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Report

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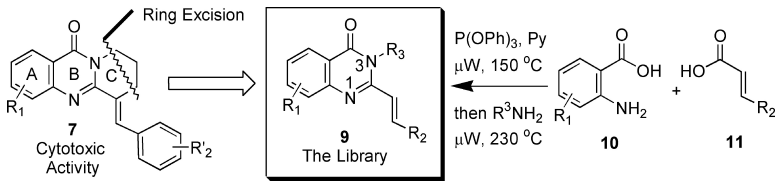

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Design and Synthesis of a Quinazolinone Natural Product-Templated Library with Cytotoxic Activity

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Quinazolinone **1** is a naturally occurring alkaloid as well as a core structural subunit in a growing class of bioactive natural products and synthetic compounds (Figure 1).[1] In addition to a variety of biological activities that quinazolinone alkaloids possess, cytotoxicity is a frequently found property in several members of this alkaloid family, as exemplified by luotonin F (**2**),[2] luotonin A (**3**),[2b,3] and fumiquinazoline A (**4**).[4] Moreover, the quinazolinone moiety has been extensively utilized as a druglike scaffold in medicinal chemistry, and as such, the quinazolinone skeleton is considered to be a privileged structure.[5] Therapeutic agents containing the quinazolinone core structure have been on the market or are in clinical trials for the treatment of cancer.[6]

We recently described an efficient microwave-assisted, three-component, one-pot reaction for the synthesis of various 2,3-disubstituted quinazolin-4-ones from readily available anthranilic acids, carboxylic acids, and amines.[7] This novel approach allows access to a broader chemistry scope relative to previously existing methods, accommodating an expanded array of carboxylic acids and amines. On the basis of this methodology, we have successfully developed efficient and concise total syntheses of quinazolinone natural products, including fumiquinazolines,[8] mackinazolinone,[9] isaindigotone,[9] and quinazolinobenzodiazepine.[10] The efficiency of this method further enabled us to quickly generate natural product-templated libraries of these natural products.[11] These libraries were screened in an MTS cell proliferation assay,[12] and remarkably, compounds represented by **7** showed promising cytotoxic activity (Figure 2), which is an unprecedented feature of such tricyclic quinazolinones.[9,13,14] Notably, this class of compounds is a hybrid chemical series of the natural products mackinazolinone (**5**) and isaindigotone (**6**), yet neither parent species (**5** or **6**) exhibits cytotoxic activity.[15]

This new finding stimulated us to generate a further round of privileged structure-based quinazolinone natural product-templated libraries with expanded diversity for the discovery of novel anticancer agents. Herein, we describe the library design, synthesis, and evaluation against cancer cell lines of our bicyclic quinazolinone natural product-templated libraries.

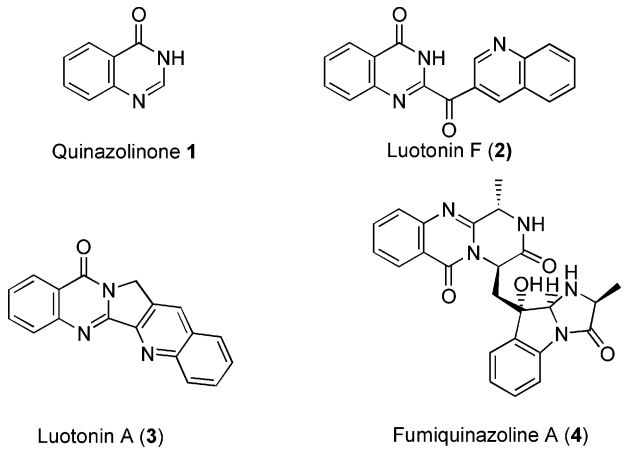

Figure 1. Quinazolinone **1** and related natural products showing cytotoxic activity.

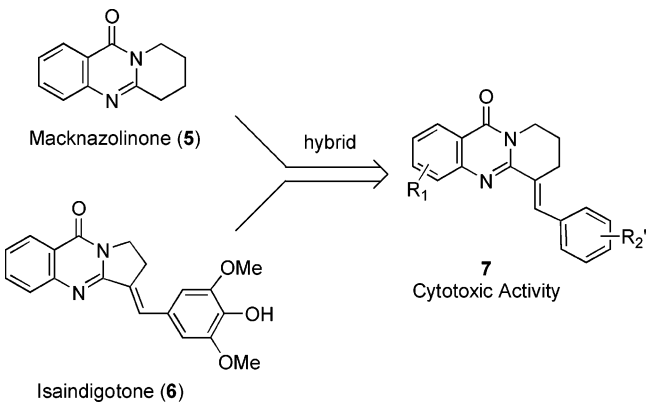

Figure 2. Hybrid structure **7** exhibiting cytotoxic activity.

