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Privileged structure-based quinazolinone natural product-templated libraries: Identification of novel tubulin polymerization inhibitors

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Abstract—A focused quinazolinone natural product-templated library was designed and synthesized. Compounds from this privileged structure-based library were identified as antimitotic agents acting through destabilization of tubulin polymerization. The results suggested that **2** could be a privileged substructure.

Since Evans et al. first introduced the concept of privileged structure in 1988, privileged structure-based drug discovery has emerged as a fruitful approach in medicinal chemistry.[1,2] Privileged scaffolds increase hit rates for biological targets of interest, leading to the discovery of other biologically active targets and generating leads with enhanced drug-like properties.[2,3] Consequently, medicinal chemists value privileged structures as core scaffolds for viable starting points in library design and synthesis. This approach permits the identification of increased numbers of active compounds from screens against a variety of receptors.

Quinazolinone **1** is a core structural subunit found in a variety of bioactive natural products (Fig. 1).[2,4] Quinazolinone alkaloids possess a diverse range of biological activities including cytotoxicity,[5] anti-inflammatory activity,[6] and cardiovascular activity.[6,7] These compounds also act as cardiotonic, antihistamine, antifungal, antiviral, antimycobacterial, and antimalarial agents,[6] and they have demonstrated psychotropic, hypnotic, and a range of other central nervous system (CNS) effects.[8] As such, the quinazolinone skeleton is considered to be a privileged structure.[2]

We recently described the efficient and concise total syntheses of fumiquinazolines, mackinazolinone (**2**), isaindigotone (**4**), and quinazolinobenzodiazepine natural products.[9] This methodology additionally enabled us to rapidly access libraries of related natural product derivatives. These compounds were screened in an MTS[10] cell proliferation assay. Remarkably, compound **5a** showed promising cytotoxic activity, which is an unprecedented feature of the natural tricyclic quinazolinones (Scheme 1, Table 1). Notably, this class of compounds is a hybrid chemical series of natural products

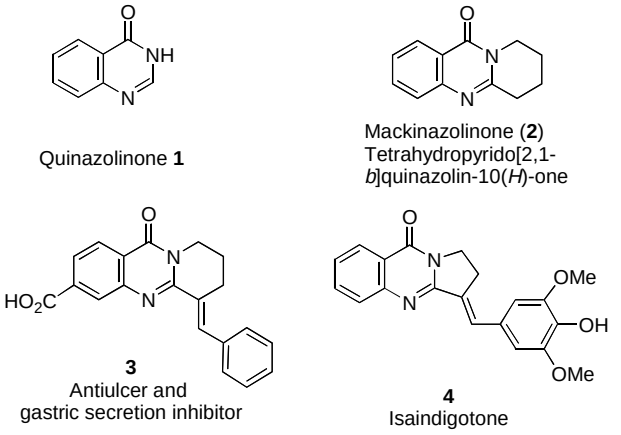

Figure 1. Naturally occurring quinazolinones and related bioactive compounds.

Keywords: Privileged structure; Quinazolinone; Natural product-templated library; Tubulin polymerization inhibitors.
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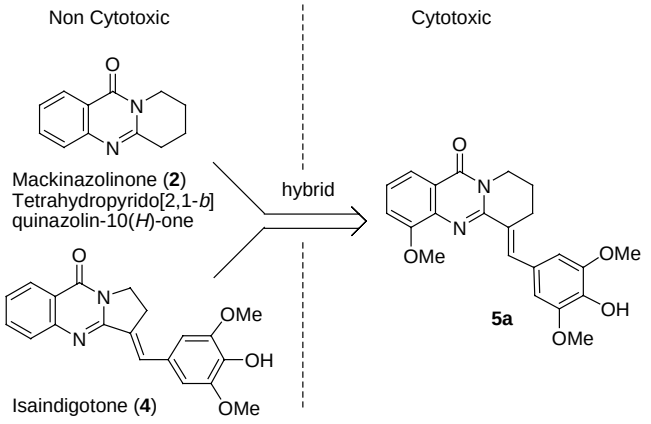
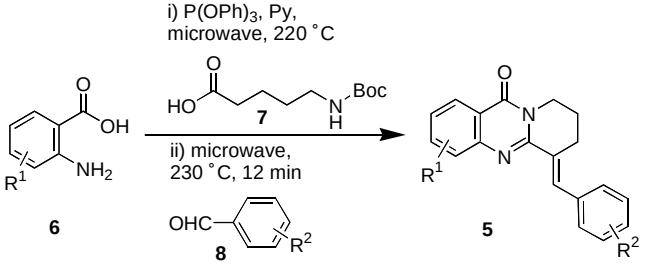


