Molecular Diversity, **5:** 153–161, 2000.
KLUWER/ESCOM

New approach for preparation of 2,3,7-trisubstituted 3,4-dihydroisoquinolinone libraries on solid phase

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Received 3 March 2002; Accepted 22 May 2002

Key words: decarboxylation, dihydroisoquinoline, solid phase

Summary

In an attempt to prepare 7-substituted 3,4-dihydroisoquinolinone family of compounds, we observed an unexpected decarboxylation. The reaction of 4-nitrohomophthalic anhydride with a Schiff base formed on solid support leads to the formation of core structure. LC-MS and ^{1}H NMR analysis confirmed the structure of unexpected intermediate. A library of 38,400 compounds was produced using this new synthetic approach.

Solid-phase combinatorial chemistry has become a powerful tool for rapid generation of a large number of organic compounds, which can speed up the process of both lead discovery and lead optimization in the pharmaceutical industry. As a result, any solid-phase synthesis, which can lead to the preparation of potentially biologically active small molecules, is being heavily investigated [1–3].

3,4-Dihydroisoquinolinone is a general motif (Figure 1, I and II) within many biologically active compounds [4–7]. It can also be found as a substructure in the more complex systems [8–9] or as a reaction intermediate [10].

The reaction of imine with cyclic anhydride has been used for the synthesis of pyrrolidines, piperidines and isoquinolines [11]. In 1996, Griffith et al. transferred this reaction to the solid phase and by further derivatization on 4-carboxy substituent, a library of 2,3,4-trisubstituted 3,4-dihydroisoquinolinones was synthesized [12]. Two alternative approaches to automated synthesis of this class of heterocycles was published recently [13]. The general structure of this series of compounds is shown in Figure 1 (I).

To increase the molecular diversity of this library and to study the effect on reactivity of various building blocks, we initiated an experiment in which the synthesis of 3,4-dihydroisoquinolinones was carried out using a micro-plate as reaction vessel (Figure 2) [14]. The reaction sequence is illustrated in Scheme 1. Tentagel-NH_2 resin with a Rink linker was chosen because of its chemical stability to the conditions used in this synthesis. We employed four different amino acids in Step 1, Fmoc-2-aminoethanoic acid (1a), Fmoc-5-aminopentanoic acid (1b), Fmoc-trans-4-(aminomethyl) cyclohexanecarboxylic acid (1c), and Fmoc-4-(aminomethyl)benzoic acid (1d), respectively. The acids were separately attached onto Tentagel-NH_2 resin using normal peptide coupling conditions (DIC/HOBT/DIEA) in dimethylformamide. The complete coupling with each amino acid was confirmed by indicator bromophenol blue [15]. The Fmoc-protecting group was then removed by treatment with 50% piperidine in dimethylformamide for 2 h at room temperature to give III. Resin bound amines III were then condensed with 8 different aldehydes (2a–2h) in dimethylformamide in the presence of dehydrating agent trimethyl orthoformate (TMOF) to provide imines IV. After washing with dimethylformamide (3x), further reaction

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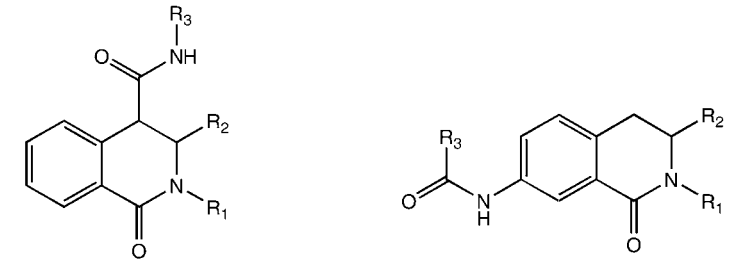


