# A Focused Library of Tetrahydropyrimidinone Amides via a Tandem Biginelli–Ugi Multi-Component Process

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**Abstract:** A second generation focused library of 30 tetrahydropyrimidinone amides was prepared. The design was based on the structure of an Hsp70 modulator. This small library demonstrates the utility of tandem multi-component reactions in structure–activity relationship studies of biological lead molecules. The tandem Biginelli–Ugi multi-component reaction facilitated the effective variation of four different substituents and allowed the synthesis of the library members in a sequence of only two one-pot reactions.

**Key words:** Biginelli reactions, Ugi reactions, cascades, Hsp70, pyrimidinones

A multi-component reaction (MCR) represents a sequence of bimolecular events leading to products that incorporate essentially all atoms of three or more starting materials.[1] MCRs are atom economical and convergent, and provide a large increase in structural complexity. Thus, these reactions are increasingly popular for the synthesis of combinatorial libraries and biological tools as well as in drug discovery efforts at pharmaceutical companies.[2,3]

The three-component Biginelli reaction[4] combines readily available aldehydes and ureas with β-keto esters, leading to the medicinally attractive tetrahydropyrimidinone scaffold.[5] Although MCRs have been combined with click chemistry,[6] Diels–Alder reactions,[7] Staudinger/aza-Wittig reactions,[8] and various cyclization strategies[9] in order to obtain an even higher degree of complexity, only a few examples of tandem[10] (i.e. cascade,[11] or domino[12]) multi-component reactions (TMCRs) like the Petasis–Ugi[13] or the Ugi–Ugi[14] reaction are known. In this context, the combination of the three-component Biginelli tetrahydropyrimidinone synthesis with the four-component Ugi condensation[15] would appear to be particularly attractive, since six individual reaction components could be combined in a two-pot sequence to yield a large increase in structural diversity. Furthermore, the formation of the typical acyclic amide backbone in the Ugi reaction is nicely complemented by the heterocyclic scaffold that emerges from the Biginelli step.

We recently reported preliminary results for a Biginelli–Ugi tandem multi-component reaction sequence and demonstrated that the products of this transformation have the ability to modulate endogenous and co-chaperone-stimulated Hsp70 ATPase activity.[16] Hsp70 molecular chaperones display an important function in cellular processes like protein folding, degradation, and transport,[17] and have been linked to a number of human diseases like cystic fibrosis,[18] Parkinson's[19] and Huntington's disease,[20] as well as cancer.[21] Only a few small molecule modulators of Hsp70 are known to date.[22] Although several compounds from a targeted library of tandem Biginelli–Ugi MCR products did not affect Hsp70 chaperone function, others impacted only the ATPase activity of Hsp70. The most interesting class of modulators, exemplified by MAL3-101 (**1**{*1*,*1*,*1*}) and MAL3-39 (**2**) (Figure 1), had no effect on the $K_{CAT}$ for ATP hydrolysis, but blocked the ability of Hsp70 co-chaperones to enhance Hsp70 ATPase activity. Compound MAL3-51 (**3**) showed no activity and was used as a negative control in these assays.[16]

These biological data encouraged us to synthesize a second generation focused library based on MAL3-101 (**1**{*1*,*1*,*1*}). Herein, we report the design and synthesis of this library and include detailed experimental protocols for the tandem Biginelli–Ugi MCR.

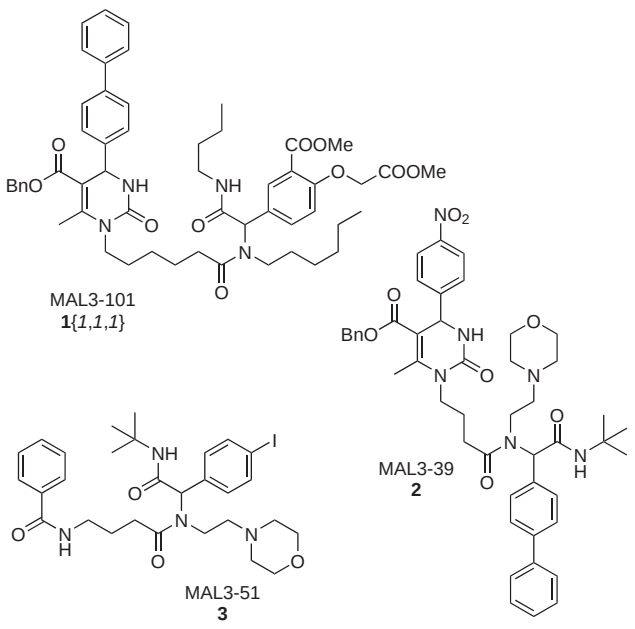


**Figure 1** Biological probe molecules with (MAL3-101 and MAL3-39) and without (MAL3-51) Hsp70 ATPase modulating activity

