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Generation of Anti-trypanosomal Agents through Concise Synthesis and Structural Diversification of Sesquiterpene Analogues

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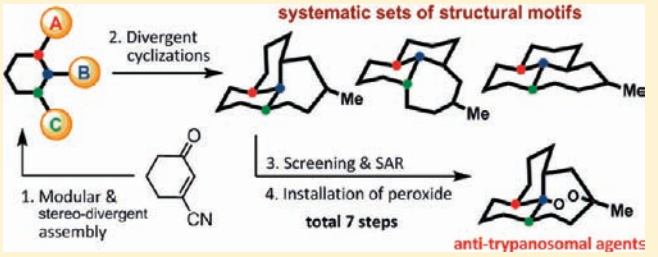
Supporting Information

ABSTRACT: To access high-quality small-molecule libraries to screen lead candidates for neglected diseases exemplified by human African trypanosomiasis, we sought to develop a synthetic process that would produce collections of cyclic scaffolds relevant to an assortment of natural products exhibiting desirable biological activities. By extracting the common structural features among several sesquiterpenes, including artemisinin, anthecularin, and transtaganolides, we designed six types of scaffolds with systematic structural variations consisting of three types of stereochemical relationships on the sp^3 ring-junctions and two distinct arrays of tricyclic frameworks. A modular and stereodivergent assembly of dienynes exploiting a versatile manifold produced a series of cyclization precursors. Divergent cyclizations of the dienynes employing tandem ring-closing metathesis reactions overrode variant reactivities of the cyclization precursors, leading to the six canonical sets of the tricyclic scaffolds incorporating a diene group. Screenings of trypanosomal activities of the canonical sets, as well as regio- and stereoisomers of the tricyclic dienes, allowed generation of several anti-trypanosomal agents defining the three-dimensional shape of the pharmacophore. The candidate tricyclic dienes were selected by primary screenings and further subjected to installation of a peroxide bridge, which generated artemisinin analogues that exhibited potent *in vitro* anti-trypanosomal activities comparable or even superior to those of artemisinin and the approved drugs, suramin and eflornithine.



INTRODUCTION

African sleeping sickness, also known as human African trypanosomiasis (HAT), is a vector-borne parasitic disease that is recognized as one of the world's most neglected diseases and causes serious medical and agro-economic problems in >30 sub-Saharan African countries.[1] The protozoan parasite *Trypanosoma brucei* is transmitted by blood-sucking tsetse flies, multiplies within the bloodstream of the mammalian host, and eventually invades the central nervous system. The disease is invariably fatal if not treated properly. In 2006, the World Health Organization estimated that HAT currently affects approximately 300 000 people annually and is responsible for 25 000 deaths each year.[2] A vaccine-based approach, however, is unlikely due to the antigenic variation of the parasite. Chemotherapeutic options are also very limited, and only four drugs are approved for HAT treatments (Figure 1): suramin (since 1916), pentamidine (1941), melarsoprol (1949), and eflornithine (1990). Chemotherapy with these outdated drugs is frequently limited in efficacy, plagued by severe side-effects, and hampered by increasing resistance of the parasites. Consequently, there is an urgent need for discovering safe and effective anti-trypanosomal drugs with novel structures and unique modes of action. With the intention of developing a new generation of lead candidates for HAT, we herein report development of a concise synthetic process leading to a collection of natural product-like scaffolds with skeletal and stereochemical variations. This collection has enabled structure−activity relationship (SAR) studies to elucidate a three-dimensional (3-D) image of the pharmacophore relevant to a series of biologically intriguing sesquiterpenes.

Aside from eflornithine, an amino acid derivative, three of the four prescription drugs for HAT disease are comprised of heteroatom-incorporated/appended aromatic rings and have sp^2-rich structural features with a substantial lack of sp^3 stereocenters. Recent studies reflect growing concern with larger numbers of aromatic rings that can negatively affect several drug-like properties, including lipophilicity and aqueous solubility, and thereby increase risks of failure in clinical trials.[3] Synthetic drugs and pharmaceutical collections of chemical libraries are typically prepared in cost- and labor-effective ways

