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Synthesis of Constrained Heterocycles Employing Two Post-Ugi Cyclization Methods for Rapid Library Generation with In Cellulo Activity

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Abstract

Benzimidazoles and quinoxalinones are present in the core of many pharmacologically relevant compounds. Several combinatorial methods have been developed to attach ring systems to both scaffolds for derivatization at select positions. Herein, we describe the development of novel constrained heterocyclic compounds attached to the N1 position of both benzimidazole and quinoxalinone scaffolds. Utilizing robust post-Ugi cyclization methods, including the Ugi-deprotection-cyclization (UDC) methodology, allows for efficient access to a new area of chemical space. Additionally, molecular modeling and in cellulo screening was employed to therapeutically validate the compounds formed with this method.

Keywords

Heterocycle Formation; MDM2/MDMX; Multicomponent Reaction; Post-Ugi Modifications; Ugi Condensation

Introduction

Benzimidazoles and quinoxalinones are important heterocycles in medicinal chemistry due to their pharmacological properties. The benzimidazole scaffold demonstrates anticancer,

Supplementary Information

The supplementary information contains all experimental details, including protocols for each synthetic step, computational modelling and the MTT assay, as well as characterization data for all key intermediates and final compounds, including ¹H and ¹³C NMR spectra.

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Conflict of Interest

The authors declare no conflict of interest.

antimicrobial, antiulcer, anti-inflammatory, antiviral, antihypertensive, antihistaminic, antithrombotic, antidiabetic, and psychoactive effects.^[1] This wide range of activity has led to numerous marketed benzimidazole-based therapeutics including bend-amustine (anticancer),^[1a] albendazole (antimicrobial),^[1f] mebendazole (antimicrobial),^[1k] omeprazole (antiulcer),^[1g] candesartan (antihypertensive),^[1k] telmisartan (antihypertensive),^[1l] and astemizole (antihistaminic).^[1m] Similar to benzimidazole, quinoxalinones display a wide range of therapeutic activity including anticancer, antimicrobial, anti-inflammatory, antiviral, antithrombotic, and antidiabetic activities.^[2]

The broad range of therapeutic applications for both heterocycles has made them attractive targets for combinatorial efforts. Previous synthetic methods have focused on adding additional rings directly to the heterocycles, or expanding at the 2, 5, or 6 position of benzimidazoles, or the 3, 6, and 7 position of quinoxalinones.^[3] Interestingly, few combinatorial syntheses have focused on the N1 position of either molecule and, to the best of our knowledge, separate heterocycles have not been attached at this position. Therefore, methods to expand into new chemical space are highly valuable to generate molecules with novel biological and therapeutic properties.

In our continuing efforts to develop efficient synthesis of drug like molecules,^[4] we have developed diverse libraries of benzimidazole and quinoxalinone fused-heterocycles through a rapid, three step Ugi condensation and post-Ugi cyclization synthetic protocol. Since its discovery in 1959,^[5] the Ugi condensation reaction has become an essential approach in combinatorial chemistry. This four-component reaction has been utilized by the pharmaceutical industry for expansive chemical library formation due to its robust and simple nature.^[6] One drawback to the Ugi condensation is the production of linear products with poor drug properties. Post-Ugi modifications, such as the Ugi Deprotection Cyclization (UDC), the Ugi Activation Cyclization (UAC),^[7] and the Ugi Deprotection + Activation Cyclization (UDAC),^[8] enhance molecular complexity and drug-like characteristics. These methods are valuable in combinatorial work enabling the formation of large libraries in minimal steps and with high overall yields.^[9] The UDC method unmasks a nucleophile through removal of a protection group,^[10] the UAC method employs a convertible isocyanide, and the UDAC method is a combination of both the UDC and UAC strategies. Through activation, the nucleophile cyclizes with an available carbonyl group to form a heterocyclic ring. Cyclizations have also been accomplished using S_NAr techniques.^[11] Particularly notable contributions have come from C. Hulme, A. Domling, and J. Zhu.^[3e, 6a, d, 12]

Previously, Hulme has described double cyclization techniques utilizing two Boc-protected amines that complete the cyclization following deprotection.^[13] We accessed these unique dual heterocycle scaffolds using two distinct reactive functions that cyclize independently. First, we employed the UDC technique, revealing a masked amine to form the benzimidazole and quinoxalinone heterocycle. Following, the second cyclization was completed with an efficient S_NAr reaction or amide bond formation. The dual cyclization further increases rigidity, improves drug-like properties, and enhances molecular complexity, resulting in the novel library formation in three efficient steps. Additionally, this method allows for supplementary synthetic modification of the cyclization products. Lastly, to

validate the utility of this method, products were virtually screened and found to inhibit the p53/MDMX protein-protein interaction, and products were shown to have in cellulo activity against a pancreatic cancer cell line.

Results and Discussion

To form the benzimidazole compounds, the Ugi reaction was performed with various carboxylic acids, isocyanides, mono-N-Boc-o-phenylenediamine, and 2-fluoro or 2-carboxy benzaldehyde (Scheme 1). This reaction formed the bond between the future benzimidazole and the second heterocycle. Deprotection of the diamine resulted in the first cyclization to complete the benzimidazole portion. The second cyclization occurred one of two ways: (1) Intermediates formed using 2-fluorobenzaldehyde were heated with Cs_2CO_3 to promote fluorine nucleophilic replacement to generate the oxindole moiety, or (2) intermediates formed using 2-carboxybenzaldehyde were heated with DIPA to generate the isoquinolindione group. Eleven examples using the protocol were generated with these starting materials (Figure 1).

