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Synthesis and biological evaluation of unnatural canthine alkaloids

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Abstract—Employing a 'one-pot' microwave-assisted protocol, unnatural canthine alkaloids with biological activities beyond the natural products have been prepared. This report describes unnatural canthine alkaloid analogs as selective, allosteric Akt kinase inhibitors.

Recently, the application of diversity-oriented synthesis to construct libraries of complex molecules, based on natural product templates, has received a great deal of attention.[1] Importantly, Shair and co-workers have reported on the biomimetic solid-phase synthesis of libraries of galanthamine-like alkaloids **1** with biological properties beyond the natural product.[2] Notably, this work resulted in the discovery, through phenotypic screens, of secramine **2**, an unnatural amaryllidaceae alkaloid, that inhibits protein trafficking (Fig. 1).[2]

Inspired by this work, an unrelated class of natural products, the canthin-6-one alkaloids **3** attracted our attention.These alkaloids represent a tetracyclic subclass of β-carbolines that possess an additional D-ring in the core canthine skeleton **4** (Fig. 2).[3] Since their discovery in 1952, only forty members of this class of alkaloids have been reported, and little structural diversity exists from both natural and synthetic sources. Notably, members of this family of alkaloids have demonstrated a wide range of pharmacological activities including antiviral and antifungal properties as well as potent cytotoxicity against a variety of cell lines rendering this framework attractive for diversity-oriented organic synthesis.[4]

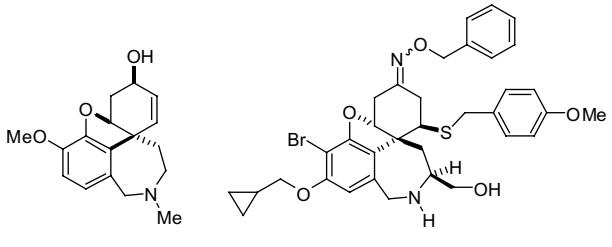

Figure 1. Galanthamine and the unnatural sercramine.

In a recent letter, our laboratory reported on 'one-pot' microwave-assisted protocol for the synthesis of the canthine alkaloid core.[5] Under this protocol, an indole-tethered acyl hydrazide **5** was treated with a 1,2-dicarbonyl compound, such as benzil **6**, in HOAc with excess NH_4OAc for 40 min in a single-mode microwave synthesizer at 220 °C to deliver the unnatural 1,2-diphenyl canthine derivative **7** in 80% yield (Scheme 1). Further expansion

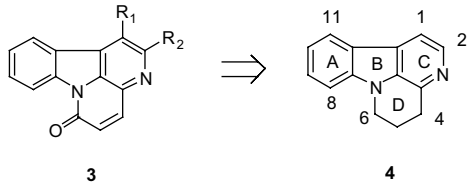

Figure 2. Canthin-6-one and canthine alkaloid core structures.

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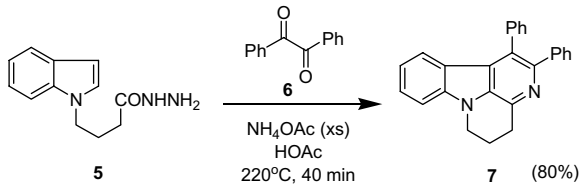


Scheme 1. 'One-pot' microwave synthesis of the canthine core.

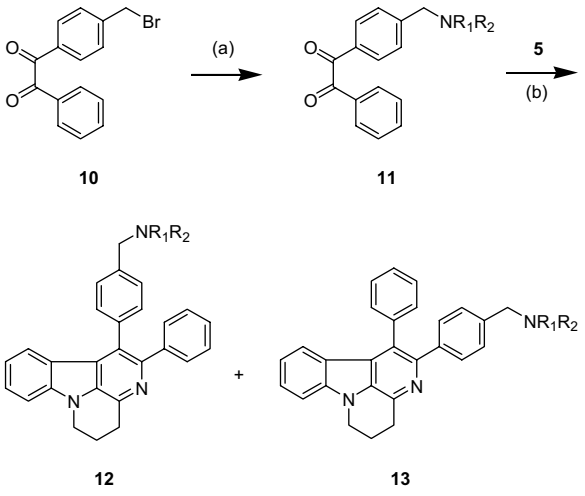
of this methodology for diversity-oriented organic synthesis (DOS) focused on functionalization of the C1/C2 positions of the canthine core, a historically difficult position to analog, utilizing a diverse collection of functionalized 1,2-dicarbonyl derivatives.[5]