Our design is depicted in Scheme 1. Excision of ring C of template **7** would give template **8**, which now possesses three sites for introduction of diversity (R^1, $R^{2\prime}$and R^3). To further increase the diversity of the library represented by 8, the phenyl ring of cinnamic acids can be generalized (R^2 of the styryl carboxylic acids **11**). Thus, the newly formed template **9** is based on a bicyclic quinazolinone structural framework that is widely distributed in natural alkaloids (more than 20 alkaloids of this class have been isolated in bacteria, fungi, and higher plants with various bioactivities).[1]

In the event, the library containing core structure **9** was prepared in one synthetic operation using our standard microwave-assisted, three-component, one-pot reactions from commercially available starting materials: anthranilic acids **10**, styryl carboxylic acids **11**, and amines **12** (Scheme 2). The library design was initiated using an integrated ArQule library design tool.[16] Starting with the construction of the virtual library ($N = 1152$) using **9** as the template, suitable commercially available building blocks were randomly selected, which included 6 anthranilic acids (with R^1 on the

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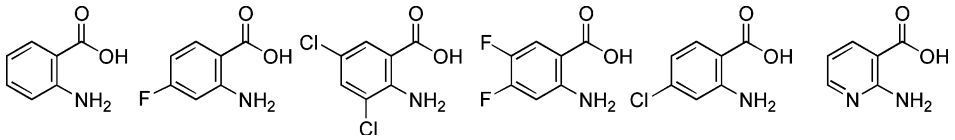
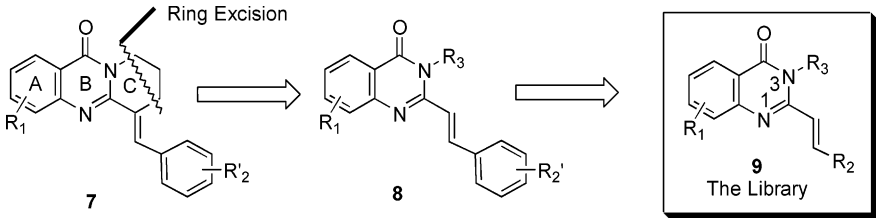
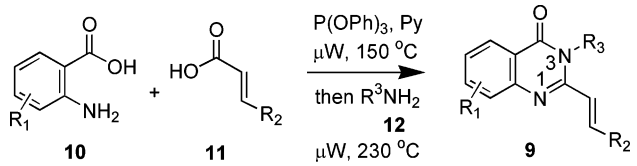


Figure 3. Selected anthranilic acids **10** for library design.

Scheme 1. Design of the Bicyclic Quinazolinone Natural Product-Templated Library



Scheme 2. Synthesis of the Bicyclic Quinazolinone Natural Product-Templated Library



phenyl ring; Figure 3), 12 styryl carboxylic acids (with R^2 connected with styryl group; Figure 4), and 16 primary amines (R^3NH_2; aliphatic, benzyl, and heterocyclic amines, and hydrazines; Figure 5). On the basis of the diversity of the final products,[17] 170 compounds were then selected for synthesis by cherry-picking from the virtual library.

The library synthesis was preformed on a microwave station integrated into a solution-phase, high-throughput, automated, synthesis platform, with the reaction scheme shown in Scheme 2.[18] Reaction of anthranilic acids **10** (0.2 mmol) with carboxylic acids **11** (0.2 mmol) in the presence of $P(OPh)_3$ (0.24 mmol) in pyridine at 150 °C for 10 min under microwave irradiation, followed by the addition of amines **12** (0.2 mmol) and microwave irradiation at 230 °C for 5 min, yielded a library of compounds **9**. These compounds were then purified on an in-house, high-throughput, purification platform by reversed-phase HPLC with mass-triggered fraction collection,[19] quantified by weight, and characterized by LC/MS to confirm the synthesis of the desired compounds and to establish purity. The passing rate for this library was 71% with 121 good compounds (amount >10 μmol, purity: ELSD >90% and UV_{214nm} >80%). Representative compounds from the library are illustrated in Figure 6. Compounds **9a**, **9c**−**d** and **9k**−**m** were randomly selected from each plate for evaluation by ^1H NMR and ^{13}C NMR to confirm both the structures and purities.

