ACS Combinatorial Science

Research Article

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Parallel Solution-Phase Synthesis and General Biological Activity of a Uridine Antibiotic Analog Library

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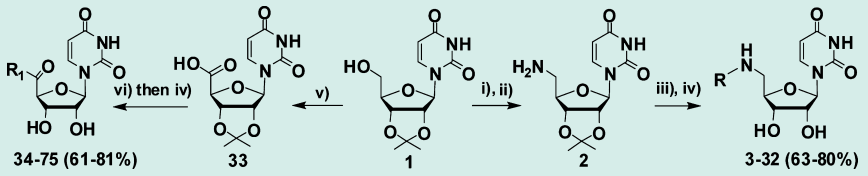
Supporting Information



ABSTRACT: A small library of ninety four uridine antibiotic analogs was synthesized, under the Pilot Scale Library (PSL) Program of the NIH Roadmap initiative, from amine **2** and carboxylic acids **33** and **77** in solution-phase fashion. Diverse aldehyde, sulfonyl chloride, and carboxylic acid reactant sets were condensed to **2**, leading after acid-mediated hydrolysis, to the targeted compounds **3**−**32** in good yields and high purity. Similarly, treatment of **33** with diverse amines and sulfonamides gave **34**−**75**. The coupling of the amino terminus of D-phenylalanine methyl ester to the free 5′-carboxylic acid moiety of **33** followed by sodium hydroxide treatment led to carboxylic acid analog **77**. Hydrolysis of this material gave analog **78**. The intermediate **77** served as the precursor for the preparation of novel dipeptidyl uridine analogs **79**−**99** through peptide coupling reactions to diverse amine reactants. None of the described compounds show significant anticancer or antimalarial acivity. A number of samples exhibited a variety of promising inhibitory, agonist, antagonist, or activator properties with enzymes and receptors in primary screens supplied and reported through the NIH MLPCN program.

KEYWORDS: *uridine antibiotic analogs, nucleoside peptides, specific or general biological activities*

INTRODUCTION

The structural complexity and varied three-dimensional characteristics of the natural products have been key elements behind their propensity to produce wide ranging and interesting biological activities. As such, the natural products have been a crucial resource for probing biology and metabolism with the goal of identifying new targets and leads for drug discovery, and a large portion of the current anti-infective and anticancer drugs are derived from natural sources.[1] Among these, nucleosides and nucleoside antibiotics show a variety of interesting biological activities resulting in numerous active drugs that are used clinically as anticancer, antiviral and antifungal agents.

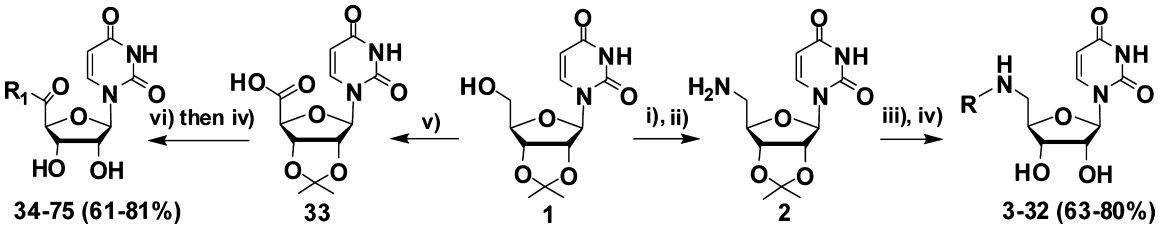
Although nucleosides are excellent synthetic templates for diversity-oriented synthesis to produce novel and richly substituted small molecules with unique directionally oriented groups to probe biological surfaces, they are relatively poorly represented in commercial and public compound libraries. Furthermore, a number of robust approaches are available, both historically and more recently using automated chemistry, that allow rapid preparation of small libraries of highly pure unique nucleosides.[2] Hence, it has been our goal to produce new libraries based on nucleoside templates for internal and external screening, particularly through the NIH Roadmap Program and the Molecular Libraries Probe Production Centers Network (MLPCN). Traditional nucleosides with an available 5′-hydroxyl that can enter nucleoside metabolic pathways, however, are problematic as biological probes due to their propensity to enter numerous nucleoside metabolic pathways and cause general toxicity through inhibition of DNA and RNA metabolism. On the other hand, there are copious examples of relatively simple to complex nucleosides that exhibit diverse alternative mechanisms of action.[3,4] Natural nucleoside antibiotics, for example, demonstrate potent and specific activities such as protein synthesis inhibition, glycosyltransferase inhibition and methyltransferase inhibition, among others.[2−6] More recently, there has been a trend to develop approaches for the synthesis and screening of this exciting class of compounds, which do not exhibit typical broad antimetabolite activities based on nucleoside phosphorylation and incorporation into nucleoside metabolic pathways. Among the nucleoside antibiotics are numerous examples of 5′-substituted peptidyl analogs modified with a diversity of amino acids, and there has been a great deal of interest in the continued isolation and synthesis of nucleoside amino acids and peptides.[7−10] Herein, our present report is dedicated to the synthesis of a small library of peptidyl uridine compounds generally inspired by several bioactive natural nucleoside antibiotics including the mureidomycins,[11] muramycins,[12] polyoxins (nikkomycin and tunicamycin),[4] and capuramycin.[4]