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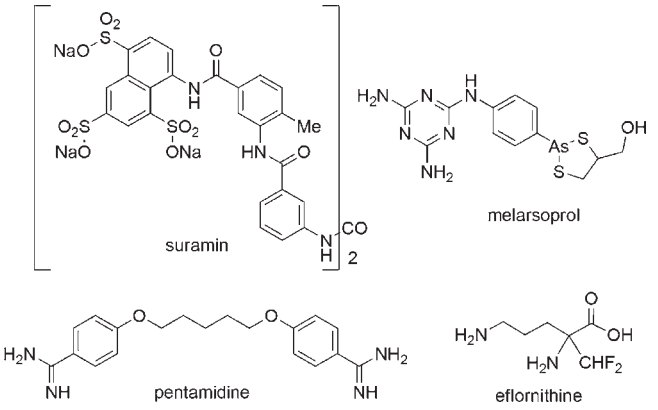

Figure 1. Four drugs approved for human African trypanosomiasis.

that exploit flat aromatic heterocyclic chemistry and consequently have disk- or rod-like shapes.[4] In contrast, natural products often possess complex and globular shapes composed of fused skeletons with fewer aromatic rings and increased numbers of stereogenic centers.[5] According to a recent analysis by Shoichet, 83% of the core scaffolds present among natural products are simply absent among commercially available molecules, demonstrating that vast areas of the biologically relevant chemical space charted by natural products remain unexploited.[6] It has been also recognized that an increased proportion of sp^3 stereocenters induces more 3-D shapes with diverse undulations on the surfaces. This improves interaction with the biological target, enhancing the potency and/or specificity of the drug candidate and increasing the probability of providing new pharmacophores and unique binding geometries.[7] Furthermore, incorporation of sp^3 centers multiplies the number of potential stereoisomers and thus generates structural variations in the compound collections. Despite this increased attention to synthetic approaches to access natural product-inspired libraries,[8−12] the *de novo* design and synthesis of optimal screening collections of small molecules bearing biological relevance and structural diversity has proved to be a formidable challenge in modern organic synthesis.

The adaptation and survival of the parasite *T. brucei* in its host involve integrated regulation of Ca^{2+}-ATPases, which are essential in calcium ion homeostasis. While several target proteins were explored for the chemotherapy of trypanosomiasis,[13] Ca^{2+}-ATPases were shown to be essential for parasite viability and regarded as targets for anti-trypanosomal drug development.[14] In fact, an anti-malarial drug, artemisinin **1** (Figure 2a),[15,16] targets PfATP6, the orthologue of the sarco-/endoplasmic reticulum Ca^{2+}-ATPase,[17] and exhibits anti-trypanosomal activity with an effective 50% inhibitory concentration (IC_{50}) of 5.8 μg/mL for *T. brucei rhodesiense*.[18] In 2007, Karioti and Tasdemir reported isolation of a novel sesquiterpene lactone, anthecularin **2**, which exhibits anti-trypanosomal activity (IC_{50} = 10.1 μg/mL for *T. brucei rhodesiense*).[19,20] In addition, structurally related transtaganolides/basiliolides **3** and **4** are reported to inhibit Ca^{2+}-ATPase, whereas their anti-protozoal activity has not been reported.[21,22] Regardless of the presence or absence of an endoperoxide bridge installed on the framework, we paid particular attention to the structural homology of these sesquiterpenes as a privileged scaffold for the development of novel anti-trypanosomal agents. We also noted the promising pharmacological properties of anti-malarial trioxane **1** bearing seven stereogenic sp^3 centers, which satisfies all the requirements for Lipinski's rule of five.[5a,23] Herein, we report the concise synthesis

