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Synthesis and evaluation of heteroaromatic 6,7-diaryl-2,3,8,8a-tetrahydroindolizin-5(1*H*)-ones for cytotoxicity against the HCT-116 colon cancer cell line

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Abstract—A heteroaromatic 6,7-diaryl-2,3,8,8a-tetrahydroindolizin-5(1*H*)-one analog library was prepared and tested for cytotoxic properties against the HCT-116 colon cancer cell line, thus providing additional information pertaining to structure–activity relationships for this class of compounds. The most active of the new analogs proved to be the C6 2-thiophene and 3-thiophene analogs with IC_{50} values of 0.27 μM and 0.60 μM, respectively.

Tyloindicine I (**1**, Fig. 1) was isolated from the aerial parts of *Tylophora indica* and described by Ali et al.[1,2] Preliminary analysis revealed potent nanomolar and cancer cell-selective cytotoxic properties most likely exerted through a novel mechanism of action.[2,3] During our efforts toward the total synthesis of tyloindicine I, we found that a reaction intermediate toward the synthesis of **1**, 6-phenyl-7-(4-methoxyphenyl)-2,3,8,8a-tetrahydroindolizin-5(1*H*)-one (racemic **2**, Fig. 1) displayed selective cytotoxicity toward colon cancer cell lines, was active in vivo in the mouse hollow fiber assay, and presumably exerts its cytotoxic activity via an unknown novel mechanism of action.[3] Related studies were reported by Sharma et al., who also investigated 2,3,8,8a-tetrahydroindolizin-5(1*H*)-ones as cytotoxic agents.[4]

Initial structure–activity studies from our laboratory focused on altering the substitution patterns of both the northern and southern aromatic rings attached to the 2,3,8,8a-tetrahydroindolizin-5(1*H*)-one core.[3] These studies revealed that the lead compound, racemic **2**, was the most active in the library and indicated that the southern aromatic ring at C6 did not accommodate

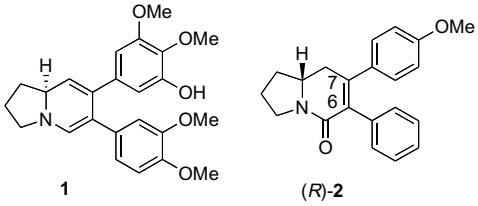

Figure 1. Tyloindicine I (**1**) and lead 7-(4-methoxyphenyl)-6-phenyl-2,3,8,8a-tetrahydroindolizin-5(1*H*)-one (**2**).

substitution well. In subsequent studies, we prepared both enantiomers of **2**, and found that only the (*R*)-**2** enantiomer is cytotoxic.[5]

In an effort to expand the structure–activity relationship information for these compounds, we sought to develop an analog library to probe the ramifications of replacing the southern phenyl ring with various heteroaromatic rings. As the C6 aromatic ring of related analogs had proved not to accommodate substitution well, we could not explore the electronic ramifications of the southern ring by substitution alone. With this limitation in mind, a 14-membered heteroaromatic 6,7-diaryl-2,3,8,8a-tetrahydroindolizin-5(1*H*)-one analog library was prepared. Ultimately, this library would serve to vary the electronic characteristics of the southern ring without increasing the steric bulkiness by adding substituents to the aromatic ring system.

Keywords: Tetrahydroindolizinones; Synthesis; Heteroaromatic; Structure–activity studies; Cytotoxicity; HCT-116 colon cancer cell line.
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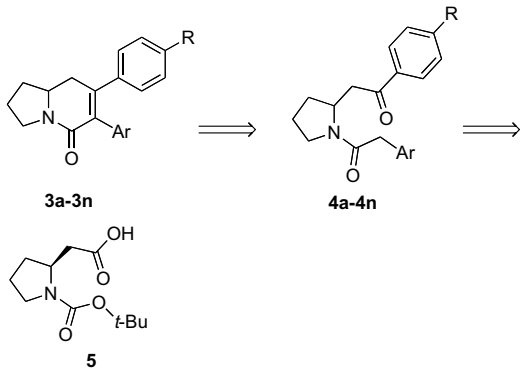
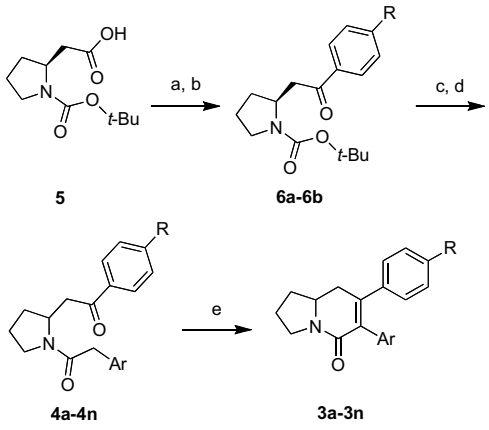


Scheme 1. Retrosynthesis for **3a**–**3n**.

The retrosynthetic analysis for the analog library is patterned after the stereospecific synthesis developed recently in our laboratories to generate both enantiomers of **2** (Scheme 1).[5] Although in this case, without any stereocontrolling element elsewhere in the molecule, the stereocenter is destroyed due to a retro-Michael–Michael racemization.[5] Regardless, the use of *N*-BOC L-homoproline (**5**) as the starting substrate was convenient and the synthesis was otherwise sound for our purposes of probing the heteroaromatics effects on biological activity. Thus, title compounds **3a**–**3n** can be envisioned as coming from amides **4a**–**4n** following an intramolecular aldol condensation of the amide with the aryl ketone. In turn, amides **4a**–**4n** may be derived from **5** through a Grignard reaction followed by a BOC-deprotection/peptide bond formation sequence.