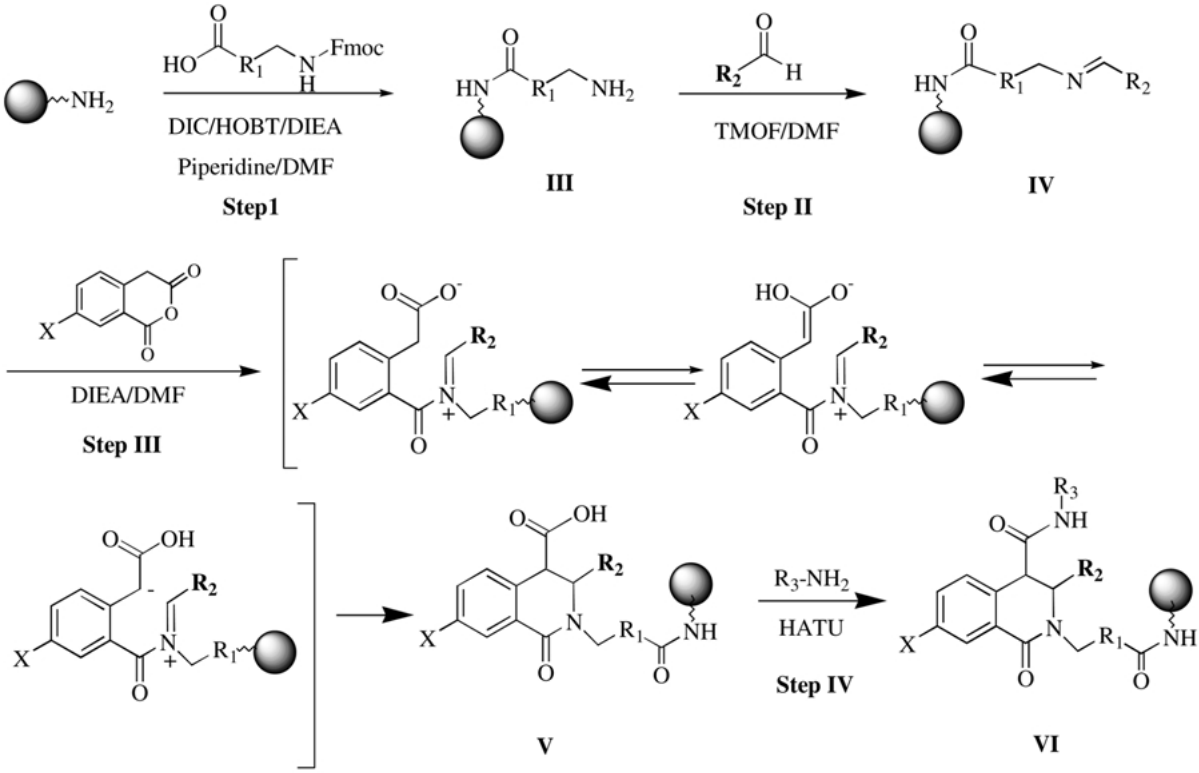
I

2,3,4-Trisubstituted 3,4-Dihydroisoquinolinone

II

2,3,7-Trisubstituted 3,4-Dihydroisoquinolinone

Figure 1. General structure of 2,3,4-trisubstituted 3,4-dihydroisoquinolinone (I) and 2,3,7-trisubstituted 3,4-dihydroisoquinolinone (II).



Scheme 1. Reaction sequence for preparation of 2,3,4-trisubstituted 3,4-dihydroisoquinolinones.

with 3 different homophthalic anhydrides (3a: 4-methoxy; 3b: 4-nitro; 3c: 4-chloro) was carried out in dimethylformamide at room temperature to provide isoquinoline V. To monitor the reaction sequence up to this point, small amounts of 96 intermediates were cleaved from the resin using TFA/H_2O (95/5) and analyzed using LC-MS. The results are shown in Figure 3. The resin was then mixed by row and evenly split into each vial by row and then activated by the treatment with O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU). Twelve different amines (4a–4l) were delivered into the corresponding row and let to react