Sixty compounds were randomly selected for screens against three cancer cell lines (NCI-H460, DU-145, SF-268) in an MTS cell proliferation assay.[20] This standard MTS assay can detect most cell growth perturbations with high sensitivity.[12] The active compounds (**9c**−**9j**) from the cell-based proliferation assay are listed in Table 1. The IC_{50} concentration ranged between 6.6 and 60 μM in the selected cancer cell lines.

Table 1. Cytotoxic Activity of **9c**−**9j** Using a Cell Proliferation Assay[a]

	cell line		
ID	NCI-H460	DU-145	SF-268
Taxol	0.012		
9c	6.61	7.98	11.7
9d	11.6	12.6	16.0
9e	12.6	16.1	19.2
9f	14.4	17.7	22.6
9g	15.1	19.4	21.2
9h	12.3	17.8	20.5
9i	36.6	51.6	59.6
9j	28.8	57.3	42.5

[a] IC_{50} in μM.[14]

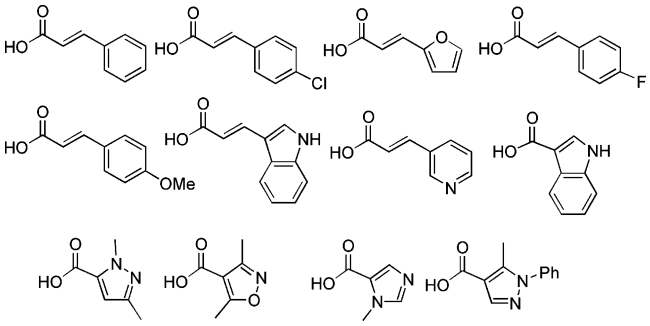


Figure 4. Selected carboxylic acids **11** for library design.

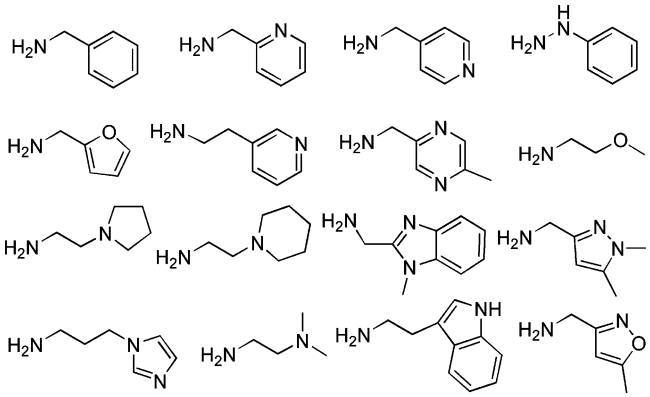


Figure 5. Selected amines **12** for library design.

Some key structural features appeared to be important in cytotoxic activity. In comparison with parent compound **7**, the major difference was that the newly designed compounds **9** also exhibited the cytotoxic activity, but it was limited only