Concurrently, our laboratory was engaged in a nascent medicinal chemistry program to develop selective Akt (PKB) kinase inhibitors. Akt is a serine/threonine kinase that recently garnered attention as a promising target for cancer therapy due to its critical role as a regulator of the cell's apoptotic machinery.[6] However, the development of small molecule Akt inhibitors for the treatment of cancer has been hindered by a lack of Akt specific (vs the AGC family of kinases) and isozyme selective (Akt1, Akt2, and Akt3) inhibitors due to high sequence identity homology.[7]

Our lead for this program, a 2,3-diphenylquinoxaline **8**, resulted from a high throughput screening effort to identify compounds capable of inhibiting the three Akt isozymes (Fig. 3).[8] Interestingly, **8** displayed a high degree of isozyme selectivity (IC_{50}s: Akt1 = 3.4 μM, Akt2 = 23.1 μM, and Akt3 >50 μM) and was found to be specific for Akt (>50 μM vs PKA, PKC, and SGK). In assays with Akt mutants lacking the PH domain, **8** displayed no inhibition; moreover, Akt inhibition by **8** was not competitive with ATP. The selectivity observed has been attributed to an allosteric mode of inhibition, for which a model has been proposed.[8] Further optimization of **8** resulted in **9**, a more potent dual Akt1/Akt2 inhibitor (IC_{50}s: Akt1 = 0.06 μM, Akt2 = 0.21 μM, and Akt3 2.2 μM) with the same allosteric mode of inhibition.[9]

Recognition of the related 1,2-diphenyl heterocyclic motif present in **7** led us to screen this unnatural canthine alkaloid in our Akt kinase assay. Unfortunately, **7**, an unsubstituted phenyl analog, displayed no inhibition (>50 μM) against all three of the Akt isozymes. For the series of inhibitors represented by **8** and **9**, the unsubstituted phenyl congeners displayed moderate micromolar levels of inhibition against Akt1. However, the addition of a benzylic amine to the 4-position of one of the phenyl rings, as in **8** and **9**, dramatically increased potency, but known GPCR privileged structures, such as the piperidinyl benzimidazolone moiety in **9**, proved optimal.[9]

Therefore, second generation libraries of unnatural canthine alkaloids were designed to incorporate basic amines into the 4-position of both the C1 and C2 phenyl rings, a previously undescribed pattern of substitution. As depicted in Scheme 2, commercially available *p*-bromomethyl benzil **10** was treated with primary and secondary amines (Fig. 4), followed by scavenging with a fluorous-tagged thiol. After Fluoro*Flash*™ SPE purification, the *p*-aminomethyl benzil analogs **11** were obtained with an average yield and purity of 93% and 95%, respectively.[10] Then, indole-tethered acyl hydrazide **5** was treated with one of the *p*-aminomethyl benzil analogs **11** in HOAc with excess NH_4OAc for 40 min in a single mode microwave synthesizer at 220 °C. This key 'one-pot' reaction produced **12** and **13** as a 1:1 mixture of regioisiomeric canthine alkaloids, resulting from the unsymmetrical benzils **9** employed, in yields ranging from 35% to 70% (un-optimized). All analogs were purified (>98%) by mass-triggered preparative HPLC;



Scheme 2. Library of unnatural canthine alkaloid analogs **12**:**13**. Reagents and conditions: (a) (i) HNR_1R_2, PS-DIEA, DCM, rt, (ii) R_f-SH, (iii) Fluoro*Flash*™ SPE, 93%; (b) **5** (1.1 equiv), NH_4OAc (10 equiv), HOAc, 220°C, 40 min, microwave, 35–70%. All compounds purified by mass-triggered HPLC.