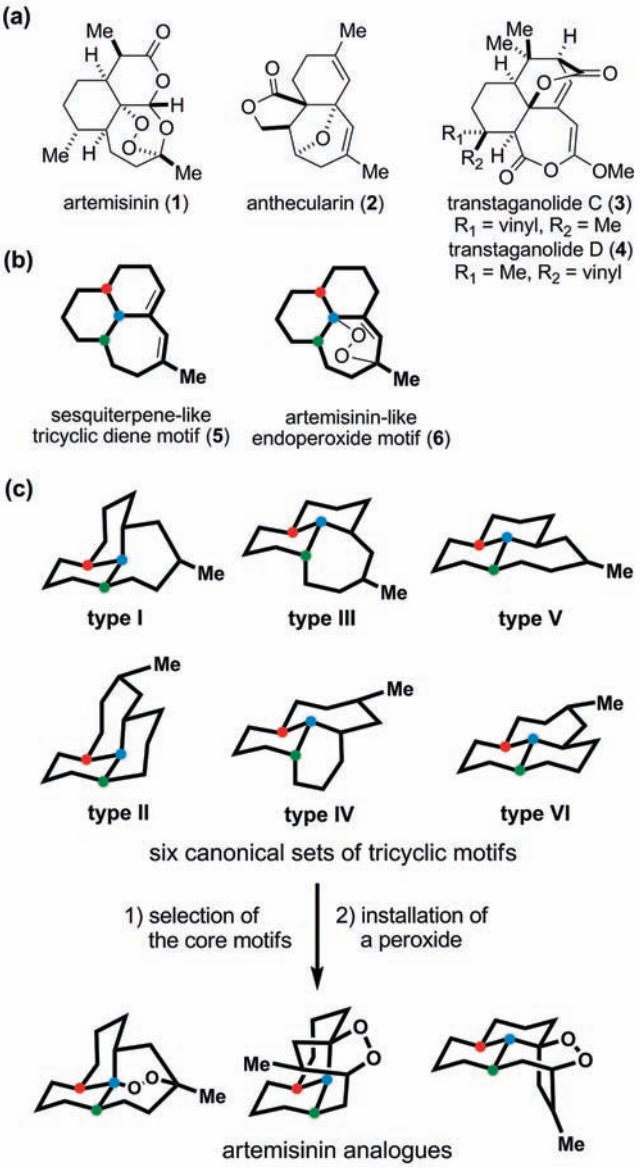

Figure 2. (a) Naturally occurring sesquiterpenes: anti-malarial drug artemisinin (**1**), anti-trypanosomal agent anthecularin (**2**), and Ca^{2+}-ATPase inhibitors, transtaganolides C and D (**3**, **4**). (b) Structural motifs: tricyclic **5** bearing a diene and tetracyclic **6** bearing a peroxide bridge. (c) Schematic illustration for structural diversification of the motifs and a synthetic approach to define the pharmacophore.

and structural diversification of structural motifs inspired by this assortment of sesquiterpenes with desirable biological properties toward the lead generation for trypanosomiasis. We also explored SARs exploiting a systematic set of small molecules with a dense matrix of stereochemical and skeletal variations.

RESULTS AND DISCUSSION

Design of Structural Motifs. Given the structural homology of the four sesquiterpenes **1**−**4** sharing closely related core skeletons, we designed a tricyclic scaffold **5** as the structural motif for generating anti-trypanosomal agents (Figure 2a,b). In addition, the presence of methyl substituents on the seven-membered rings in **1** and **2** as well as diene functionalities in **2** and **3** inspired us to

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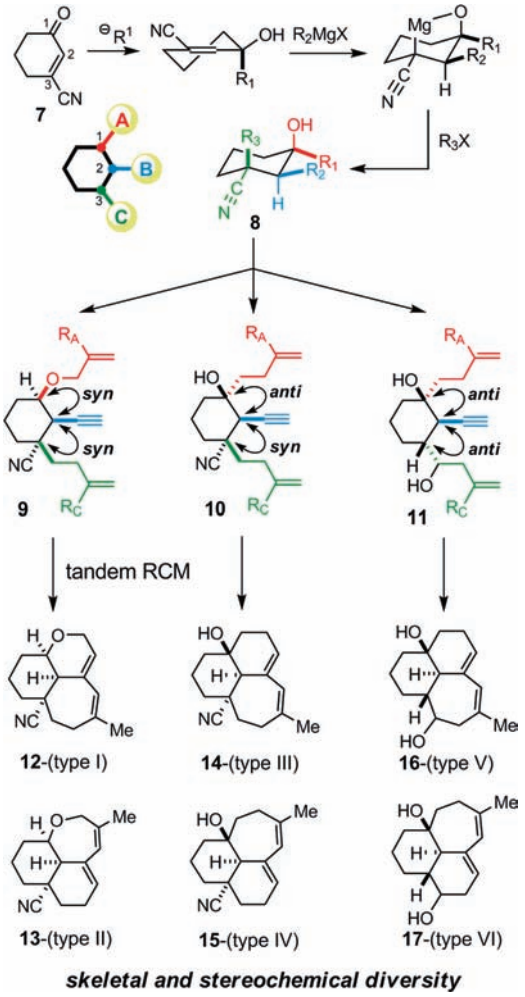
Scheme 1. Schematic Illustration of Synthetic Strategies To Generate Six Types of Sesquiterpene Analogues



