Three-Component Combinatorial Synthesis of a Substituted 6*H*-Pyrido[2′,1′:2,3]imidazo-[4,5-*c*]isoquinolin-5(6*H*)-one Library with Cytotoxic Activity

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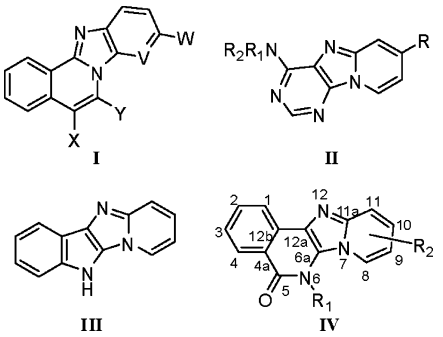
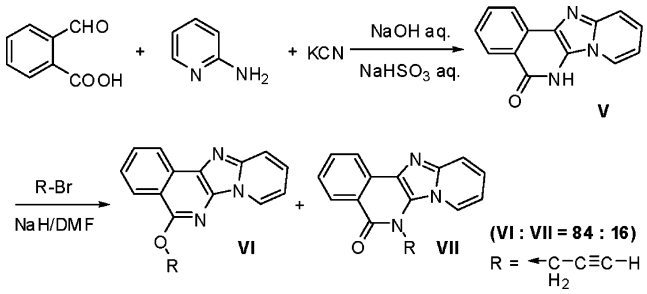
As a structural component of key bioactive molecules, a fused imidazo[1,2-*a*]heterocycles moiety was widely incorporated in the design of multiple biologically active agents. Consequently, the synthesis of various fused imidazo[1,2-*a*]heterocycles, such as benzimidazo[2,1-*a*]isoquinolines (**I**),[1] pyrido[1,2-*e*]purines (**II**), as anticancer agents,[2] a potent antihypertensive compound pyridino[1,2-*a*]imidazo[5,4-*b*]-indole (**III**),[3,4] and dimeric analogs of 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole, which would interact with DNA,[5] are reported. This type of structural moiety is also represented by the launched drugs zolimidine, zolpidem, and alpidem, and it can also be found in many other pharmacologically active compounds.[6]



Novel fused heterocyclic ring systems are often considered important scaffolds in medicinal chemistry; therefore, the search for the efficient and combinatorial methodologies to allow for convenient synthesis of new scaffolds attracts both organic and medicinal chemists. We designed a new type of fused substituted 6*H*-pyrido[2′,1′:2,3]imidazo[4,5-*c*]isoquinolin-5(6*H*)-ones (**IV**) that may be used for identification of novel scaffolds of diverse biological activity. To the best of our knowledge, only a single example for the formation of this heterocyclic system by a three-component reaction is reported in the literature, namely, the synthesis of pyrido-[2′,1′:2,3]imidazo[4,5-*c*]isoquinolin-5(6*H*)-one (**V**) by condensation of 2-aminopyridine, phthaldehydic acid, and potassium cyanide, with only 35% yield. Moreover, the R group at position 6 could be introduced by alkyl halides in a sodium hydride−DMF mixture method, but it is reported that under these conditions an 84:16 mixture of *O*- and *N*-alkylated products **VI** and **VII** was obtained (Scheme 1),[7] which makes it hard to diversify the library.

Scheme 1. Synthesis of Pyrido[2′,1′:2,3]imidazo[4,5-*c*]-isoquinolin-5(6*H*)-one and its Alkylation Products



To introduce a substituted group at the 6N position to construct the 6*H*-pyrido[2′,1′:2,3]imidazo[4,5-*c*]isoquinolin-5(6*H*)-ones library, a novel three-component reaction was investigated. In this report, we explored a strategy for parallel synthesis of substituted 6*H*-pyrido[2′,1′:2,3]imidazo[4,5-*c*]-isoquinolin-5(6*H*)-one analogues that were prepared by a one-pot reaction of 2-formylbenzoic acid and 2-aminopyridines with isocyanides. Since the designed products easily precipitated from the reaction mixture, higher purity products could be obtained by simple filtration and washing without any further purification. The key aspect of the synthetic strategy includes the Groebke reaction,[6,8,9] using bifunctional reagents.[10]