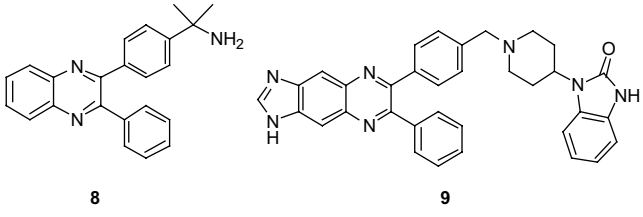


Figure 3. HTS screening lead and optimized dual Akt1/Akt2 inhibitor.

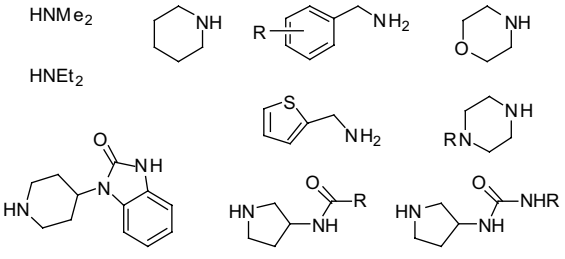
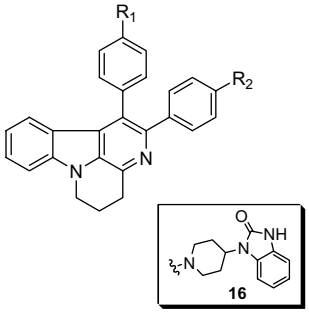


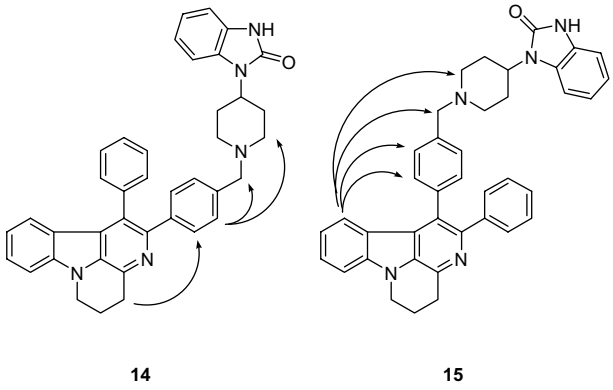
Figure 4. Primary and secondary amines.



14 R_1 = H, R_2 = **16**

15 R_1 = **16**, R_2 = H

Figure 5. Unnatural canthine alkaloids that inhibit Akt.



14 **15**

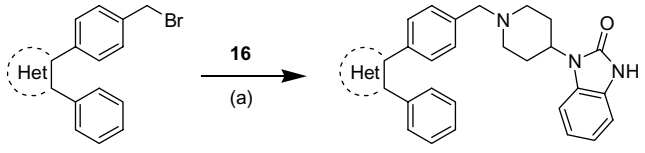
Figure 6. ROE correlations to assign structures to **14** and **15**.

however, separation of the individual regioisomers proved problematic.[11] Therefore, we elected to perform initial screening on the 1:1 mixtures of regioisomeric canthines.

With the exception of **14**:**15**,[12] derived from the incorporation of the piperidinyl benzimidazolone moiety in **9**, all of the analogs screened against the three Akt isozymes were inactive (Fig. 5). This was a surprising result, as the analogous congeners in the quinoxaline series **8** and **9**, were active compounds. As a 1:1 mixture of regioisomers, **14**:**15** displayed Akt isozyme selectivity (IC_{50}s: Akt1 = 1.8 μM, Akt2 = 2.8 μM, and Akt3 >50 μM) and an almost 10-fold increase in Akt2 inhibition, relative to **8**. As observed with the quinoxaline series, inhibition was PH-domain dependent and noncompetitive with ATP.[8,9] Though clearly an allosteric inhibitor, the divergent SAR from the quinoxaline series potentially suggests an alternative allosteric binding site.