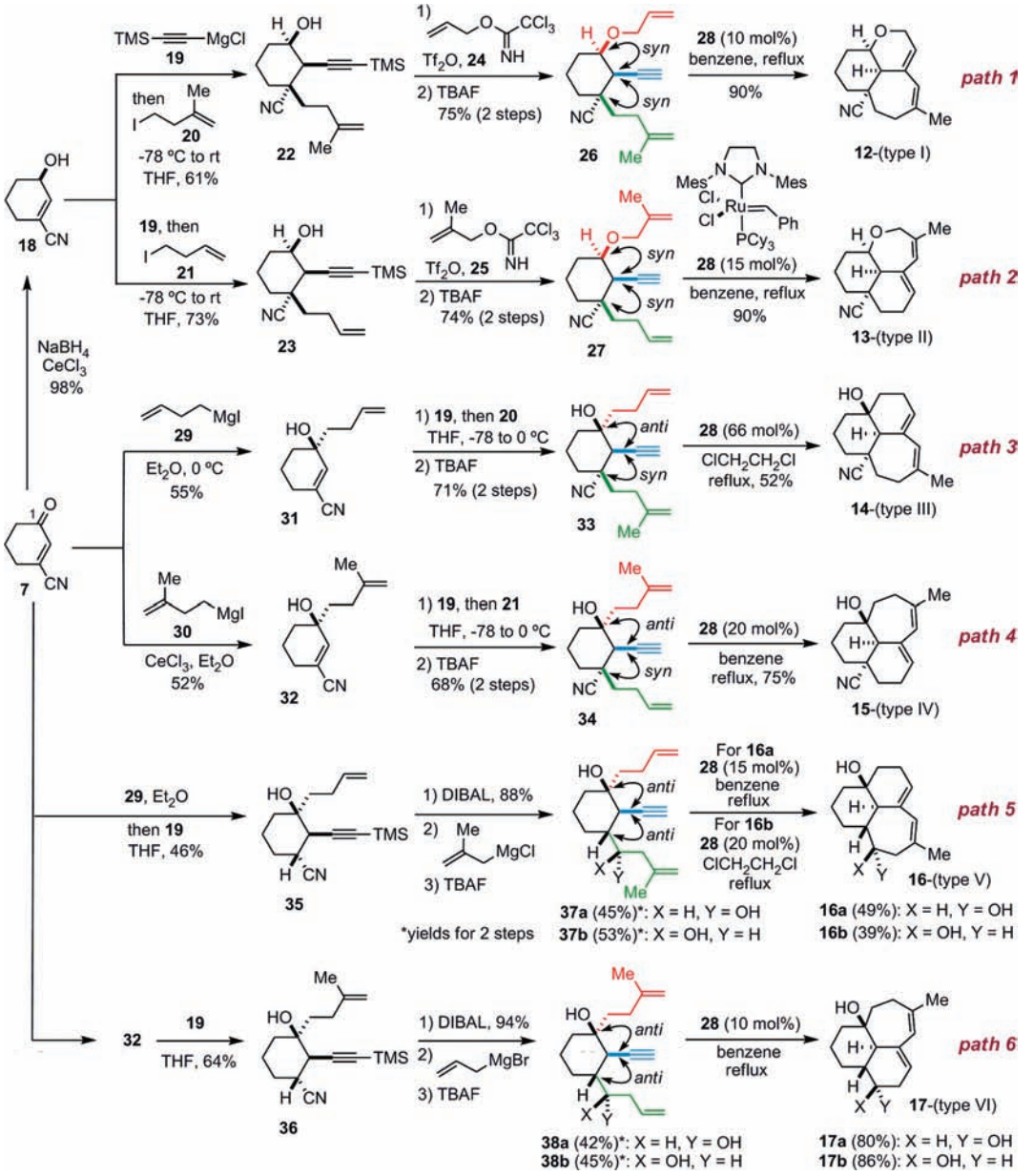
install methyl and diene groups on the scaffold. More importantly, in order to systematically generate 3-D structural diversity, we designed six types of tricyclic frameworks as canonical sets of structural motifs as illustrated in Figure 2c. To this end, we planned to diversify two factors that regulate molecular architecture: (1) three variations of stereochemical relationships (*cis-cis*, *trans-cis*, and *trans-trans*) for the three consecutive sp^3 ring-junctions highlighted in red, blue, and green and (2) two distinct arrays of the seven-membered ring against the ring-junctions. By gaining access to a series of tricyclic sesquiterpene analogues bearing rigid skeletons, we planned to perform SAR studies estimating the optimum stereochemical properties and cyclic arrays of the pharmacophore concurrent with screening for antitrypanosomal activities. Moreover, we envisaged further manipulations of the optimal structural motifs selected in the primary screenings. As illustrated in Figure 2b,c, installation of a peroxide bridge exploiting the diene groups embedded in the tricyclic cores would produce the artemisinin-like tetracyclic motif **6** by incorporating two additional sp^3 ring-junctions. This would lead to the development of a flexibly expandable synthetic process to afford multiple types of targeted motifs with elevated oxidation levels and structural diversities and complexities.