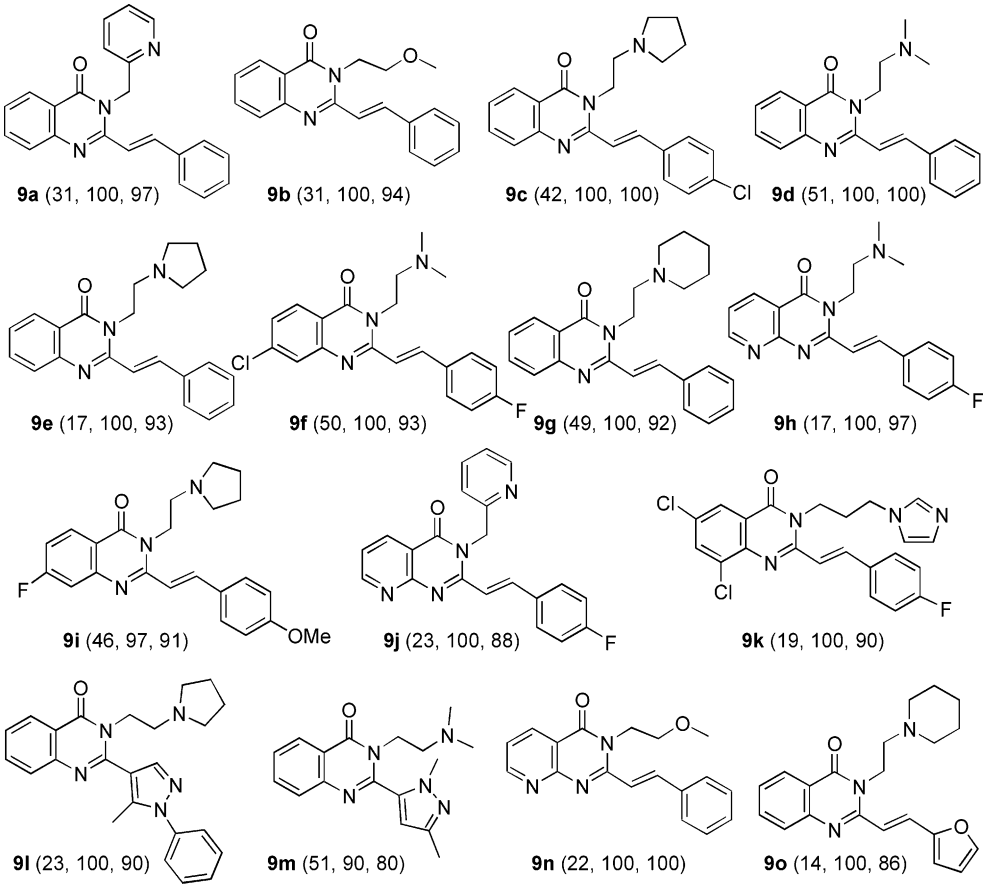


Figure 6. Representative compound structures of the library. The numbers in parentheses are the isolated yields (%) via high-throughput HPLC purification, ELSD (%), and UV_{214nm} (%) after purification.

to those with a basic nitrogen in R^3. For example, compounds bearing a basic nitrogen on the R^3 component displayed cytotoxic activity (products **9c**−**g**), but compounds **9** with nonbasic amines (product **9b**) and hydrazines (data not shown) did not. In addition, compounds with R^1 groups that were either neutral or bearing an electron-withdrawing group in the anthranilic acids (products **9c**−**g**) or hetero anthranilic acids (N in the aromatic ring; products **9h**, **9j**) exhibited cytotoxic activity. It is also notable that compounds containing cinnamic acid residues (R^2 = phenyls, **9c**−**j**) demonstrated cell killing ability, whereas the compounds with a heterocyclic ring on R^2 did not (products **9l**, **9m**, and **9o**).

In summary, we have designed and prepared a quinazolinone natural product-templated library and identified a group of 2-styrylquinazolinone compounds possessing a defining structural feature, a 3-substituted aliphatic chain bearing a basic nitrogen, as cytotoxic agents. This work has also demonstrated that a natural product-templated library can serve as a useful starting point for hit identification in drug discovery. Moreover, as the diversity of compounds accessed in template **9** is further expanded, new opportunities may present themselves in the search for more potent cytotoxic agents that may prove to be useful as anticancer hits and leads. Research on the design and synthesis of quinazolinone natural product-templated libraries and identification of novel cytotoxic agents is ongoing, and the results will be reported in due course.

Acknowledgment. The authors thank Dr. Jeffrey Link and Mr. Ted Manley for microwave technical support; and Dr. Craig Thompson, Mr. Bill Dahlberg, and Ms. Hannah Neumeier for assay support.

Supporting Information Available. Experimental procedure, yields, NMRs, purities (UV_{214nm} and ELSD), and MS spectral data for compounds **9a**, **9c**−**9d**, and **9k**−**n**; yields, purities (UV_{214nm} and ELSD), and MS spectral data for all 60 compounds selected for biological screening. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) We employed a Biotage Smith Synthesizer integrated into the ArQule AMAP high-throughput parallel chemistry platform.

(19) The purification of crude products were performed on the Preparative HPLC. The preparative HPLCs were conducted using a ProntoSIL 120-10-C18 column (50 × 20 mm). The flow rate was at 40 mL/min utilizing an acetonitrile/water mobile phase and 0.1% trifluoroacetic acid as a modifier with mass-triggered fraction collection. The crude and purified products were characterized by LC/MS with an integrated LC/MS system of a Shimadzu LC instrument and Micromass DMZ MS instrument. The HPLC was conducted using a Zorbax SB-C8 column (4.6 × 30 mm 3.5μm) with a flow rate at 3.0 mL/min, an acetonitrile/water mobile phase, and 0.05% trifluoroacetic acid as a modifier. For detailed information, see: (a) Kyranos, J. N.; Cai, H.; Zhang, B.; Goetzinger, W. K. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 719. (b) Goetzinger, W.; Zhang, X.; Bi, G.; Towle, M.; Cherrak, D.; Kyranos, J. N. *Int. J. Mass. Spectrom.* **2004**, *238*, 153.

(20) The three cancer cell lines represent diverse tissue sources. Two sets of assays ($n = 2$) were run with 20 data points using Taxol as a positive control.

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