To form the quinoxalinone compounds, a similar procedure was utilized but the starting carboxylic acid was substituted with various glyoxylic acids to obtain the larger ring (Scheme 2). Again, the Ugi condensation formed the bond between the two heterocycles. Removal of the Boc group resulted in cyclization to form the quinoxalinone heterocycle. Then, one of the two previously mentioned cyclization methods were used to form the oxindole and isoquinolindione heterocycles. A further 5 examples were produced using the protocol with glyoxylate starting materials (Figure 2).

This method is optimal for large library formation because it permits further derivatization on benzimidazoles and quinoxalinones. The 2, 5, and 6 positions of the benzimidazoles and the 3, 6, and 7 positions of the quinoxalinones are all available for common derivatization techniques, and additionally, it is combined with the oxindole or isoquinolindione groups for further expansion. This results in the ability to add diversity to the dual-heterocycle core and leads to the potential of expansive library generation.

We investigated a method to increase molecular diversity by further modifying the original products (Scheme 3). Starting with P72-2 or P73-2, the ester was hydrolyzed and subsequently reacted with amines using EDC-based peptide coupling. This step produced four additional examples (Figure 3). From this study, the newly reported benzimidazoles were found compatible with traditional expansions of benzimidazoles at position 2.

Select final compounds were screened in a virtual library and found to bind to MDMX, an important negative regulator of the p53 tumor suppressor (Figure 4).^[14]

The p53/MDMX protein-protein interaction is a highly sought after drug target for cancer therapeutics, but to date no drug targeting the interaction has obtained FDA approval. Therefore, combinatorial libraries with compounds capable of disrupting protein-protein interactions are highly valuable therapeutic starting points. Modeling was focused on the MDMX hydrophobic pocket (Figure 4B, C), which is the region that binds p53.^[15] Modeling results showed two distinct binding modes of the compounds (Figure 4D-G).

While the location of the bulky isopropyl and tertbutyl groups was consistent regardless of substitution, it appeared the lower halves of the compounds switched between two independent binding modes. The benzimidazole compounds favored binding to a narrow, deep non-polar groove. On the other hand, the quinoxalinone compounds favored a flipped orientation, with the nonpolar portion positioned further in the deep hydrophobic pocket and the polar moiety oriented towards the solvent.

In cellulo screening further validated the utility of these novel heterocycles. Select compounds were screened using an MTT assay in MIA PaCa cells.^[16] Compounds showed micromolar activity against this pancreatic cancer cell line, supporting the therapeutic utility of the compounds (Table 1).

Conclusions

In conclusion, we have developed an efficient three-step protocol to access a previously undescribed dual heterocycle library. This new method can expedite the formation of compound libraries by producing compounds with multiple points of diversity in a small number of convergent steps. Our method expands on traditional post-Ugi modifications by creating a second heterocycle through an additional cyclization, further promoting diversity and drug like properties for compound libraries. We have shown, through computational modelling, the compounds developed are capable of binding to MDMX by exploiting a deep non-polar groove and a deep hydrophobic pocket on the protein. Additionally, the compounds show cellular activity in the mid micromolar range, supporting the utility of this methodology to produce starting points for a focused drug library.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Scope of the benzimidazole dual-heterocycle compounds. ^aIsolated yield for Ugi reaction. ^bIsolated yield for two cyclization steps.

Figure 2. Scope of the isoquinolinedione dual heterocycle compounds. ^aIsolated yield for Ugi reaction. ^bIsolated yield for two cyclization steps.

Figure 3. Scope of expanded benzimidazole dual heterocycle compounds. ^aIsolated yield for Ugi reaction. ^bIsolated yield for two cyclization steps.

Figure 4.

(A) Structures of hits from computational screening against MDMX. (B) Binding cleft on MDMX where compounds are theorized to interact, the non-polar groove is highlighted (C) Depiction of the deep hydrophobic pocket on MDMX. (D) P66-6 theoretical binding in MDMX (E) P69-4 theoretical binding in MDMX (F) P70-1 theoretical binding in MDMX (G) P71-1 theoretical binding in MDMX. PDB #2Z5S

Scheme 1.

Synthetic method for the synthesis of benzimidazole dual-heterocycle compounds a) MeOH, rt, 12hr; b) 10% TFA/DCE, 120 °C MW, 10 min; c) Cs₂CO₃, DMF, 150 °C MW, 10 min; d) DIPA, DMF, 160 °C MW, 10 min.

Scheme 2.

Synthetic method for the synthesis of isoquinolinedione dual-heterocyclic compounds. a) MeOH, rt, 12hr; b) 10% TFA/DCE, 120 °C MW, 10 min; c) Cs₂CO₃, DMF, 150 °C MW, 10 min; d) DIPA, DMF, 160 °C MW, 10 min.

Scheme 3.

Method to expand benzimidazole dual-heterocyclic compounds. a) NaOH, THF, H₂O, 100 °C MW, 15 min; b) EDC, HoAt, DMAP, DCM, rt, 12 hr.

Table 1.

Compound Activity in MIA PaCa Cells.

Entry	Compound	IC50 (µg/mL)
1	P66-6	> 50
2	P66-7	> 50
3	P66-8	29.53
5	P69-1	24.97
6	P69-4	19.33
7	P69-6	20.81
8	P69-8	19.91
9	P69-10	> 50
10	P70-1	27.35
11	P72-2	37.60
12	P74-3	32.73