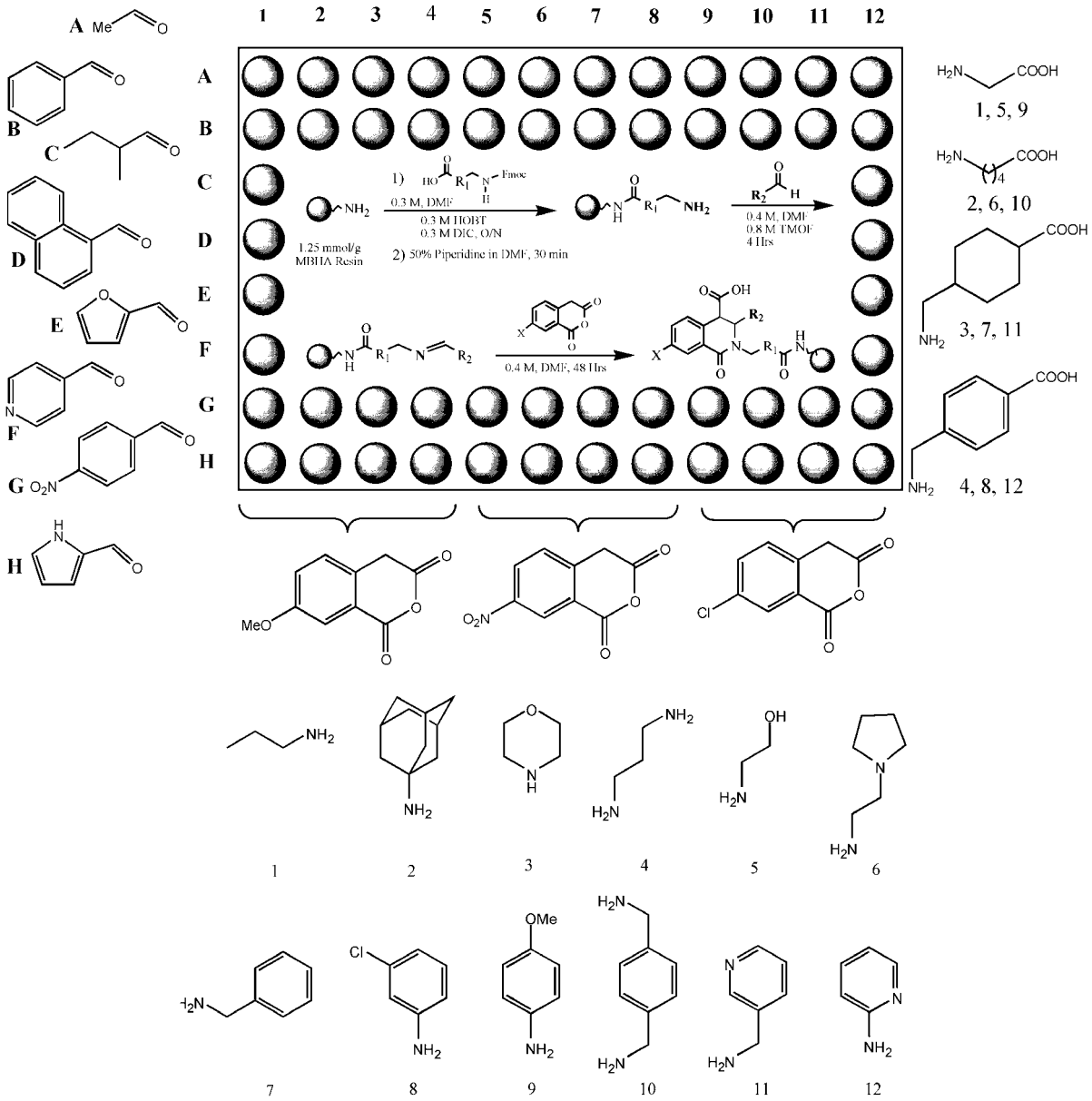


Figure 2. Array of building blocks. Aldehydes were distributed by the rows (A–H), amino acids by columns (three columns per amino acid, e.g. 1,3,5), anhydrides by columns (four columns specified in the figure), amines by the columns (1–12).

overnight. The final compounds were cleaved from the resin using TFA/H_2O (95/5) and analyzed by LC-MS.

The analysis of intermediates revealed unexpected results. All samples derived from acetaldehyde (**2a**) and pyrrole 2-carboxaldehyde (**2h**) did not provide a single product. Among the additional 72 compounds, all with 4-methoxyhomophthalic anhydride and 4-chlorohomophthalic anhydride gave the expected product with 4-carboxy substituent as a single compound. Separation of diastereomers was usually observed due to the presence of two chiral centers in the molecules (two examples are shown in Figures 4A and B). The reactions with 4-nitrohomophthalic anhydride provided single compounds, with 44 less in the molecular weight than the expected 4-carboxy substituted ones, and no separation of diastereomers was observed (one example is shown in Figure 4C). It can be explained by 4-decarboxylation due to the pres-

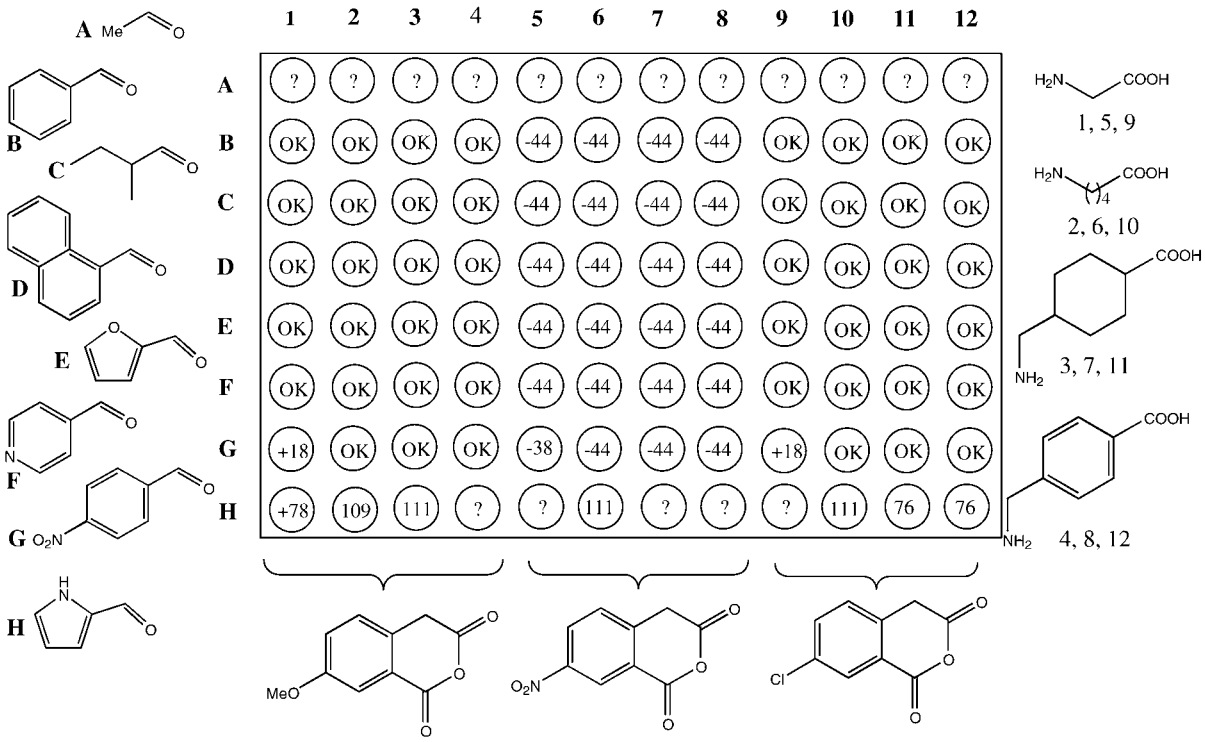


Figure 3. The LC-MS analytical results of the intermediates. '?' represents that a mixture of products was obtained; 'OK' represents that the peak of desired product was obtained; '–(+)number' represents that single compounds with certain number less (more) in the molecular weight than the expected 4-carboxy substituted ones were obtained.

ence of 4-nitro substitution stabilizing the formation of phenylmethyl anion. Among the most compounds in which glycine was used as a building block, although the 'N+1' peak was observed, the 'N+1–17' peak is predominant, which can be attributed to the loss of NH_3 in glycine amide part under current ionization condition.

Further indirect evidence of decarboxylation was provided by the analytical results of final products **VI**. All compounds with 4-methoxyhomophthalic anhydride and 4-chlorohomophthalic anhydride, except those with acetaldehyde and pyrrole 2-carboxaldehyde, gave desired amide products with corresponding amines. All intermediates with 4-nitrohomophthalic anhydride were not changed after the condensation.