Most syntheses of nucleoside peptides have involved either the coupling of an amino or hydroxyl group of a nucleoside to

Received: November 15, 2013
Revised: February 17, 2014
Published: March 25, 2014

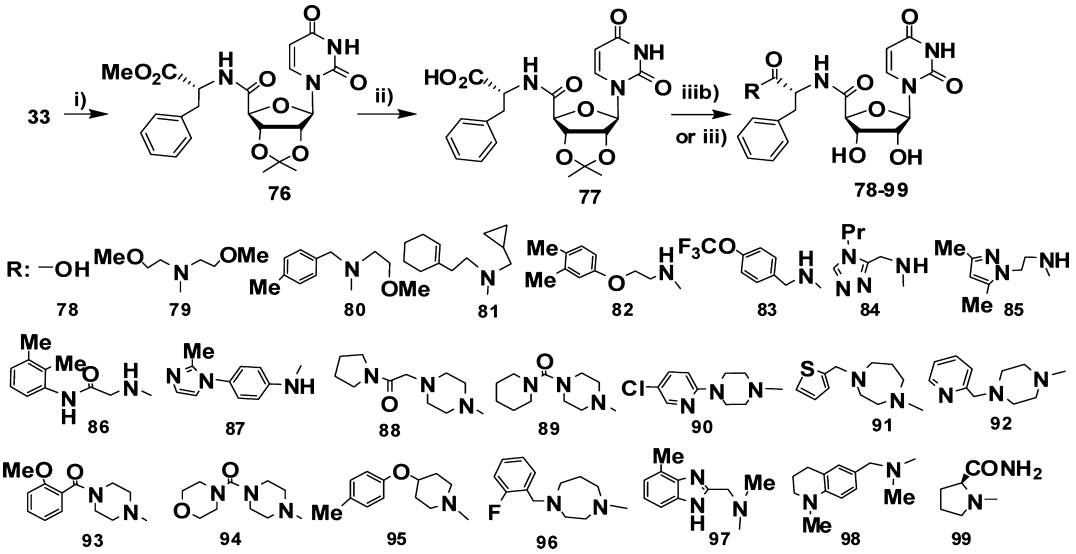
dx.doi.org/10.1021/co4001452 | ACS Comb. Sci. 2014, 16, 232−237

Scheme 1[a]



[a](i) (a) TsCl, pyridine; (b) NaN_3, DMF, 50 °C. (ii) NH_4HCO_2, Pd−C 10%, MeOH. (iii) R = a part f rom an aldehyde/MeOH, 0−40 °C, $NaBH_4$; R = a part from amino acid/HATU, DIEA, CH_3CN; R = a part from sulfonyl chloride/DMF, $CsCO_3$. (iv) 50% HCO_2H, 70 °C. (v) TEMPO-iodobenzene diacetate. (vi) R_1 = amine derivative, HATU, DIEA, CH_3CN or R_1 = sulfonamide, DCC, DMAP, CH_2Cl_2.