Scheme 1. Hybrid structure **5a**, which demonstrates cytotoxic activity where the natural products **2** and **4** do not.

mackinazolinone (**2**) and isaindigotone (**4**), yet neither parent species (**2** or **4**) exhibits cytotoxic activity.

It has been reported that compound **3** (Fig. 1), which has the same core scaffold as **5a**, is an effective inhibitor of gastric acid secretion and ulcer formation,[11] while we found that **5a** demonstrated cytotoxic activity.[9a] These intriguing results prompted us to generate a focused quinazolinone natural product-templated library of **5**, based on natural product mackinazolinone (**2**) as a template, with extended diversity on two phenyl rings (i.e., R^1 and R^2) (Scheme 2). We expected that the screening of this library might be useful in identifying the target of the cytotoxic action of **5** and uncover other novel anticancer agents.[12] In addition, a determination that tetrahydropyrido[2,1-*b*]quinazolin-10(*H*)-one (**2**) is a privileged substructure might, in turn, support our future library design and synthesis for other phenotypic screens. Herein, we describe the mackinazolinone natural product-templated library design, synthesis, and biological evaluation against cancer cell lines and target identification.[13]

The library containing core structure **5** can be synthesized in one step using our standard microwave-assisted three-component one-pot reactions from readily available starting materials—anthranilic acids **6**, *N*-Boc amino acid **7**, and aldehydes **8**.[14] Toward this end, library design was first conducted using the integrated ArQule library design tool.[15] Starting with the construction of the virtual library ($N = 900$), suitable building blocks were selected, which were derived from 30 anthranilic acids (with R^1 on the phenyl ring), 1 *N*-Boc amino acid, and 30 benzaldehydes (with R^2 on the phenyl ring) (Scheme 2). Based on the diversity of the final products, 67 compounds for the library synthesis were then selected by cherry-picking from the virtual library.[16]



Scheme 2. Synthesis of the mackinazolinone natural product-templated library.

The library synthesis was preformed on a microwave station integrated into a solution-phase high-throughput automated synthesis platform (Scheme 2).[17] Reaction of anthranilic acids **6** (200 μmol) with *N*-Boc amino acid **7** (200 μmol) in the presence of $P(OPh)_3$ (240 μmol) in pyridine at 220 °C for 10 min under microwave irradiation, followed by addition of the benzaldehydes **8** (240 μmol), microwave irradiation at 230 °C for 12 min yielded compounds **5**. These compounds were then purified on our high-throughput purification platform by reverse-phase HPLC with mass-triggered fraction collection,[18] quantified by weight, and characterized by LC/MS to confirm the syntheses of the desired compounds and to establish purity. The passing rate for this library was 66% with 44 good compounds (>10 μmol, ELSD >90% and UV_{214nm} >80%). Representative compounds from the library are given in Figure 2. Compounds **5a**, **5c**–**5e**, and **5g**–**5j** were randomly selected for evaluation by ^1H NMR and ^{13}C NMR to confirm both the structures and purities.

Table 1. Cytotoxic activity of **5** in a cell proliferation assay (IC_{50} in μM)

Compound	Cell line					pH3
	NCI-H460	A549	DU-145	MDA-MB-231	SF-268	
Taxol	0.012	0.013	—	—	—	0.031
5a	0.33	0.69	0.51	1.30	0.79	0.54
5h	0.24	0.55	0.79	2.09	0.83	0.26
5k	1.50	3.17	2.63	11.20	3.48	1.64
5j	1.36	5.49	3.11	7.72	4.76	1.16
5l	3.91	9.43	14.20	>20	14.10	—
5g	4.31	10.60	10.30	>20	11.20	10.60
5f	1.98	11.70	8.47	>20	6.72	2.38
5p	8.63	>20	18.30	>20	18.60	—
5m	3.20	>20	14.70	>20	11.50	—
5n	3.71	>20	10.30	>20	>20	—
5o	14.90	>20	>20	>20	>20	—