Design of a Synthetic Process Generating Stereochemical and Skeletal Variations. To obtain concise and stereocontrolled access to the systematic sets of the tricyclic motif **5**,[24] we sought to develop a synthetic process, as outlined in Scheme 1, fulfilling the following strategic requirements: (1) diversification of stereochemical relationships of the sp^3 ring-junctions into three types and the cyclic arrays into two types and (2) rapid and efficient construction of varied molecular architectures employing robust annulations to override the variances of the substrates. To this end, we designed oxonitrile **7** as a versatile manifold to assemble three building blocks on the three consecutive sites (A−C) of the six-membered ring in a modular fashion.[25] Addition of an R_1 group to the carbonyl group would produce a hydroxylalkenenitrile that effects chelation-controlled conjugate additions of acetylides as the R_2 group by applying Fleming's protocol.[26] Subsequent alkylation of the resulting *C*-magnesiated nitrile would proceed with retention of configuration to afford product **8** bearing R_1−R_3 groups with high levels of stereoselectivities. According to the sequential installations of R_1−R_3 groups, dienyne **10** with an *anti-syn* stereochemical relationship could be synthesized. Moreover, we conceived a stereodivergent method to access **9**-(*syn-syn*) and **11**-(*anti-anti*), exploiting the hydroxyl and nitrile groups installed on **8**. Either alkylation of the secondary alcohol or introduction of an R_1 group having a terminal olefin as a Grignard reagent at site A would establish *syn* or *anti* stereochemical relationships with the acetylene group at site B. Similarly, installation of an olefin group as the R_3 group or manipulation of the nitrile group would construct requisite stereochemical relationships between sites B and C.

To construct the six structural motifs **12**−**17**, we planned tandem ring-closing olefin metathesis reactions of dienynes,[27,28] leading to tricyclic systems with concomitant incorporation of diverse functionalities into the skeletons (Scheme 1). We performed divergent cyclizations by controlling the modes of tandem cyclizations of dienynes, leading to products with distinct cyclic arrays. A less-substituted olefin facilitates initial ruthenium carbene complex formation. Therefore, the tandem ring-closure of dienynes with sterically differentiated olefins would proceed in a regio-controlled manner via cyclization from sites A to B followed by B to C leading to a tricyclic diene, whereas a reversed substitution pattern of olefins at sites A and C could invert the cyclization mode to produce a distinct tricycle. The overall synthetic process consisted of (1) preparation of manifold and building blocks (build), (2) modular and stereodivergent assembly of the three components on the manifold (couple), and (3) divergent cyclizations of dienynes (pair). This strategy demonstrates the applicability of the build−couple−pair (BCP) strategy[9d,25,29] in library developments to generate scaffold diversity in a programmable fashion.