Scheme 2[a]



[a](i) D-Phenylalanine, HATU, DIEA; (ii) NaOH, dioxane; (iii) (a) *R* = amine derivative, HATU, DIEA, CH_3CN, (b) 50% HCO_2H.

the carboxyl group of a blocked amino acid, or displacement of a leaving group on a nucleoside by the amino group of an amino acid. Also, oxidation of the 5′-hydroxymethylene of nucleosides to 5′-carboxylates is an essential step in the preparation of a number of biologically active peptides.[12−15] With these approaches in mind, we have designed and prepared diverse nucleoside antibiotic-like small molecule libraries under the PSL program of the NIH Roadmap Initiative to probe specific or general biological activities. Very recently, we have reported an initial phase of this program and certain analogs have shown interesting and diverse biological activities in preliminary MLPCN screening.[16] In continuation of this effort, we now report a facile synthesis of a novel uridine analog library derived from amine **2** and carboxylic acid intermediates **33** and **77** (Schemes 1 and 2) using parallel solution phase chemistry.

Both **2** and **33** are suitable precursors for the synthesis of a variety of biologically active nucleoside analogs[12−27] dating back to the first report describing the structures and synthesis of polyoxin analogs.[28] Hence, the 5′-amino and 5′-carboxylic acids groups of the nucleoside ribose moiety of **2** and **33** as well the carboxylic acid group of **77** were chosen as three sites of diversification through robust reductive amination or sulfonation reactions as well as current and robust peptide coupling chemistry to prepare the target library of analogs **3**−**99** (Schemes 1 and 2). The resulting nucleoside peptide library was expected to show reasonable stability to dissolution, storage and screening as evidenced for similar aminoacyl functions found in various nucleoside antibiotics such as puromycin, gougerotin, amicetin, and blasticidin S, all known inhibitors of protein synthesis. In general, to allow the efficient and relatively larger scale preparation of small molecule libraries, solid or solution-phase organic synthesis has been adapted for use with a variety of automated equipment such as liquid handlers, and techniques, such as the fluorous-tag approach and solid-phase extraction.

For our approach, we chose to use solution-phase organic synthesis due to several factors. First, there are a variety of robust reactions for both reductive amination and peptide bond forming reactions that lead to relatively pure products in high yields while precluding difficult purification of products. Hence, we felt that a solid phase approach to improve yields via reagent cycling as well as purity through reaction efficiency and release of purified material from a solid substrate was not necessary. Furthermore, our funded grant required repositing approximately 20 mg of material with the MLSMR, as well as a tacit commitment to supply additional material to interested researchers upon request. Hence, we targeted 50−100 mg of pure product to be able to fulfill this request as well as to maintain a supply of material for internal screening purposes. The ease and speed of the reported reactions, purification and good yields certainly justifies our choice although we would not