Structure of 2-(7-nitro-1-oxo-3-phenyl-2,3,4-dihydroisoquinolyl) ethanamide as a product of solid-phase reaction (Tentagel-NH_2 resin) using glycine, benzaldehyde and 4-nitrohomophthalic anhydride as building blocks was proven by mass spectroscopy and NMR spectrum analysis.

Previous studies suggested that the presence of electron-deficient substituent on the phenyl ring is able to stabilize the formation of phenylmethyl anion, which leads to the decarboxylation of phenylacetic acid [16–17]. So it is reasonable to assume that we stimulated the 4-decarboxylation in **I** by using 4-nitro substituted homophthalic anhydrides. This decarboxylation might happen before or after the ring closure reaction.

To form a third site for diversity, we reduced the nitro group to amino group by Tin (II) chloride and acceptably pure compound was obtained (Figure 4D), in this case, MBHA resin was chosen because of its chemical stability to the reduction condition [18]. Then a set of carboxylic acids was used to react with the 7-amino group to give amide products, which can be obtained after anhydrous hydrogen fluoride cleavage [19]. Consequently, an efficient route for the solid-phase synthesis of 2,3,7-trisubstituted 3,4-dihydroisoquinolinones (Figure 1, II) through 4-decarboxylation has been developed, which has 2 diversity sites in the 2-piperidinone part of **I** and a new one on the phenyl ring.

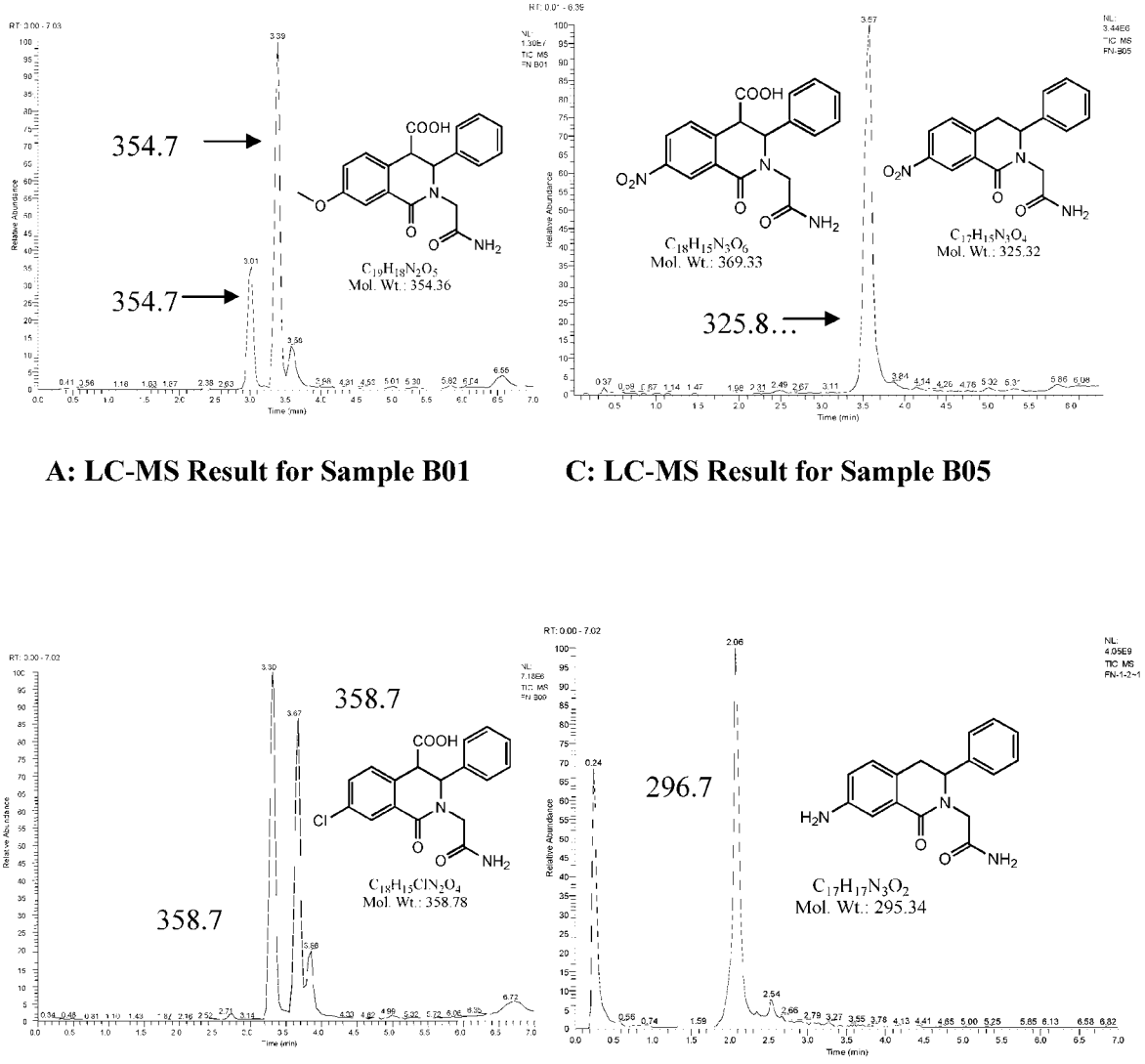
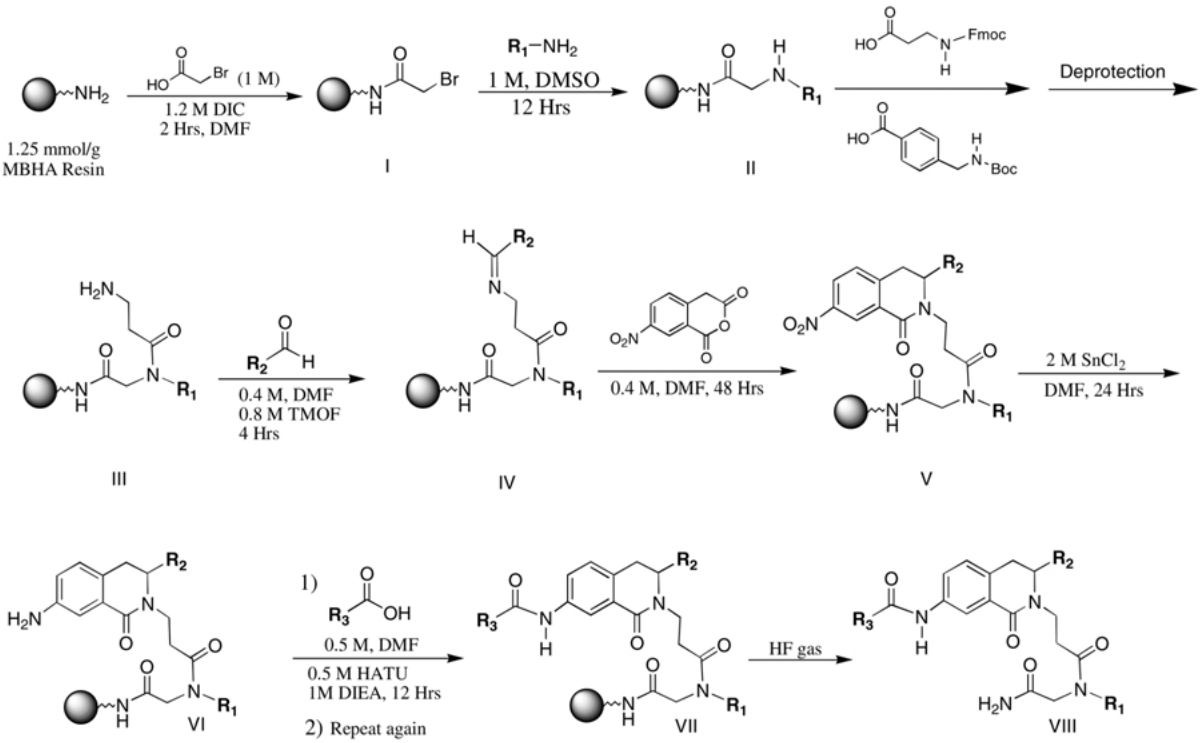