These data also suggested that the source of Akt inhibition might be solely due to the piperidinyl benzimidazolone moiety, **16**, in combination with a 1,2-diphenyl heterocyclic motif. In order to evaluate this possibility, a library of 48 compounds was prepared wherein various heterocycles **17** with a 1,2-diphenyl motif were functionalized with **16** to provide **18** (Scheme 3). When screened in our Akt kinase assay, none of these closely related analogs displayed any inhibition of the individual Akt isozymes.

With data suggesting that the canthine scaffold, in concert with **16**, provided a truly novel allosteric Akt kinase



17 **18**

Scheme 3. Library of functionalized 1,2-diphenyl heteroaromatic scaffolds. Reagents and conditions: (a) (i) **16**, PS-DIEA, DCM, rt, (ii) R_f-SH, (iii) Fluoro*Flash*™ SPE, 94%.

inhibitor, HPLC conditions were rapidly developed to separate the two regioisomeric canthines **14** and **15**. Once separated, standard 1-D NMR techniques failed to distinguish the two regioisomers as well as a variety of 2-D heteronuclear NMR techniques (HMQC and HMBC). Ultimately, the regioisomers were distinguished by ROE correlations (illustrated by arrows in Fig. 6) and further confirmed by HMBC 3-bond ^{1}H-^{13}C correlations and structures were assigned as shown in Figure 5.[13]

With pure regioisomers **14** and **15** in hand, they were reassayed in our Akt kinase screen in order to determine if the inhibitory activity observed was due to only one or both regiosiomers. As shown in Table 1, both **14** and **15** were essentially equipotent against both Akt1 and Akt2. Moreover, both **14** and **15** were PH domain dependent and noncompetitive with ATP and therefore, represent only one of the three known templates to provide this type of allosteric inhibition of the Akt kinase.[8,9]

Based on these data, **14** and **15** were studied for their ability to induce apoptosis in a LnCaP tumor cell line, as measured by an increase in caspase-3 activity. When LnCaP cells were treated with 15 μM **14** or **15**, there was no observable increase in caspase-3 activity. However, when co-administered with TRAIL (TNF-α related apoptosis inducing ligand), a modest 1.8- to 2.2-fold increase in caspase-3 activity was observed relative to control.[8,9] This was an attractive starting point for further studies.

The results obtained from these small libraries argue well for the synthesis of additional libraries based on the canthine core with increased structural diversity. Currently, libraries incorporating our 'one-pot' protocol

Table 1. Akt inhibition by unnatural canthine alkaloids **14** and **15**

Compound	Akt1 IC_{50} (μM)[a]	Akt2 IC_{50} (μM)[a]	Akt3 IC_{50} (μM)[a]
14	1.34	1.65	>50
15	1.47	2.28	>50

All compounds >50 μM versus PKA, PKC, SGK, and d-PH mutants

[a] Average of three measurements.

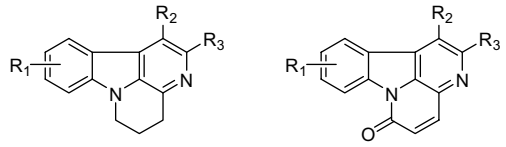


Figure 7. Generic canthine libraries with 3-position diversity.

with three-position diversity are being prepared and the resulting biological activities of unnatural canthine alkaloids will be reported in due course (Fig. 7).

In summary, unnatural canthine alkaloids have been discovered with biological properties beyond the natural product by application of microwave-assisted organic synthesis (MAOS). Unnatural canthine alkaloids **14** and **15** were shown to be potent and selective Akt kinase inhibitors that induced apoptosis in LnCaP cells. Moreover, these unnatural molecules were dependent on the PH domain of the Akt kinase for enzyme inhibition and noncompetitive with ATP, suggesting a unique, allosteric mode of inhibition. Clearly, the melding of natural product templates with known pharmacophores (privileged structures) can result in attractive lead structures for medicinal chemists to pursue and refine.