Synthesis of Tricyclic Skeletons. On the basis of our strategic plan, we carried out the synthesis of the six distinct tricycles incorporating internal dienes starting from manifold **7** (Scheme 2). First, we developed synthetic pathways 1 and 2, leading to type I and II dienes, **12** and **13**, with *cis-cis*-fused tricyclic skeletons. The C1 carbonyl group of **7** was reduced with $NaBH_4$−$CeCl_3$ to yield racemic hydroxylalkenenitrile **18**. The chelation-controlled conjugate addition of acetylide **19** and subsequent stereocontrolled alkylation of the resulting anion with **20** produced **22** in 61% yield. Because allylations of the alcohol **22** under basic conditions caused side reactions such as isomerization of the acetylene into an allene, we employed acid-catalyzed conditions exploiting trichloroacetimidate **24**. Subsequent removal of the trimethylsilyl group gave the dienyne **26** in 75%

Scheme 2. Systematic Synthetic Process Leading to Six Types of Tricyclic Dienes (Type I–VI)



yield (two steps). The dienyne **27**, with a 2-methylallyl ether at site A and a 3-butenyl group at site C, was synthesized in a similar fashion. Upon treatment of **26** with 10 mol % Grubbs second-generation catalyst **28** in benzene under reflux for 3.5 h, tandem ring-closing metathesis (RCM) of the dienyne system proceeded smoothly to afford the desired tricyclic diene **12** in 90% yield. Accordingly, ring-closure of **27** produced tricyclic diene **13** in excellent yield (90%). The modes of ring-closure were successfully controlled by exploiting the group-selective ruthenium carbene complex formation of monosubstituted olefins in the presence of disubstituted olefins. Thus, we achieved divergent and stereocontrolled access to tricyclic dienes **12** and **13** having *cis-cis*-fused ring-junctions in five steps from **7**.

Next, pathways 3 and 4 were explored to access *trans-cis*-fused tricyclic type III and IV dienes, **14** and **15**, respectively. Addition of Grignard reagent **29** to **7** afforded alcohol **31** in 55% yield.[30] Chelation-controlled conjugate addition–alkylation and subsequent removal of the silyl group produced **33** in 71% yield (two steps) as the major product.[31] Similar stepwise transformations gave **34**, in which the substitution pattern of terminal olefins at sites A and C was exchanged compared to that of **33** employing Grignard reagent **30** and alkyl iodide **21** in place of **29** and **20**, respectively. While ring-closure of **33** required a high catalyst loading (66 mol %) and resulted in a modest yield (52%) of the desired **14** with *trans-cis* ring-junctions, cyclization of **34** with 20 mol % catalyst produced **15** in satisfactory yield (75%). The structure of **15** was confirmed on the basis of X-ray analysis.[32]

Next, tricyclic dienes, **16** and **17**, with *trans-trans* ring-junctions were synthesized via pathways 5 and 6. Three-component assembly of **7**, **29**, and **19**, followed by protonation of the resultant carbanion in one pot, stereoselectively produced **35**

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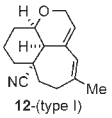
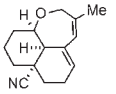
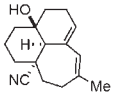
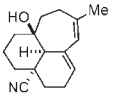
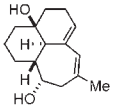
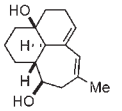
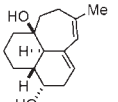
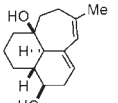
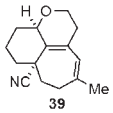
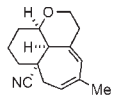
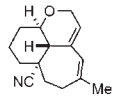
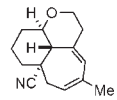
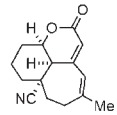
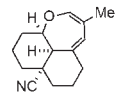
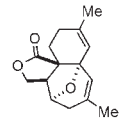
Table 1. ***In Vitro*** **Anti-trypanosomal Activities of Synthetic Sesquiterpene Analogues and Approved Drugs against** ***T. brucei brucei*** **GUTat 3.1**[a]

entry	compound	IC_{50} (μg/mL) anti-trypanosomal activity	IC_{50} (μg/mL) cytotoxicity	selectivity index (SI)
1	 **12**-(type I)	0.55	59.9	109
2	 **13**-(type II)	1.1	>100	>90.9
3	 **14**-(type III)	2.42	76.5	31.7
4	 **15**-(type IV)	4.96	24.3	4.9
5	 **16a**-(type V)	1.92	75.9	39.5
6	 **16b**-(type V)	>12.5	ND[a]	(-)
7	 **17a**-(type VI)	>12.5	ND[a]	(-)
8	 **17b**-(type VI)	>12.5	ND[a]	(-)
9	 **39**	1.98	45.4	22.9
10	 **40**	4.21	49.8	11.8
11	 **41**	1.02	40.0	39.2
12	 **42**	4.62	31.4	6.8
13	 **47**	3.0	3.73	1.2
14	 **48**	1.89	19.9	20
15	pentamidine[b]	0.00158	5.71	3600
16	suramin[b]	1.58	>100	>63
17	eflornithine[b]	2.27	>100	>44
18	 anthecularin (**2**)	10.1[c]		