dx.doi.org/10.1021/co4001452 | ACS Comb. Sci. 2014, 16, 232−237

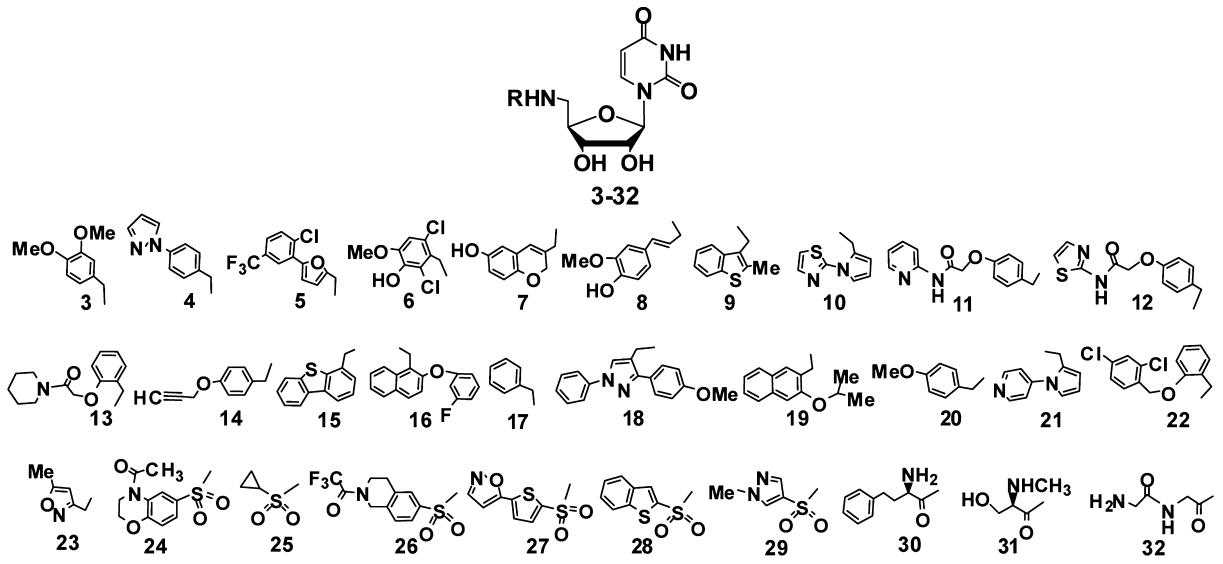


Figure 1. Structures of compounds **3**−**32**.

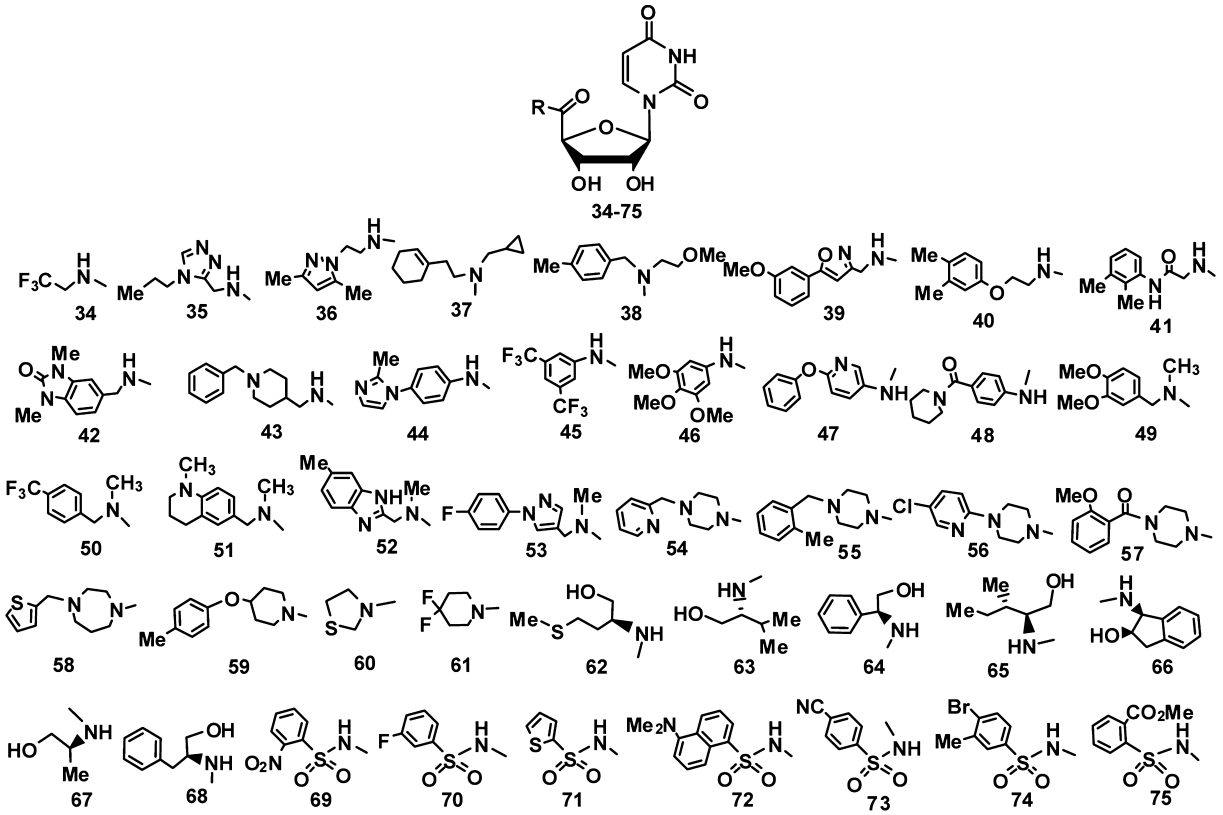


Figure 2. Structures of compounds **34**−**76**.

preclude solid phase approaches for further development of this chemistry.