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The desired tetrahydropyrimidinone scaffold was obtained from the Biginelli reaction of benzyl acetoacetate (**5**), one of twelve aldehydes **4**{*1–12*}, and 6-ureidohexanoic acid (**6**) in THF in the presence of a catalytic amount of HCl. 6-Ureidohexanoic acid was used as a bifunctional building block that served as the urea component in the Biginelli reaction and as the acid component in the subsequent Ugi condensation. In order to allow a detailed structure-activity relationship (SAR) analysis, the library was synthesized in the indexed[23] library format, in analogy to our earlier library synthesis of peptide bond isosteres.[3c] Only a single change to the substituents on MAL3-101 was made in any given sequence. Previously, our targeted library[16] of tetrahydropyrimidinone amides varied the oligomethylene chain of the ureido carboxylic acid, and we found that a five carbon linker provided the most active derivatives. Therefore, only 6-ureidohexanoic acid (**6**) was used in this focused library. In order to investigate the importance of the 4-biphenyl substituent on the activity of **1**{*1,1,1*} as an Hsp70 modulator, the aldehyde component **4**{*1–12*} was varied. To probe the importance of aromaticity and conformation at this position, analogues were prepared using 4-cyclohexylbenzaldehyde **4**{*2*}, 4-*tert*-butylbenzaldehyde **4**{*3*}, 2-naphthaldehyde **4**{*4*} and 2-phenylbenzaldehyde **4**{*5*}. Based on the activity of the related MAL3-39, 4-nitrobenzaldehyde **4**{*6*} and benzaldehydes **4**{7–9} containing electron-withdrawing substituents were incorporated. Finally, in an effort to understand the size requirements at this position, and with a goal of reducing molecular weight and lipophilicity, smaller benzaldehydes and aliphatic aldehydes **4**{*10–12*} were chosen as reactants (Figure 2). Twelve tetrahydropyrimidinones **7**{*1–12*} were thus obtained in very high purity. In most cases, precipitation from a mixture of diethyl ether and hexanes proved to be sufficient for purification (Scheme 1). Yields ranged from very modest in the case of the aliphatic aldehydes **4**{*11,12*} to high for all aromatic derivatives **4**{*1–10*} (Table 1).

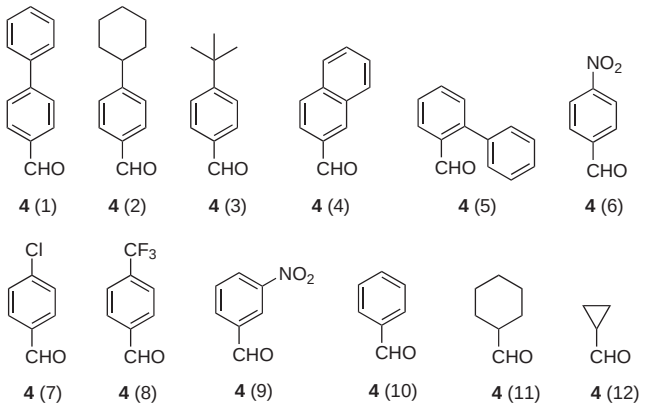
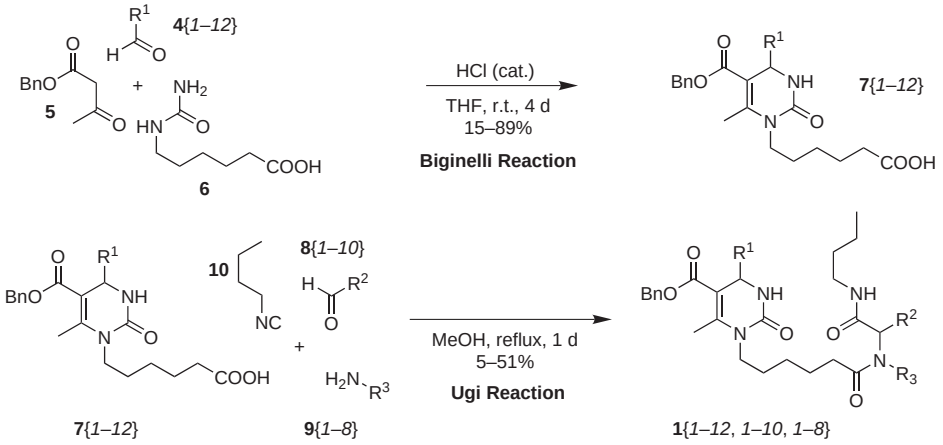