[a] Culture of trypanosome ($2.0-2.5 \times 10^4$ trypanosomes/mL for GUTat 3.1 strain) was used. The cytotoxicities were evaluated with MRC-5 cells, and the selectivity index (SI) for trypanosomiasis was calculated as (IC_{50} for MRC-5)/(IC_{50} for *T. brucei brucei*). ND means "not determined". [b] Existing anti-trypanosomal drugs. [c] Reported in ref 19 against *T. brucei rhodesiense*.

in 46% yield. Reduction of the nitrile **35** gave an aldehyde in 88% yield, which was treated with 2-methylallyl Grignard reagent to yield a diastereomeric mixture of diols in an approximately 1:1 ratio. Separation of the diastereomers and removal of the silyl group produced the dienynes **37a** and **37b**. The dienynes **38a** and **38b** with the substitution patterns of terminal olefins at sites A and C exchanged were synthesized in a similar fashion except for stepwise additions of the Grignard reagents (**7** → **32** → **36**). Whereas tandem ring-closures of **37a** and **37b** to afford the tricyclic dienes **16a** and **16b** resulted in moderate yields (39−49%), cyclizations of **38a** and **38b** proceeded smoothly to produce **17a** and **17b** in good yields (80−86%). Structures of **37a** and **17b** were unambiguously determined on the basis of X-ray analysis of their derivatives.[24] Thus, the six types of tricycles bearing internal dienes were successfully constructed in a systematic fashion. We developed this synthetic process to demonstrate the synergistic effects of stereoisomerism of sp^3 ring-junctions and the divergent cyclizations of dienynes to generate 3-D structural diversity in conformationally rigid tricycles. This synthetic strategy created structural diversity without a substantial increase in the molecular weights of the six canonical tricycles (average MW ≈ 235), consistent with a key aspect of Lipinski's rule: restriction of molecular weight range (MW < 500).[23] This is in contrast to the typical strategy in medicinal and combinatorial chemistry of appending building blocks to a common skeleton to efficiently generate analogues of a target structure.