Figure 4. Examples of LC-MS results.

The scheme of library production is shown in Scheme 2. The list of building blocks is provided in Table 1 [20].

We applied a combination of 'tea-bags' and standard deep-well polypropylene microtiter plates in our parallel synthesis [13]. In this technology, most steps of synthesis are carried out in the small packages of resin encapsulated in the polypropylene mesh or so-called 'teabags'. Until the last step, the resin from the 'bags' which have satisfactory purity of intermediates is transferred to microtiter plates. Reaction is then carried out using a highly automated method.

As shown in Scheme 2, resin in 'teabags' was first coupled with bromoacetic acid, followed by 25 different primary amines and then 2 different amino acids to give intermediate III (Table 1). The whole peptoid part in intermediate III worked as R1 building block. After deprotection of Fmoc group, condensation with 16 different aldehydes (Table 1) and further reaction with 4-nitrohomophthalic anhydride gave isoquinoline intermediates V. At this stage, a comprehensive QC analysis for intermediates was carried out. A small amount of resin was sampled out of each intermediate teabag and placed in a well of



Scheme 2. The production route for library of 2,3,7-trisubstituted 3,4-dihydroisoquinolinones.

a microtiter plate separately. All the intermediates were reduced by tin (II) chloride solution and acylated with benzoic acids using HATU as activating reagent. The final products were cleaved from the resin using gaseous hydrogen fluoride and analyzed using LC-MS. The intermediates that gave the corresponding final product with >85% purity were carried on to the next production step. In our case, 720 of 800 intermediates passed this QC criterion. As an example, the LC chromatograms of 25 final compounds, which has 25 different amines and β-Ala as building block 1, 2-pyridinecarboxaldehyde as building block 2 and benzoic acid as building block 3, are shown in Figure 5.

After reduction, resin was evenly distributed as slurry into individual wells of microtiter plates, in which the incorporation of 48 different carboxylic acids took place. The final compounds were obtained in the 'one well, one compound' format after cleavage by gaseous hydrogen fluoride.

There are two QC criteria for every reaction plate. First, to make sure the purity of final products, we analyzed 12.5% of samples by using ELSD-HPLC (Evaporative Light Scattering Detection); their average purity must be over 75% to meet the requirement. Second, every sample was analyzed by SCIEX MS through direct injection to make sure that we get the expected compounds, 75% of samples must have the peak of desired product and the intensity of 'N+1' peak must be over 10%. Only the plates, which meet both requirements, can be considered as passing plates. In our case, 328 out of 350 reaction plates met both criteria. As an example, the LC traces of 48 final compounds, which has 3-(aminomethyl)pyridine and β-Ala as building block 1, 2-pyridinecarboxaldehyde as building block 2 and 48 carboxylic acids as building block 3, is shown in Figure 6.