**Figure 2** Aldehydes **4**{*1–12*} as building blocks for the Biginelli reaction

**Table 1** Synthesis of Tetrahydropyrimidinones **7**{*1–12*}

| Compound | Isolated yield (%) | Purity (%)[a] |
|---|---|---|
| **7**{*1*} | 76 | >99 |
| **7**{*2*} | 51 | >99 |
| **7**{*3*} | 58 | >99 |
| **7**{*4*} | 81 | 97 |
| **7**{*5*} | 82 | >99 |
| **7**{*6*} | 78 | 99 |
| **7**{*7*} | 42 | >99 |
| **7**{*8*} | 75 | 97 |
| **7**{*9*} | 45 | >99 |
| **7**{*10*} | 89 | >99 |
| **7**{*11*} | 15 | >99 |
| **7**{*12*} | 23 | 98 |

[a] Determined by LC-MS-ELSD analysis.




**Scheme 1** Synthesis of tetrahydropyrimidinone amides **1**{*1–12,1–10,1–8*} in a Biginelli–Ugi TMCR

Next, the twelve tetrahydropyrimidinones **7**{*1–12*} were used as building blocks for the four-component Ugi reaction. Out of the four variable building blocks for this step, only the isocyanide component **10** was kept constant. Ten aldehydes **8**{*1–10*} were used as starting materials, replacing the 5-formyl-2-methoxycarbonylmethoxybenzoic acid methyl ester building block that was incorporated into the MAL3-101 lead structure. The choice of building blocks was based on an effort to understand the contribution of the functional groups within the benzoic acid methyl ester moiety to activity (e.g. **8**{*1–5*}), and to optimize properties such as molecular weight, lipophilicity, and solubility (**8**{*6–9*}). Consideration of the corresponding substituent in MAL3-39 dictated the incorporation of 4-biphenylcarboxaldehyde **8**{*10*} (Figure 3). The amine component was also varied to inclue linear (**9**{*1,2*}) and branched aliphatic amines (**9**{*3*}) as well as aromatic and heteroaromatic amines containing methylene and ethylene linkers (**9**{*4–8*}) (Figure 4). These building blocks were chosen to interrogate the steric and electronic requirements for activity (e.g. **9**{*1–6*} as well as to incorporate basic functionality into this position as is present in MAL3-39 (**9**{*7,8*}).