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12. Following the experimental procedure outlined in Refs. 5 and 9, **14** and **15** were obtained in a combined 48% isolated yield. Analytical data for **14**: Analytical LCMS, a single peak at 2.176 min. (MeCN/H_2O/0.05%TFA), 4 min gradient, >99% pure; ^1H NMR ($CDCl_3$, 600 MHz), δ 9.09 (br s, 1H), 7.72 (t, J = 7.78 Hz, 7.73 Hz, 1H), 7.62 (d, J = 8.48 Hz, 1H), 7.45 (m, 3H), 7.42 (d, J = 5.89 Hz, 2H), 7.41 (d, J = 5.89 Hz, 2H), 7.39 (obs d, 1H), 7.33 (d, J = 8.16 Hz, 1H), 7.31 (t, J = 7.61 Hz, 6.98 Hz, 2H), 7.18 (t, J = 8.84 Hz, 7.79 Hz, 1H), 7.01 (br m, 3H), 4.60 (br s, 1H), 4.44 (t, J = 7.00 Hz, 5.62 Hz, 2H), 4.24 (br s, 2H), 3.71 (br t, 2H), 3.59 (br d, 2H), 2.88 (m, 4H), 2.62 (t, J = 5.46 Hz, 6.79 Hz, 2H), 1.94 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 154.5, 143.8, 139.6, 138.8, 134.7, 134.1, 132.5, 131.8, 131.5, 131.2, 131.1, 130.1, 129.7, 129.4, 129.2, 128.4, 127.8, 125.1, 122.2, 122.0, 121.9, 120.9, 110.4, 110.3, 109.9, 60.0, 51.7, 47.3, 41.2, 26.0, 23.7, 21.8; HRMS calcd for $C_{39}H_{36}N_5O$ (M+H), 590.2914; found 590.2926; Analytical data for **15**: Analytical LCMS, a single peak at 2.389 min (MeCN/H_2O/0.05%TFA), 4 min gradient, >99% pure; ^1H NMR ($CDCl_3$, 600 MHz), δ 8.40 (br s, 1H), 7.72 (t, J = 7.95 Hz, 8.1 Hz, 1H), 7.60 (d, 8.79 Hz, 1H), 7.57 (d, 7.35 Hz, 2H), 7.47 (d, J = 7.95 Hz, 1H), 7.43 (d, J = 7.35 Hz, 2H), 7.36 (d, J = 8.21 Hz, 1H), 7.31 (d, J = 7.91 Hz, 2H), 7.28 (obs, 1H), 7.23 (t, J = 7.60 Hz, 8.97 Hz, 2H), 7.21 (obs, 1H), 7.12 (t, J = 7.30 Hz, 1H), 7.09 (t, J = 7.90 Hz, 1H), 7.07 (d, J = 8.21 Hz, 1H), 4.71 (m, 1H), 4.44 (t, J = 5.43 Hz, 2H), 4.33 (s, 2H), 3.73 (t, J = 5.56 Hz, 2H), 3.69 (br d, J = 11.4 Hz, 2H), 2.98 (q, J = 13.36 Hz, 12.65 Hz, 2H), 2.85 (t, J = 12.4 Hz, 2H), 2.62 (m, 2H), 2.03, (d, J = 13.17 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C) δ 153.2, 142.0, 140.7, 140.5, 137.0, 131.5, 131.3, 130.2, 129.4, 128.4, 128.0, 127.9, 127.8, 127.5, 125.6, 122.9, 120.6, 120.2, 119.9, 119.5, 110.8, 108.8, 108.2, 58.5, 50.8, 46.5, 40.3, 25.2, 24.7, 21.1; HRMS calcd for $C_{39}H_{36}N_5O$ (M+H), 590.2914; found 590.2921.
13. All NMR experiments conducted on a Varian Inova 600 MHz spectrometer. Additional details available upon request.