Reductive amination is an efficient method that is readily adaptable to parallel format and, hence, we adapted this reaction to couple compound **2**, synthesized in three steps (Scheme 1) starting from **1** according to the modified reported method,[26] with twenty-one commercially available aldehydes. Successful couplings were achieved in methanol in the presence of molecular sieves to efficiently drive intermediate imine formation. It must be noted that the use of molecular sieves was crucial in terms of yield improvement and reaction time. The reactions were accomplished on a Radleys 12-place carousel reaction station at room temperature, although occasionally reactions were facilitated with less soluble aldehydes by warming for the first ten minutes at 40 °C. The resulting aldimines were carefully treated in situ with solid sodium borohydride for one-half hour and the reaction was then preadsorbed and dried on silica gel without further workup followed by automated flash chromatography purification. Similarly, direct acid-mediated deprotection of the acetonide protecting group using 50% formic acid furnished the desired final compounds **3**−**23** (Figure 1) in good yields.

dx.doi.org/10.1021/co4001452 | *ACS Comb. Sci.* 2014, 16, 232−237

Table 1. Examples from PubChem Bioactivity Analysis (Primary Screening)

compound	SID (sample identification number)	biological activity
69	134215029	agonist of the DAF-12 from the parasite H. glycines
56, **74**, and **71**	121286507, 134215027, and 134215024	inhibitors of Dengue virus 2 by using the cytopathic effect assay
54	124753399	active in an assay that monitors the cell−cell fusion activity of HIV-1 Envs with a firefly luciferase readout
33		activator of alpha dystroglycan glycosylation.
48 and **49**	121286505 and 121286489	inhibitors of the orphan nuclear receptor subfamily 0, group B, member 1 (DAX1; NR0B1)
49	121286489	inhibitor of Crimean−Congo Hemorrhagic Fever viral ovarian tumor domain protease
57	121286503	identified as a positive allosteric modulators of the human cholinergic receptor, muscarinic 4
40 and **64**	121286502 and 121286496	agonists of the mouse 5-hydroxytryptamine (serotonin) receptor 2A
51	121286501	inhibitor of protein arginine methyltransferase 1
		inhibitor of microRNA-mediated mRNA deadenylation
		modulator of interaction between CendR and NRP-1
3, **7**, **27**, and **65**	93577277, 93577279, 92764715, and 121286497	D3 dopamine receptor antagonist
3	93577277	inhibitor of hepatitis C virus (HCV) with an IC_{50} of 6.3 μM
16 and **22**	121284933 and 121284939	inhibitor of human tyrosyl-DNA phosphodiesterase 1
9	121284937	inhibitor of sentrin-specific protease 8
15	121284934	inhibitor of RAD52 protein
28	92764714	PAX8 inhibitor using PAX8 luciferase reporter gene assay
6	92764711	activator (reactivators) of BRM function
		activator of methionine sulfoxide reductase A
4	92764709	inhibitor of HIV-1 virion infectivity factor protein

To achieve our small libraries **24**−**32** (Figure 1), **34**−**75** (Figure 2), and **79**−**99** (Scheme 2), a solution-based methodology to efficiently generate our library was pursued. The 24-position MiniBlock XT solution phase vessel was utilized. The reaction vessel chosen was a 17 × 110 mm test tube carousel because of its compatibility with our Tecan automated liquid handler (dispensation, retraction, and aspiration) and a Genevac evaporator. Thus, synthesis of sulfonylamide uridine analogs **24**−**29** (Figure 1) was achieved by reacting intermediate **2** with six sulfonyl chlorides. The reactions were carried out in *N*,*N*-dimethylformamide (DMF) at room temperature using cesium carbonate as base followed by hydrolysis of the isopropylidene blocking groups as described above. Peptide coupling of **2** to three amino acid derivatives using HATU [(2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (1 equiv) and *N*,*N*-diisoproplyethylamine (DIPEA, 1.5 equiv) in acetonitrile for three hours furnished, after direct acid-mediated deprotection, the desired amide compounds **30**−**32** in good yields. The same conditions were applied to prepare amides **34**−**68** (Figure 2) from intermediate **33** which was synthesized in 88% yield according to the method previously described[29] (Scheme 1). For the preparation of sulfonamide analogs **69**−**75** (Figure 2), we found that the combination of dicyclohexylcarbodiimide (DCC) and one full equivalent of DMAP in methylene chloride gave facile coupling between carboxylic acid **33** and seven sulfonamides.