The synthesis toward **3a**–**3n** was initiated with the Weinreb amidation of (*S*)-2-(1-(*tert*-butoxycarbonyl)-pyrrolidin-2-yl)acetic acid (**5**, Scheme 2). The subsequent Grignard addition into the Weinreb amide supplied aryl ketones **6a**–**6b**. An acidic BOC-deprotection of ketones **6a**–**6b** formed stable hydrochloride amine salts, which were subjected to an EDCI coupling protocol with the



Scheme 2. Synthesis of **3a**–**3n**.[5] Reagents and conditions: (a) *N*,*O*-dimethylhydroxylammonium chloride, EDCI, NMM, dry DCM; (b) Mg^0 turnings, cat. I_2 crystals, *p*-bromoanisol for **6a** or *p*-bromothioanisole for **6b**, dry THF; (c) 4 M HCl/dioxane; (d) heteroaryl acetic acid, EDCI, NMM, dry DCM; (e) 7.7% $KOH_{(EtOH)}$, reflux.

appropriate heteroaryl acetic acid to provide the aldol precursors **4a**–**4n**. The final step called for a basic intramolecular aldol condensation to construct the heteroaromatic library of analogs **3a**–**3n**. As previously illustrated, the acidic BOC-deprotection and basic intramolecular aldol conditions led to the retro-Michael–Michael racemization.[5] The yields and substitution patterns for the heteroaryl analog library developed in Scheme 2 are shown in Table 1. In addition, the phenyl parent compounds **4a** and **4i** were carried through the synthesis as well so as to have a standard for cytotoxicity comparison purposes.

The heteroaromatic indolizidine analog library was tested for cytotoxicity against the HCT-116 colon cancer cell line. The results are summarized in Table 2. It is clear that the pyridyl analogs **3b**–**3d** of the parent compound **3a** are the least active of the compounds tested, with the 2- and 3-pyridyl derivatives having much reduced activity. The latter two compounds were not pursued further as the thiomethyl derivatives. The thiomethyl complement of the 4-pyridyl derivative **3j**, also had the lowest activity of the thiomethyl subgroup of

Table 1. Synthetic yields for analog library[5]

Analog	R	Ar	Yield (%)
6a	OMe	n/a	80[a]
6b	SMe	n/a	77[a]
Methoxy acylation			
4a	OMe	Phenyl	74[a]
4b	OMe	2-Pyridine	78[a]
4c	OMe	3-Pyridine	See **3c**[b]
4d	OMe	4-Pyridine	45[a]
4e	OMe	2-Thiophene	93[a]
4f	OMe	3-Thiophene	86[a]
4g	OMe	2-Furan	77[a]
4h	OMe	3-Furan	59[a]
Methoxy intramolecular aldol condensation			
3a ((+/−)-**2**)	OMe	Phenyl	30
3b	OMe	2-Pyridine	89
3c	OMe	3-Pyridine	57[b]
3d	OMe	4-Pyridine	82
3e	OMe	2-Thiophene	68
3f	OMe	3-Thiophene	79
3g	OMe	2-Furan	48
3h	OMe	3-Furan	65
Thiomethyl acylation			
4i	SMe	Phenyl	58[a]
4j	SMe	4-Pyridine	44[a]
4k	SMe	2-Thiophene	87[a]
4l	SMe	3-Thiophene	77[a]
4m	SMe	2-Furan	77[a]
4n	SMe	3-Furan	53[a]
Thiomethyl intramolecular aldol condensation			
3i	SMe	Phenyl	66
3j	SMe	4-Pyridine	52
3k	SMe	2-Thiophene	51
3l	SMe	3-Thiophene	81
3m	SMe	2-Furan	36
3n	SMe	3-Furan	60

[a] Yield over two steps.
[b] Yield over three steps.

Table 2. Cytotoxicity of the heteroaromatic indolizidine analog library against the HCT-116 colon cancer cell line

Analog	R	Ar	IC_{50} (μM)
3a ((+/−)-2)	OMe	Phenyl	0.39
3b	OMe	2-Pyridine	104
3c	OMe	3-Pyridine	79
3d	OMe	4-Pyridine	19
3e	OMe	2-Thiophene	5.0
3f	OMe	3-Thiophene	1.5
3g	OMe	2-Furan	6.0
3h	OMe	3-Furan	3.0
3i	SMe	Phenyl	1.0
3j	SMe	4-Pyridine	6.0
3k	SMe	2-Thiophene	0.27
3l	SMe	3-Thiophene	0.60
3m	SMe	2-Furan	3.0
3n	SMe	3-Furan	1.1

compounds. In general, there was little difference in activity among the furan and thiophene derivatives of the parent or thiomethyl compounds, although the thiomethyl thiophene derivatives **3k** and **3l** were the most active of all the derivatives tested and were equal in potency to the parent compound **3a**.

In conclusion, a heteroaromatic analog library was designed and prepared using a synthetic sequence developed in our laboratories.[5] Since the southern aromatic ring did not accommodate substitution well, we could not explore the electronic ramifications of the southern ring by substitution, and therefore prepared a heteroaromatic analog library. The evaluation of said compound library (**3a**–**3n** in Table 2) revealed that the combination of a C6-thiophene substituent with a C7-(4-thiomethyl)phenyl moiety provided analogs (**3k** and **3l**) that displayed cytotoxicities comparable to the lead compound **3a**.

Acknowledgments

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5. Kimball, F. S.; Turunen, B. J.; Ellis, K. C.; Himes, R. H.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 4367, All intermediates and analogs in Table 1 were prepared utilizing the experimental conditions reported in this paper. The spectroscopic data for all compounds in Table 1 were found to be in agreement with their structures.