First, as a model reaction, phthaldehydic acid and 2-aminopyridines reacted with benzyl isocyanide to form a yellow precipitate. In the IR spectrum, the peak at 1645 cm^{-1} suggested the presence of a high-conjugated lactam group. The HRMS data supported that both the elemental composition and molecular weight of the precipitated product are consistent with the designed compound. Detailed analysis of ^1H and ^{13}C NMR confirmed the structure of compound **4**{*1,1*}, the signals of which revealed the presence of three sets of 19 aromatic carbons (13 of which are protonated), one methylene group [δ_H 5.92(s, 2H) and δ_C 45.59], and one carbonyl group [δ_C 160.73]. The COSY and HSQC NMR were used for further structural assignment: eight aromatic protons, on positions 1−4 and 8−11 at [δ_{H-1} 8.46 (d, J = 7.4 Hz, 1H) and δ_{C-1} 121.77], [δ_{H-2} 7.86 (m, 1H) and δ_{C-2} 133.51], [δ_{H-3} 7.60 (m, 1H) and δ_{C-3} 127.29], [δ_{H-4} 8.56 (d, J = 7.4 Hz, 1H) and δ_{C-4} 129.10], [δ_{H-8} 8.15 (d, J = 7.2 Hz, 1H) and δ_{C-8} 123.46], [δ_{H-9} 6.59 (td, J = 7.0, 1.2 Hz, 1H) and δ_{C-9} 112.43], [δ_{H-10} 7.08 (ddd, J = 9.2, 6.7, 1.1 Hz, 1H) and δ_{C-10} 124.07], and [δ_{H-11} 7.66 (d, J = 9.1 Hz, 1H) and δ_{C-11} 118.01], proton on monosubstituted phenyl ring at [δ_H 7.24−7.39 (m, 5H) and $\delta_{C1'\&5'}$ 125.61 × 2, $\delta_{C2'\&4'}$ 129.18 × 2, $\delta_{C3'}$ 127.55].

To establish a general synthetic method, the solvent was screened, and methanol was selected to be used in the reaction because of its perfect solubility characteristics for both reaction intermediate and product. When the temper-

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Scheme 2. Model Reaction

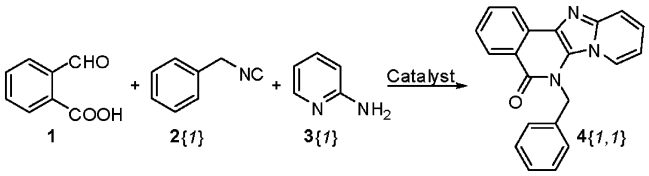


Table 1. Model Reaction, Condition, and Yield

entry	temp	catalyst	yield (%)[a]
1	55 °C	$Sc(OTf)_3$	50.8
2	55 °C		74.3

[a] Yield of crystalline compound isolated by filtration (no recovery from mother liquor); hence, the total yield may be higher.

ature was increased to 55 °C, the solubility of the iminium intermediate formed by 2-formylbenzoic acid and 2-aminopyridine was improved. Because the rate of Groebke reaction is pH-dependent, it would be catalyzed by Bronsted acid[6,9] or Lewis acid, as $Sc(OTf)_3$.[8] However, in the simulation reaction, it was found that there was no significant improvement when 0.05 mol equiv of scandium triflate was used as the catalyst compared to the catalyst-free condition. On the contrary, use of $Sc(OTf)_3$ increased the solubility of the final product in methanol which led to a relative low yield (Scheme 2 and Table 1).

On the basis of the above observations, a possible mechanism for this three-component reaction was postulated that proceeds via an iminium species **A** which is attacked by the isocyanide to give nitrilium ion **B**, the pyridine nitrogen of **B** is in a favorable position for a 5-*exo*-dig cyclization, and followed by addition of the carboxylic acid oxygen to the imino carbon giving the assumed intermediate **C**; the resulting internal ester **C** rearranges by acyl transfer to generate the lactam **4**{*1,1*} (Scheme 3). Thus, we regarded this reaction as a special type of the U3CR.

To delineate this approach, particularly in regard to library construction, this methodology was evaluated by using different isocyanides and 2-aminopyridines. Six commercially or synthetically available isocyanides **2**{*1*−*6*} and six substituted 2-aminopyridine **3**{*1*−*6*} were chosen for the library validation (Figure 1). The phenylisocyanide **2**{*6*} did not give satisfactory results presumably because of the intermediate **D**, which is difficult to form according to the proposed mechanism. And, also, the substrate 2-amino-5-cyanopyridine **3**{*6*} did not undergo this reaction. We

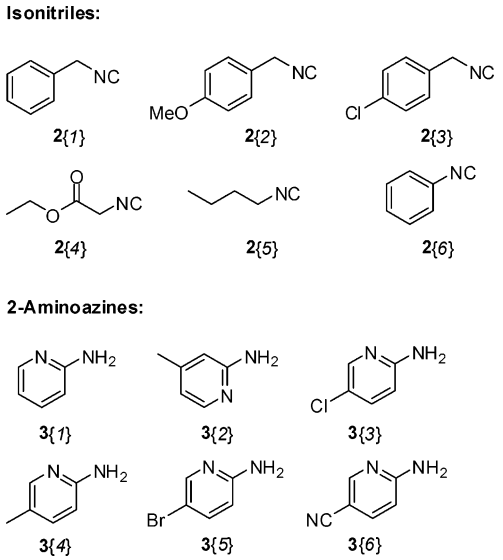


Figure 1. Diversity reagents evaluated in this work.

reasoned that this was the result of the strong electron-withdrawing effects on the pyridine ring. Finally, in this work, 2-formylbenzoic acid (**1**), five isocyanides **2**{*1*−*5*}, and five substituted 2-aminopyridines **3**{*1*−*5*} were selected, and the library synthesis was performed on a 12-reaction setup in a parallel synthesizer (Radleys Discovery Technology, Carousel 12 Place Reaction Station); the reaction mixture was added into the 1 mL Supelco filtration tube and then drained on a 12-port SPE vacuum manifold (VisiprepDL, Supelco Inc., Milwaukee, WI), rinsed with MeOH(1 mL × 2) and drained, to give the final products.

