acid series. In the sulfonamide series, the order of preference for sulfonamide substituent was consistent, benzyl being greater than phenyl, and phenyl greater than *p*-toluyl (Table 2b).

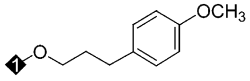
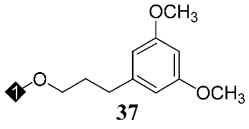
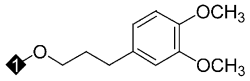
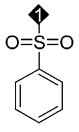
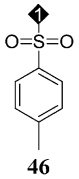
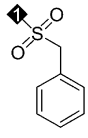
We have demonstrated the utility of a simple, inexpensive parallel synthesis set up that has allowed us to

Table 2. SAR examples of pipecolic acid sulfonamide series

(a)

R1 \ R2	 **32**	 **33**	 **34**
 **45**	**78** 740 nM	**79** 3800 nM	**80** 8000 nM
 **46**	**81** 1400 nM	**82** 4400 nM	
 **48**	**83** 320 nM	**84** 420 nM	**85** 2700 nM

(b)

R1 \ R2	 **35**	 **37**	 **38**
 **45**	**86** 260 nM	**87** 480 nM	**88** 180 nM
 **46**	**89** 220 nM	**90** 400 nM	
 **48**	**91** 92 nM	**92** 120 nM	**57** 47 nM

rapidly generate and identify extremely potent compounds from hundreds of sulfonamides and urea rotamase inhibitors. The set up has also enabled us to rapidly develop structure–activity relationships in these series. Compounds are generated in quantity and purity that are suitable for biological evaluation. This rather economical and simple set up makes it possible to implement parallel synthesis combinatorial libraries as a routine tool used by all medicinal chemists.

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