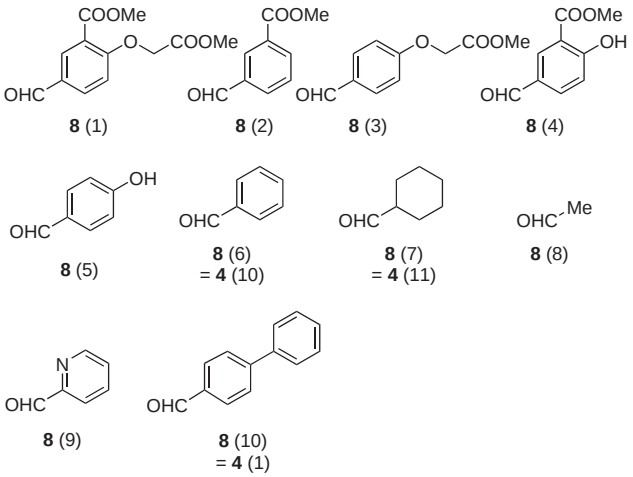



**Figure 3** Aldehydes **8**{*1–10*} as building blocks for the Ugi reaction

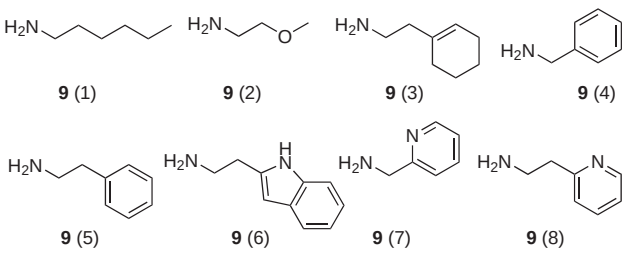


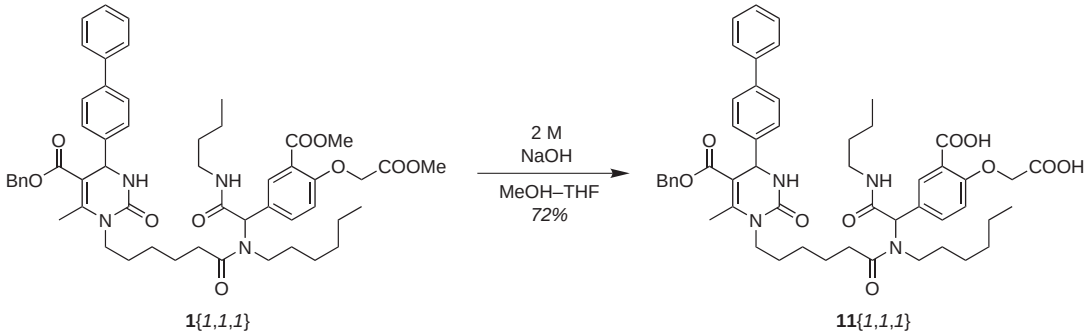

**Figure 4** Amines **9**{*1–8*} as building blocks for the Ugi reaction

In contrast to the Biginelli reaction, the Ugi step required more vigorous reaction conditions. The four components were heated at reflux in MeOH for 24 hours to give the desired tetrahydropyrimidinone amides **1**{*1–12,1–10,1–8*} in an average purity of 96% based on ELSD analysis after purification on an ISCO Companion chromatography system (Table 2). The final compounds were obtained in an average yield of 25% and in amounts ranging from 32 to 242 mg for most library members and up to 3.00 g for **1**{*1,1,1*}.

**Table 2** Synthesis of Tetrahydropyrimidinone Amides **1**{*1–12,1–10,1–8*}[a]

| Compound | Yield of Ugi reaction (%) | Purity (%)[b] |
|---|---|---|
| **1**{*1,1,1*} | 19 | >99 |
| **1**{*2,1,1*} | 38 | 92 |
| **1**{*3,1,1*} | 18 | >99 |
| **1**{*4,1,1*} | 27 | 93 |
| **1**{*5,1,1*} | 23 | 95 |
| **1**{*6,1,1*} | 35 | 94 |
| **1**{*7,1,1*} | 32 | 97 |
| **1**{*8,1,1*} | 15 | 87 |
| **1**{*9,1,1*} | 28 | 95 |
| **1**{*10,1,1*} | 35 | 97 |
| **1**{*11,1,1*} | 18 | 97 |
| **1**{*12,1,1*} | 36 | 87 |
| **1**{*1,2,1*} | 30 | 97 |
| **1**{*1,3,1*} | 6 | >99 |
| **1**{*1,4,1*} | 17 | 95 |
| **1**{*1,5,1*} | 35 | 95 |
| **1**{*1,6,1*} | 51 | 99 |
| **1**{*1,7,1*} | 33 | 98 |
| **1**{*1,8,1*} | 28 | 93 |
| **1**{*1,9,1*} | 51 | 98 |
| **1**{*1,10,1*} | 37 | >99 |
| **1**{*1,1,2*} | 32 | 99 |
| **1**{*1,1,3*} | 16 | 88 |
| **1**{*1,1,4*} | 9 | >99 |
| **1**{*1,1,5*} | 8 | >99 |
| **1**{*1,1,6*} | 11 | 99 |
| **1**{*1,1,7*} | 5 | 99 |
| **1**{*1,1,8*} | 9 | 98[c] |
| **3** | 75 | 99[c] |

[a] Labels within parentheses refer to aldehyde **4**, aldehyde **8**, and amine **9** components, respectively.
[b] Determined by LC-MS-ELSD analysis.
[c] Purity by UV at 210 nm.




**1**{*1,1,1*} **11**{*1,1,1*}

**Scheme 2** Synthesis of the free acid **11**{*1,1,1*} via hydrolysis of **1**{*1,1,1*}.

In order to investigate if the diester **1**{*1,1,1*} is the active component in the biological assay for Hsp70 chaperone function or if it is hydrolyzed to the diacid **11**{*1,1,1*} under the aqueous assay conditions, **11**{*1,1,1*} was also synthesized by hydrolysis of **1**{*1,1,1*} (Scheme 2).

In summary, we have synthesized a second generation focused library of 30 tetrahydropyrimidinone amides **1**{*1–12,1–10,1–8*} based on the structure of MAL3-101 that was previously found to block the ability of Hsp70 cochaperones to enhance Hsp70 ATPase activity without affecting the $K_{CAT}$ for ATP hydrolysis. This library demonstrates the utility of TMCRs in SAR studies of biological lead molecules. The tandem Biginelli–Ugi MCR facilitated the effective variation of four different substituents and allowed the synthesis of the library members in a sequence of only two one-pot reactions. Detailed studies on the ability of the tetrahydropyrimidinone amides **1**{*1–12,1–10,1–8*} to modulate Hsp70 ATPase activity are ongoing and will be reported in due course.

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