All compounds in this library were screened for cytotoxic activity against A549, one non-small-cell lung cancer cell line, with SRB assay; compounds **4**{*2,1*}, **4**{*2,3*}, and **4**{*2,4*} exhibited potent inhibiting effect with IC_{50} values of 1.82, 10.69, and 6.61 μM, respectively.

Some key structural features appeared to be important in cytotoxic activity. In comparison with compound **4**{*2,1*}, the major difference was the 4-methoxybenzyl group, which is essential to the activity, and the introduction of a halogen or methyl substitution to the position 9 decreases the inhibitory potency The bioactivity is also dramatically reduced when position 10 is substituted.

In summary, a convenient and efficient synthetic route for solution-phase combinatorial synthesis of diverse sub-

Scheme 3. Proposed Reaction Mechanism

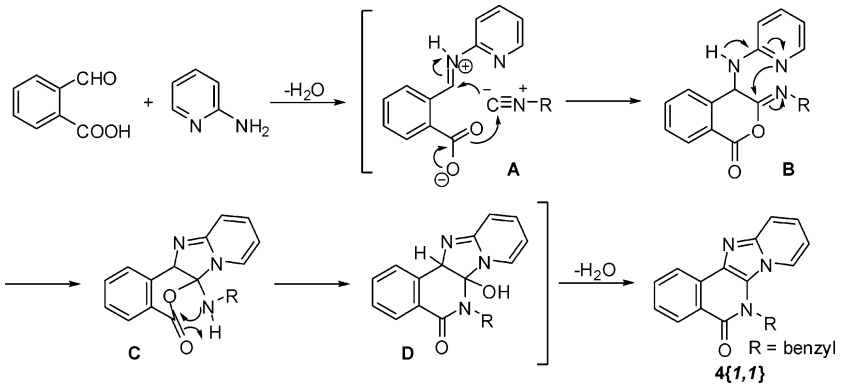
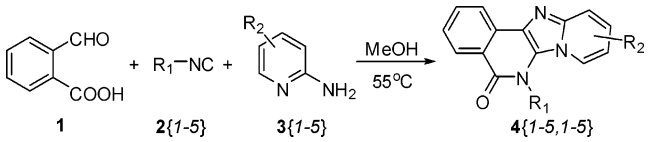


Table 2. Library of Substituted 6*H*-Pyrido[2′,1′:2,3]imidazo-[4,5-*c*]isoquinolin-5(6*H*)-ones and its Inhibition (%) of A549 Cells at 12.5 μM Concentration



1 2{*1-5*} 3{*1-5*} 4{*1-5,1-5*}

	yield (%)[a]	purity (%)	A549 inhibition (%)		yield (%)[a]	purity (%)	A549 inhibition (%)
4{*1,1*}	74.3	100	7.5	**4**{*3,4*}	71.8	100	17.4
4{*1,2*}	53.8	100	7.8	**4**{*3,5*}	50.1	100	22.0
4{*1,3*}	52.2	100	26.6	**4**{*4,1*}	46.7	100	13.4
4{*1,4*}	67.7	97	8.0	**4**{*4,2*}	50.1	100	8.7
4{*1,5*}	54.2	100	25.3	**4**{*4,3*}	43.0	100	22.4
4{*2,1*}	50.4	100	65.0	**4**{*4,4*}	79.7	100	28.1
4{*2,2*}	57.6	100	18.0	**4**{*4,5*}	39.4	100	20.1
4{*2,3*}	50.0	100	54.5	**4**{*5,1*}	82.5	100	11.3
4{*2,4*}	65.6	100	52.3	**4**{*5,2*}	41.3	100	12.2
4{*2,5*}	36.0	93	48.6	**4**{*5,3*}	58.5	100	11.8
4{*3,1*}	70.1	100	22.0	**4**{*5,4*}	64.9	100	10.2
4{*3,2*}	68.8	100	9.9	**4**{*5,5*}	62.5	100	11.7
4{*3,3*}	70.4	100	17.8				

[a] Yield of crystalline compound isolated by filtration (no recovery from mother liquor); hence, the total yield may be higher.

stituted a 6*H*-pyrido[2′,1′:2,3]imidazo[4,5-*c*]isoquinolin-5(6*H*)-ones library was developed. All the products could be purified by using a simple isolation method, and three of them showed potent antitumor activity in vitro. These findings encourage us to generate a further round of libraries with expanded diversity for the discovery of novel anticancer agents; research on the mechanisms and SARs of these compounds is ongoing, and the results will be reported in due course.

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Supporting Information Available. Experimental procedure and compound characterization data as yields, melting point, ^{1}H NMR, MS analysis, purities (UV_{214nm}) of all 25 compounds; typical compounds were characterized by ^{13}C NMR, MS (HREI and LREI), and IR analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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