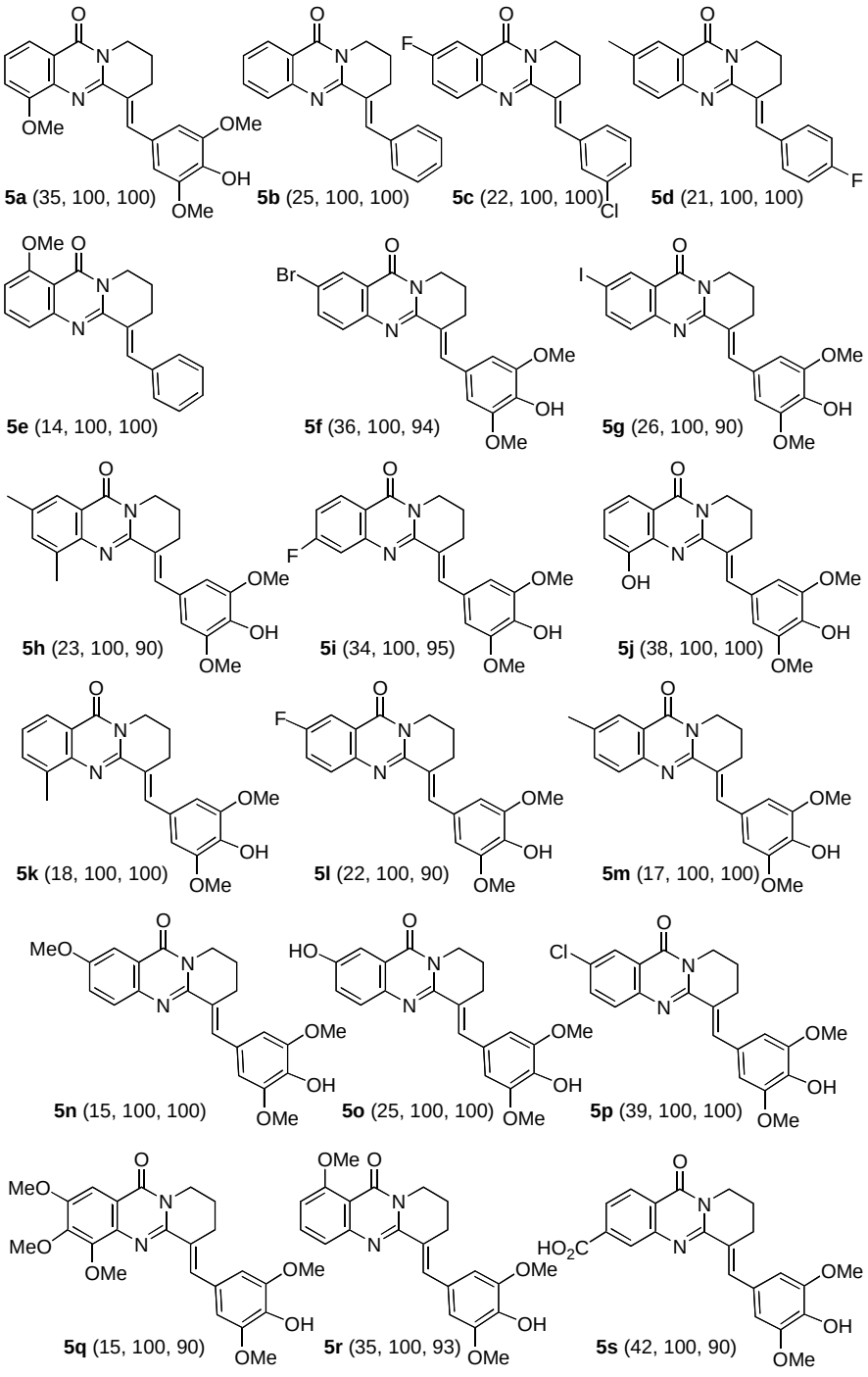


Figure 2. The representative compound structures from the library. The numbers in parentheses are the isolated yields (%) via high-throughput HPLC purification, ELSD (%), and UV_{214nm} (%) after purification.

