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Functionalized Spiroindolines with Anticancer Activity through a Metal-Free Post-Ugi Diastereoselective One-Pot Cascade Reaction

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Abstract

A post-Ugi diastereoselective one-pot cascade reaction requiring no metal catalyst was developed. The reaction scope was wide with mild conditions and good yields. A collection of spiroindolines was prepared by the protocol and screening tests in several difficult-to-inhibit cancer cell lines were conducted. The relationship of structure and anticancer activities was promising and in the Huh7 cell lines compound **16 j** is more potent than Vinblastine. The cyclization design strategy could be applicable to other multicomponent reactions (MCRs) for synthesizing bioactive and drug-like heterocycles.

Keywords

cyclization reactions; multicomponent reactions; spiroindolines; synthetic methods; Ugi reaction

Spiroindoline derivatives including pharmaceuticals, natural products, and privileged scaffolds are important heterocyclic compounds and exhibited a wide range of biological properties as shown in Figure 1. Vinblastine **1** is a legendary marketed drug for the treatment of a variety of cancers.^[1] Perophoramidine **2**, a natural product, demonstrated an exciting cytotoxicity against the HMT 116 colon carcinoma cells.^[2] Communesin F **3** and its

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Conflict of interest

The authors declare no conflict of interest.

analogues were isolated from marine sources and showed significant antileukemic and insecticidal activities,^[3] and aspidophytine **4** showed broad anti-insecticidal activities.^[4]

Recently, privileged scaffolds represented by spiroindolines **5** and **6** were prepared through a multi-component post-Ugi cascade reaction with an improved two or three step operation.^[5] However, rhodium(II)^[5a] or gold-catalysts^[5b] were required in the synthesis. The cost-effective and environment-friendly conditions, short reaction steps, and simple work-ups are still desirable.^[6]

During the past few years, we have been focusing on the use of the Ugi four-component reaction (U-4CR) for the synthesis of nitrogen-containing heterocycles in a cost-effective manner without medal catalyst.^[7] In the construction of the pyrrolopyridinone core as shown in Scheme 1, the unpurified Ugi product **7** was used for the pyrrole cyclization reaction under the previously optimized conditions (i.e., 10 % TFA/DCE solution, 150 °C, microwave irradiation, 10 min) to give benzimidazoisquinoline **8**.^[8] A C–C bond between the carbonyl (ketone) carbon atom and the C-3 carbon atom of the pyrrole moiety, rather than a C–N bond between the carbonyl (ketone) carbon and the nitrogen atom of the pyrrole, was formed in situ under acidic conditions. We therefore designed and prepared Ugi indole analogue **9**, which has reduced nucleophilicity of the indole C-3 carbon upon the introduction of the Boc-protecting group to the indole nitrogen. The C-2 carbon position was activated with the substitution of a nitrogen atom on the C-3 carbon atom of the indole moiety. We envisaged that if the cyclization reaction occurs at the C-2 carbon atom of the indole moiety, the resulting iminium cation **9a** could lead to sequential cyclization of the nitrogen atom of another amide with the iminium cation to produce highly functionalized spiroindoline **10**.

We initially investigated the possibility of using *tert*-butyl 3-amino-1*H*-indole-1-carboxylate **13** as a starting material in an Ugi reaction with 2-oxo-2-phenylacetic acid **11a**, aldehyde **12a**, and isocyanide **14a** to form the Ugi product **15a** (in Scheme 2). With compound **15a**, the previously optimized acidic conditions (10 % TFA/DCE solution, 150 °C, microwave irradiation, 10 min) was tested.^[8] Unprecedentedly, the diastereoselective formation of the fully functionalized spiroindoline **9a** occurred without a medal catalyst in one pot. Other diastereomers were not detected in the HPLC-MS determination and column purification. As expected, a *5-exo-dig* indole cyclization likely occurred to produce proposed intermediate **9a**, which underwent a second sequential cyclization and aromatization to give compound **16a** in a diastereoselective manner in 53 % yield (Scheme 2). The relative stereochemistry of compound **16a** was unambiguously determined to be the *trans*-conformation by X-ray crystallography (Figure 2).^[9]