When **33** was coupled to D-phenylalanine methyl ester, **76** (Scheme 2) was formed in 81% yield. Saponification with NaOH gave carboxylic acid intermediate **77**, and subsequent treatment with 50% formic acid quantitatively afforded final nucleoside analog **78**. The carboxylic acid group of **77** was designed as a site of further diversification through peptide coupling chemistry to prepare the targeted compounds **79**−**99** (Scheme 2) following the same conditions described for **30**−**32**.

All the synthesized analogs were characterized by proton NMR, HPLC, and mass analysis. Their purity was found to range from 90 to 100% and the average purity was 97%.

BIOLOGICAL EVALUATION

Compounds **3**−**32**, **34**−**75**, and **78**−**99** were screened in vitro against human brain tumor and leukemia cell lines at a fixed concentration of 8 μM. Only compound **66** showed modest toxicity in these cells with an inhibition of: 75%, 66%, 39% and, 43% for brain tumor and leukemia (Jurkat, NALM-16, Raji and Reh cells) respectively. Compound **44** was found to have a slight inhibition (47%) when all synthesized compounds were screened against the malaria strain 3D7 of *Plasmodium falciparum* at a fixed concentration of 7 μM. In addition, the synthesized analog library has been submitted in the MLPCN to be screened against a wide range of biological assays (see www.ncbi.nlm.nih.gov/pcsubstance search term Robert Reynolds). Certain analogs (Table 1) exhibited a variety of interesting activities in primary screens. For example, compounds **3**, **7**, **27**, and **65** were found to be antagonists of the D3 dopamine receptor which represents a very important target for the treatment of several neuropsychiatric disorders. Compound **3** was also identified as an inhibitor of hepatitis C virus with an IC_{50} of 6.3 μM. Analogs **56**, **71**, and **74** were identified as inhibitors of dengue virus type 2. Protein arginine methyltransferase 1 (PRMT1) activity has been associated with cardiovascular, malignant, infectious, and autoimmune disease, and; quinoline carboxamide **51** was active as a PRMT1 inhibitor in a high-throughput assay for PRMT1-specific inhibition. The same compound was identified as an inhibitor of microRNA-mediated mRNA deadenylation by a fluorescence polarization assay. Compound **51** was also active as a modulator of the interaction between "C-end Rule" (CendR) peptides and Neuropilin-1 (NRP-1), which is a pleiotropic cell surface receptor with multiple ligands that plays an essential role in angiogenesis, cardiovascular development, regulation of vascular permeability and development of the nervous system.

dx.doi.org/10.1021/co4001452 | ACS Comb. Sci. 2014, 16, 232−237

BIOLOGICAL ASSAYS

The antitumor assays were performed following procedures previously described.[30] The antimalarial assay was realized using the protocol published by Guiguemde et al.[31]

CONCLUSION

A general automated solution-based methodology from three diversity positions was explored, optimized and used to synthesize a 94-membered library. Equipment such as a multichannel liquid handler, vacuum centrifuge and automated chromatography allowed the automation of solution-phase chemistry and assisted in the preparation of high quality products. No marked antimalarial or anticancer activity was witnessed and all the prepared analogs have been submitted for screening in the MLPCN. Preliminary screening has indicated a variety of interesting activities and full evaluation of the libraries can be followed via the SID numbers listed in Table 1 or by visiting PubChem Substance.

ASSOCIATED CONTENT

Supporting Information

Additional material as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This investigation was supported by NIH Grant 1P41 GM086163-01 (Pilot-Scale Libraries Based on Nucleoside Templates for the ML Initiative, Robert C. Reynolds, P.I.). We thank James M. Riordan, Jackie Truss, Mark Richardson and David Poon of the Molecular and Spectroscopy Section of Southern Research Institute for analytical and spectral data. We also thank Kip Guy and Anang Shelat at St. Jude for preliminary single dose screening data for human brain tumor, leukemia and malaria cell lines.

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