In summary, an efficient route for the solid-phase synthesis of 2,3,7-trisubstituted 3,4-dihydroisoquinolinones through 4-decarboxylation has been developed. This route allows to bring together three different types of building blocks to construct a 2,3,7-trisubstituted 3,4-dihydroisoquinolinone template with a set of diverse substituents at various positions. A library of 38,400 compounds derived from 50 R^1s, 16 aldehydes (R^2) and 48 carboxylic acids (R^3) has been produced. After final QC, about 82% of compounds met the QC requirement.

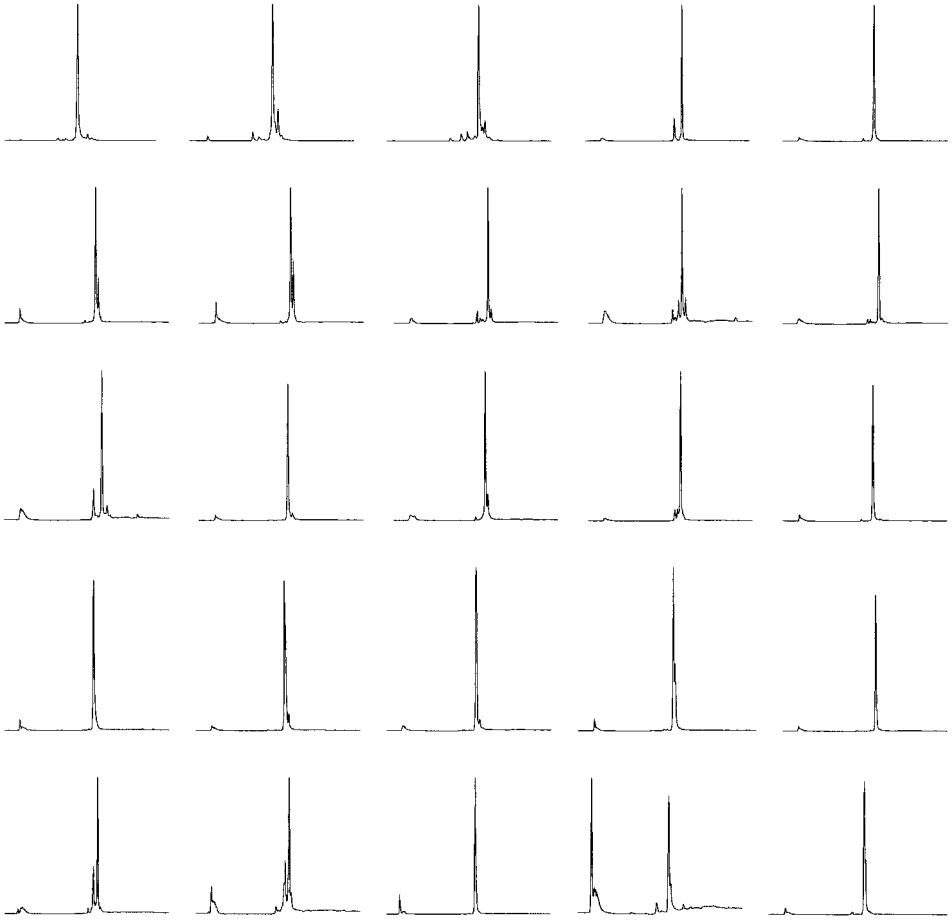


Figure 5. The LC traces of 25 final compounds, which has 25 different amines and β-Ala as building block 1, 2-pyridinecarboxaldehyde as building block 2 and benzoic acid as building block 3.

References

1. Lam, K. S., Lebl, M. and Krchnak, V., *The 'One-Bead-One-Compound' combinatorial library method*, Chem. Rev., 97 (1997) 411–448.
2. Lebl, M., *Solid-phase synthesis of combinatorial libraries*, Curr. Opin. Drug Discovery Dev., 2 (1999) 385–395.
3. (a) Dolle, R. E. and Nelson, K., *Comprehensive survey of combinatorial library synthesis: 1998*, J. Comb. Chem., 1 (1999) 235–282.
   Dolle, R. E., *Comprehensive survey of combinatorial library synthesis: 1999*, J. Comb. Chem., 2 (2000) 383–433.
4. Grunewald, G. *et al.*, *Synthesis and evaluation of 3-trifluoromethyl-7-substituted-1,2,3,4-tetrahydro-isoquinolines as selective inhibitors of phenylethanolamine N-methyltransferase versus the α2-Adrenoceptor*, J. Med. Chem., 42 (1999) 3315–3323.
5. Suto, M. J., Turner, W. R., Arundel-Suto, C. M., Werbel, L. M. and Sebolt-Leopold, *Dihydroisoquinolinones: The design and synthesis of a new series of potent inhibitors of poly(ADP-ribose)polymerase*, Anti-Cancer Drug Des., 6 (1991) 107–117.
6. Hutchinson, J. H., Cook, J. J., Brashear, K. M., Breslin, M. J., Glass, J. D., Gould, Robert J., Halczenko, W., Holahan, M. A., Lynch, R. J., Sitko, G. R., Stranieri, M. T. and Hartman, G. D., *Non-peptide glycoprotein Iib/IIIa antagonists. 11. Design and in vivo evaluation of 3,4-dihydro-1(1H)-isoquinolinone-based antagonists and ethyl ester prodrugs*, J. Med. Chem., 39 (1996) 4583–4591.
7. Maeda, H., Suzuki, M., Sugano, H., Yamamura, M. and Ishida, R., *Synthesis and central nervous system actions of thyrotropin-releasing hormone analogs containing a 1-oxo-1,2,3,4-3,4-dihydroisoquinolinone moiety*, Chem. Pham. Bull., 36 (1988) 190–201.
8. Robert, K. Y., Zee-Cheng and Cheng, C. C., *N-(aminoalkyl)imide antineoplastic agents. Synthesis and biological activity*, J. Med. Chem., 9 (1985) 1216–1222.
9. Ares, J. J., Kador, P. F. and Miller, D. D., *Synthesis and biological evaluation of irreversible inhibitors of aldose reductase*, J. Med. Chem., 29 (1986) 2384–2389.
10. Anderson, W. K., Heider, A. R., Raju, N. and Yucht, J. A., *Synthesis and antileukemic activity of bis(((carbamoyl)oxy)methyl)-substituted pyrrolo(2,1-a)iso quinolines, pyrrolo(1,2-a)quinolines, pyrrolo(2,1-*