It was anticipated that analogous tetracyclic spiroindoline **16a** could afford a broad range of bioactive compounds with potential applications in anticancer research.^[10] To synthesize a small library of spiroindolines for biological screenings, we further optimized reaction conditions for high yield. A variety of different acids, bases, temperatures, and reaction times (Table 1) were investigated. The U-4CR proceeded well in methanol to give Ugi adduct **15a**.^[7] The acidic conditions with 5 % HCl in AcOH or 10 % TFA/DCE (DCE = 1,2-dichloroethane) was examined at different temperatures. Gratifyingly, the microwave

irradiation at 100 °C in 10 % TFA/DCE for 10 min could afford the desired product **16a** in 92 % yield. The higher temperatures at 150 and 120 °C led to a sharp reduction of yields to 53 and 63 %, respectively. It is noteworthy that the reaction failed to afford the desired product **16a** when it was conducted without microwave irradiation at room temperature or in refluxing solvent (Table 1, entries 7 and 8). We also investigated the cyclization reaction with a variety of different organic and inorganic bases under microwave irradiation conditions (Table 1, entries 9–16). Interestingly, organic bases still could afford compound **16a** (entries 10–13), but the yields were much lower. The use of inorganic bases failed to afford compound **16a** (entries 15–16). Hence, the optimized reaction conditions are shown in entry 3: the reaction was heated with microwave irradiation at 100 °C for 10 min in 10 % TFA/DCE.

Encouraged by the remarkable yield under the optimized conditions, we investigated the scope of this transformation by varying starting materials. In all cases, the initial Ugi product **15** was used directly in the next step without purification after the removal of solvent under a gentle stream of nitrogen. A variety of different starting materials were employed under the optimized conditions for the construction of structurally diverse tetracyclic spiroindolines **16a–n** with yields in the range of 65–89 % (Table 2), indicating good functional-group tolerance. It is noteworthy that the final products were readily purified by column chromatography over silica gel in high purity (>95 %).

Based on the above experimental results, we have postulated a plausible mechanism for the diastereoselective cyclization reaction, which is shown in Figure 3. An enantiomer, the *R*-intermediate (**17a**, not isolated), would undergo a nucleophilic attack of the iminium cation from the nitrogen atom in the remaining amide. Under the acidic conditions, a double bond was clearly formed spontaneously or subsequently with concomitant dehydration to give the final *trans* compound **16a** (*R,R*). The diastereoselective formation of the *trans* product could be explained by the favored lipophilic interaction between two benzene ring moieties substituted on two chiral centers. In addition, two oxygen atoms would have lipophobic and steric hindrance to the benzene group as shown in compound **16a'** (*S,R*). The reaction is expected proceed under thermodynamic control and the relative energy was calculated for compound **16a'** (*S,R*) with 8.42 kcal mol⁻¹ higher over **16a** (*R,R*) with 0.0 kcal mol⁻¹.^[11]

To evaluate potentials for developing a drug candidate from compounds **16a–n**, a thymidine incorporation assay was used to measure cancer cell viability upon treatment. The cancer cell lines PANC-1, Huh7, MDAMB468 and MHCC97-H were selected, which are some of the more difficult-to-inhibit cell lines in the National Cancer Institute's 60 human tumor cell lines panel. Compounds **16b**, **16e–h**, and **16j** exhibited respectful anticancer activities in the human liver cancer Huh7 cell lines with IC₅₀ less than 10 μM in Table 3. Remarkably, compound **16j** inhibited the Huh7 cell line proliferation with IC₅₀ of 2.0 μM, in contrast vinblastine **1** was only active on the same cell line with an IC₅₀ of 45.6 μM. Compounds **16a** and **16f** were also active against the triple negative breast cancer MDAMB468 cell lines with IC₅₀ of 6 μM and 8 μM, respectively. Compounds **16c** and **16h** inhibited the cancer cell proliferation in another liver cancer MHCC97-H cell lines with IC₅₀ of 5 μM and 8 μM, respectively.