The 44-membered library was screened in a search for the biochemical target and mechanism of action. Compounds that halt the cell cycle are some of the most successful and widely prescribed chemotherapeutics in use today. In general, these compounds can be divided into two classes: tubulin polymerization modulators and DNA replication antagonists. Tubulin modulating drugs, such as Taxol® and vinblastine, act by disrupting tubulin polymerization dynamics leading to a cellular arrest at the M phase of the cell cycle.[19] After a prolonged period of mitotic arrest, the cells ultimately undergo apoptosis (programmed cell death), which is thought to be the mode of clinical efficacy for these drugs.[20] Agents that induce mitotic arrest via disruption of tubulin dynamics or via other mechanisms (such as Eg5 inhibition) are sought-after, and one review article indicates there are over 21 distinct new chemical entities of this type currently in clinical trials as well as at least 11 compounds in preclinical trials.[21]

The library of **5** was first screened against five cancer cell lines (NCI-H460, A549, DU-145, MDA-MB-231, and

SF-268) and one primary cell culture (human mammary cell, HMEC) in an MTS cell proliferation assay. The active compounds with IC_{50} data are summarized in Table 1. The IC_{50} concentrations of active compounds were less than 20 μM in most cancer cell lines tested. The IC_{50} in HMEC cells was greater than 20 μM (data not shown), indicating that compounds **5** target cancer cells specifically. Interestingly, the highly oxygenated substitution pattern of the pendant phenyl ring of **5** (4-hydroxy-3,5-dimethoxy) played an important role in the activity. Conversely, the compounds with other functional groups on R^2 (**5c**–**5e**) or H (**5b**) (data not shown) did not exhibit any cytotoxic activity. Regarding the SAR of the R^1 moiety, the compounds with substituents on position 3, in general, are more active than those of substituents on other positions (**5a**, **5h**, **5j**, and **5k** vs **5l**–**5p**, **5r**). Given that compound **5b** possesses the parent structure found in **3**, which was effective at inhibiting gastric acid secretion and ulcer formation, and given the identification of the cytotoxicity of compounds **5** in our hands, we believe that **2** may be a privileged substructure in its own right.

The active compounds from cell-kill screens were progressed to the phosphohistone H3 (pH3) assay (Table 1) to evaluate whether they induce mitotic arrest.[22] The results demonstrated that these compounds induced mitotic arrest in a dose-dependent fashion with the EC_{50} of pH3 from 0.3 to 11 μM, suggesting that the mechanism of the cell proliferation perturbation is very likely caused by mitotic arrest.

Tubulin polymerization dynamics within the cell are critical for completion of mitosis and are frequently targeted by agents that induce mitotic arrest.[23] The tubulin polymerization assay was used to determine whether these compounds perturbed tubulin dynamics. All of active compounds in the MTS assay were selected for screening in tubulin polymerization assay. They all showed inhibitory activity to tubulin polymerization. The representative data are shown in Figure 3. These data strongly supports the notion that this class of compounds interferes directly with tubulin polymerization by binding to the tubulin and that this interaction is the likely cause of mitotic arrest.

In summary, we designed and prepared a focused mackinazolinone natural product-templated library and identified that tetrahydropyrido[2,1-*b*]quinazolin-10(*H*)-one (**2**) may be a privileged substructure. Screens with pH3 assay and tubulin assay suggested that this class of compounds are antimitotic agents and exert their action through the inhibition of tubulin polymerization. Research on the design and synthesis of privileged structure-based quinazolinone natural product-templated libraries and identification of novel antimitotic agents is ongoing and the results will be reported in due course.

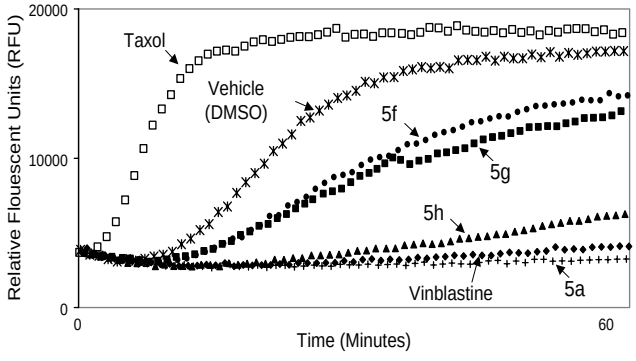


Figure 3. The tubulin assay with selected compounds.

Acknowledgments

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