R1	AMINES
1	N,N-Dimethylethylenediamine
2	2-Methoxyethylamine
3	Benzylamine
4	3-(Trifluoromethyl)benzylamine
5	Cyclopropylamine
6	Propylamine
7	Allylamine
8	3-Methoxybenzylamine
9	2-(4-Methoxyphenyl)ethylamine
10	2,3-Dimethoxybenzylamine
11	2,4-Dichlorophenethylamine
12	N,N-Diethyl-1,3-propanediamine
13	3-Ethoxypropylamine
14	N,N-Di-N-butylethylenediamine
15	1-(2-Aminoethyl)piperidine
16	1-(3-Aminopropyl)imidazole
17	4-(2-Aminoethyl)morpholine
18	2-(Aminomethyl)-1-ethyl-pyrrolidine
19	2-(2-Aminoethyl)pyridine
20	3-(Aminomethyl)pyridine
21	Acethydrazide
22	Methyl hydrazinocarboxylate
23	Piperazine
24	1-Amino-4-methylpiperazine
25	*

R1	AMINO ACIDS
1	Fmoc$^{\beta}$-Alanine
2	Fmoc-4-Aminomethylbenzoic acid

R2	ALDEHYDES
1	4-(Trifluoromethyl)benzaldehyde
2	4-(3-Dimethylaminopropoxy)benzaldehyde
3	4-Acetamidobenzaldehyde
4	3-(3,4-Dichlorophenoxy)benzaldehyde
5	3,5-Dimethoxybenzaldehyde
6	2-Imidazolecarboxaldehyde
7	4-Methylbenzaldehyde
8	3,4-Difluorobenzaldehyde
9	3-Cyanobenzaldehyde
10	Benzaldehyde
11	1-Napthaldehyde
12	2-Quinolinecarboxaldehyde
13	2-Pyridinecarboxaldehyde
14	4-(Methylthio)benzaldehyde
15	3-Pyridinecarboxaldehyde
16	4-tert-Butylbenzaldehyde

R3	CARBOXYLIC ACIDS
1	4-(Trifluoromethoxy)Benzoic Acid
2	2,6-Difluorobenzoic Acid
3	2-Pyrazinecarboxylic Acid
4	2-Furoic Acid
5	2,3,5,6-Tetrafluoro-P-Toluic Acid
6	3,4-Difluorobenzoic Acid
7	4-Formylphenoxyacetic Acid
8	2-(Trifluoromethyl)Cinnamic Acid
9	Diethylphosphonoacetic Acid
10	2-Fluoro-3-(Trifluoromethyl)Benzoic Acid
11	2-Fluorobenzoic Acid
12	4-Cyanobenzoic Acid
13	4-Acetylphenoxyacetic Acid
14	1-Phenyl-1-Cyclopropanecarboxylic Acid
15	Phthalide-3-Acetic Acid
16	Mesitylglyoxylic Acid
17	6-Methylchromone-2-Carboxylic Acid
18	2-Naphthoxyacetic Acid
19	3,5-Bis(Trifluoromethyl)Benzoic Acid
20	2-Chloronicotinic Acid
21	Fumaric Acid
22	2-Methylpyrazine-5-Carboxylic Acid
23	2-Bromo-5-Methoxybenzoic Acid
24	4-Iodobenzoic Acid

R3	CARBOXYLIC ACIDS
25	2-Bromobenzoic Acid
26	4-Methyl-1,2,3-thiadiazole-5-carboxylic acid
27	3,4,5-Trimethoxycinnamic Acid
28	2-(Methylthio)benzoic Acid
29	3-(Trifluoromethyl)phenylacetic Acid
30	2-Methylcyclopropanecarboxylic acid
31	2-Methylvaleric acid
32	Methoxyacetic acid
33	2-Propylpentanoic acid
34	3,5,5-Trimethylhexanoic acid
35	Vinylacetic acid
36	3-Cyclopentylpropionic acid
37	Hexanoic Acid
38	Tetrahydro-2-furoic acid
39	2-Nonenoic acid
40	Cyclohexanepropionic acid
41	Octanoic acid
42	3-Methoxycyclohexanecarboxylic acid
43	4-Methyl-1-cyclohexanecarboxylic acid
44	3-Methylthiopropionic acid
45	3-Methoxypropionic acid
46	Cyclopentylacetic acid
47	2-Norbornaneacetic acid
48	(Methylthio)acetic acid

Table 1. List of building blocks for library of 2,3,7-trisubstituted 3,4-dihydroisoquinolinones