In conclusion, we have developed an unprecedented one-pot Ugi-post cascade reaction that proceeds under metal-free conditions for the diastereoselective construction of highly functionalized tetracyclic and drug-like spiroindolines. This method can be expanded for synthesizing structurally diverse spiroindolines by varying starting materials. The isolated final products as well as the proposed reaction mechanism are consistent with the hypothesis and design (Scheme 1). The cascade cyclization design approach would be highly applicable to other multi-component reactions such as Passerini,^[12] Petasis,^[13] Betti,^[14] Kabachnik–Fields,^[15] Mannich,^[16] among others. With the mild reaction conditions for affording products in good yield and commercially available starting materials, we have quickly prepared a small library of structurally diverse spiroindolines. The screening results are promising, particularly with low micromolar inhibition IC₅₀ (**16j**) in the Huh7 cell lines, which is more potent than Vinblastine **1**, a well-known anti-cancer drug. Further efforts are ongoing studying the mechanism of action of lead compound **16j** and the optimization of its potency and drug properties. Efforts will be also directed toward the application of the cascade cyclization design strategy to the total synthesis of natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Natural and privileged spiroindolines.

Figure 2.
X-ray crystal structure of compound **16 a**.

Figure 3.
Proposed synthetic mechanism leading to compound **16a**.

Scheme 1.
Approach to indole cyclization.

Scheme 2.
Synthetic strategy to prepare compound **16a**.

Table 1Optimization of reaction for compound **16a**.

Entry	Solvent and conditions	T [°C]	Time [min]	Yield [%] ^[a]
1	10 % TFA/DCE	MW 80	10 min	65
2	10 % TFA/DCE	MW 90	10	73
3	10% TFA/DCE	MW 100	10	92
4	10 % TFA/DCE	MW 120	10	63
5	10 % TFA/DCE	MW 100	20	81
6	5 % HCl/AcOH	MW 100	10	42
7	10 % TFA/DCE	r.t.	720	NR
8	10 % TFA/DCE	reflux	360	NR
9	DMF/DBU (2 equiv.)	MW 150	10	trace
10	DMF/DBU (2 equiv.)	MW 150	20	12
11	DMF/DBU (2 equiv.)	MW 150	30	18
12	DMF/DBU (2 equiv.)	MW 180	30	32
13	DMF/DIPA (2 equiv.)	MW 150	30	24
14	DMF/TEA (2 equiv.)	MW 150	30	NR
15	DMF/K ₂ CO ₃ (2 equiv.)	MW 150	30	NR
16	DMF/NaOH (2 equiv.)	MW 150	30	NR

^[a]Yield of isolated product.

MW = microwave irradiation, NR = No reaction.

Table 2Scope of the Ugi/domino cyclization route leading to spiroindolines **16 a–n**.^[a] ^[a]Yield of isolated product for one-pot procedure.

Table 3

Anticancer activities IC₅₀ (μM) of compounds **16a–n** for cancer cell lines.^[a]

Entry	Cpd.	PANC-1	Huh7	MDAMB468	MHCC97-H
1	16 a	35 ± 2.6	19 ± 2.5	6±1.1	20 ± 1.5
2	16 b	22 ± 4.6	7±2.1	18 ± 2.1	17 ± 2.2
3	16 c	31 ± 5.6	30 ± 1.2	29 ± 2.1	5±1.3
4	16 d	39 ± 4.2	16 ± 1.3	10 ± 1.1	34 ± 5.6
5	16 e	10 ± 4.2	8±0.8	15 ± 2.3	10 ± 1.2
6	16 f	30 ± 2.2	4±1.1	8±1.2	13 ± 1.8
7	16 g	32 ± 1.4	5±1.1	18 ± 2.2	39 ± 5.1
8	16 h	16 ± 5.1	4±1.2	15 ± 1.4	8±1.2
9	16 i	67 ± 2.0	63 ± 1.1	48 ± 2.2	65 ± 1.2
10	16 j	18 ± 8.2	2±1.5	21 ± 2.2	15 ± 2.5
11	16 k	18 ± 6.5	55 ± 3.8	12 ± 0.4	32 ± 4.2
12	16 l	79 ± 1.2	77 ± 2.2	53 ± 3.3	77 ± 4.7
13	16 m	67 ± 3.1	42 ± 3.2	42 ± 2.5	38 ± 4.8
14	16 n	56 ± 4.5	29 ± 1.1	31 ± 3.2	30 ± 2.8

^[a]All MTT assays were repeated three times by using six samples per assay.