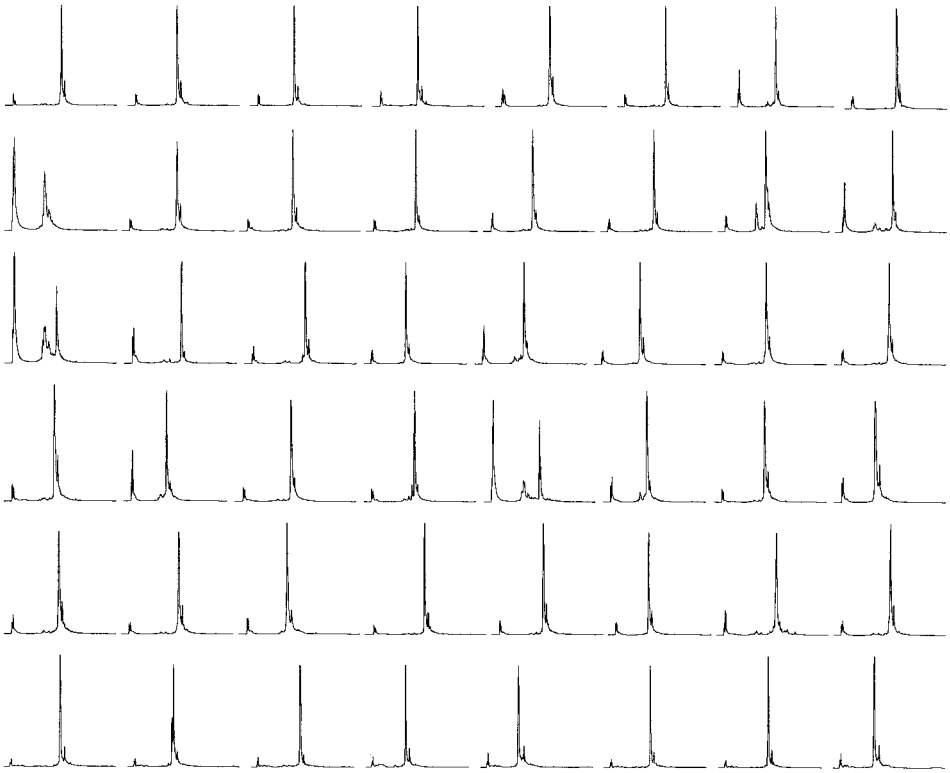


Figure 6. LC traces of 48 final compounds with 3-(aminomethyl)pyridine and β-Ala as building block 1, 2-Pyridinecarboxaldehyde as building block 2 and 48 carboxylic acids as building block 3.

a)isobenzazepines, pyrrolo(1,2-a) benzazepines, J. Med. Chem., 31 (1988) 2097–2102.

11. Cushman M. and Madaj, E. J., *A study and mechanistic interpretation of the electronic and steric effects that determine the stereochemical outcome of the reaction of schiff bases with homophthalic anhydride and 3-phenylsuccinic anhydride*, J. Org. Chem., 52 (1987) 907–915.
12. Griffith, M. C., Dooley, C. T., Houghten, R. A. and Kiely, J. S., 'Solid-phase synthesis, characterization, and screening of 43,000-compound 3,4-dihydroisoquinolinone combinatorial library', *Molecular Diversity and Combinatorial Chemistry*, ACS, Washington, DC, p. 50, 1996.
13. Solid-phase synthesis of large tetrahydroisoquinolinone arrays by two different approaches, Lebl, M., Krchnak, V., Ibrahim, G., Pires, J., Burger, C., Ni, Y., Chen, Y., Podue, D., Mudra, P., Pokorny, V., Poncar, P. and Zenisek, K., *Synthesis*, Stuttgart, 1971–1978, 1999.
14. Krchnak, V., Weichsel, A. S., Lebl, M. and Felder, S., *Automated solid-phase organic synthesis in micro-plate wells. Synthesis of N-(alkoxyacyl)amino alcohols*, Bioorg. Med. Chem. Lett., 7 (1997) 1013–1016.
15. Krchnak, V., Vagner, J. and Lebl, M., *Noninvasive continuous monitoring of solid-phase peptide synthesis by acid-base indicator*, Int. J. Pept. Protein Res., 32 (1988) 415–416.
16. Buncel, E., Venkatachalam, T. K. and Menon, B. C., *Carbanion mechanisms. Part 14. A spectrophotometric study of 4-nitro-, 2,4-dinitro-, and 2,4,6-trinitrobenzyl carbanions. Decarboxylation of (nitrophenyl)acetate anions*, J. Org. Chem., 49 (1984) 413–417.
17. Trahanovsky, W. S., Cramer, J. and Brixius, D. W., *Oxidation of organic compounds with cerium (IV). XVIII. Oxidative decarboxylation of substituted phenylacetic acids*, J. Amer. Chem. Soc., 96:4 (1974) 1077–1081.
18. We observed that the Rink linker could be cleaved by HCl formed in tin(II) chloride solution, resulting in lower yield (10%).
19. Lebl, M., Pires, J., Poncar, P. and Pokorny, V., *Evaluation of gaseous hydrogen fluoride as a convenient reagent for parallel cleavage from the solid support*, J. Comb. Chem., 1 (1999) 474–479.
20. To reduce the list of potential building blocks to a manageable size, large number of building blocks are sieved through several layers of filters, such as availability, compatibility and molecular weight. Building blocks that fit these criteria are computationally evaluated using our two-step diversity analysis process to ensure the library produced is maximally diverse. For further details, see: R. S. Pearlman and K. M. Smith, Drugs Future, 23 (1998), 885.