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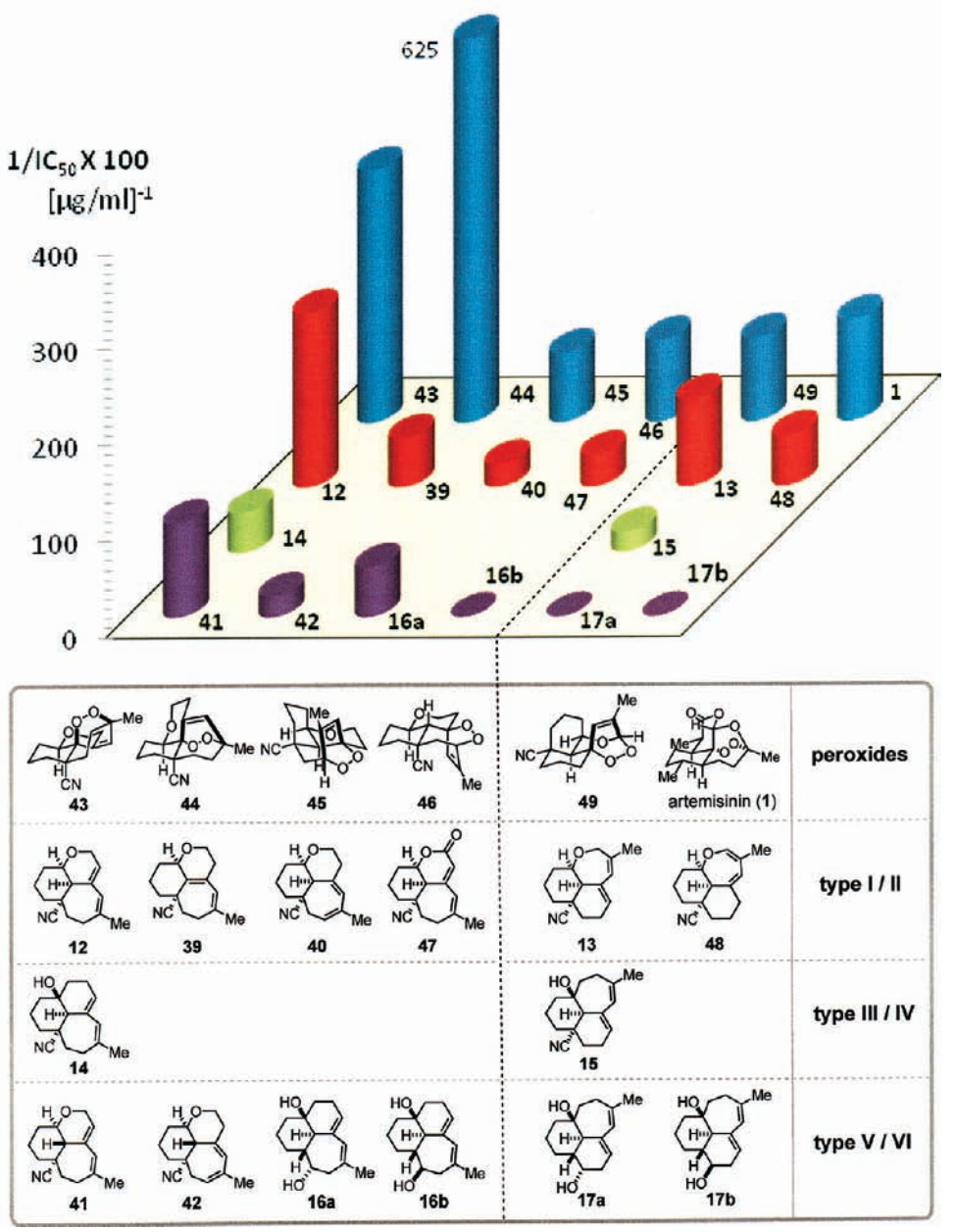

Figure 3. SAR study of synthetic sesquiterpene analogues for *in vitro* anti-trypanosomal activities. The dashed line divides types of the canonical scaffolds except for the lane of peroxides: left of the line are the potencies for type I, III, and V dienes; right of the line are those for type II, IV, and VI dienes.

Anti-trypanosomal Activities of the Six Canonical Sets of Tricyclic Dienes. With the six kinds of tricyclic dienes **12**−**17** in hand, *in vitro* anti-trypanosomal activities were evaluated employing a GUTat 3.1 strain of *T. brucei brucei* (Table 1, entries 1−8).[33] The *cis-cis*-fused **12** (type I) possessing a cyclic ether and a nitrile exhibited the most potent activity (IC_{50} = 0.55 μg/mL), and **13** (type II) with its distinct cyclic array also showed convincing activity (IC_{50} = 1.1 μg/mL). The *trans-cis*-fused dienes **14** (type III) and **15** (type IV) having a tertiary alcohol and a nitrile showed moderate activities. Regarding the type V and VI dienes, **16** and **17**, bearing *trans-trans*-fused skeleton with two hydroxyl groups, only **16a** having a 1,4-*anti*-diol exhibited substantial activity (IC_{50} = 1.92 μg/mL), whereas the other three dienes **16b**, **17a**, and **17b** showed negligible activities. Comparisons of the anti-trypanosomal activities between the six types of canonical scaffolds are graphically illustrated in Figure 3, clearly showing that type I and II dienes, **12** and **13**, exhibited dominant potencies over the others. On the basis of the evaluation of cytotoxities of the synthetic molecules against a mammalian cell line (MRC-5 cells), we calculated a selectivity index [SI = (IC_{50} for MRC-5 cells)/(IC_{50} for *T. brucei* strain)] as a means to assess combined potencies of both anti-trypanosomal and cytotoxic activities, as shown in Table 1. It is worth mentioning that the dienes **12** (type I) and **13** (type II) possessed SIs comparable to those of two approved drugs for HAT, suramin and eflornithine (Table 1, entries 16 and 17).

Diversification of Dienes 12 and 13 and Further SAR Studies. Taking the preliminary SAR study into account, we

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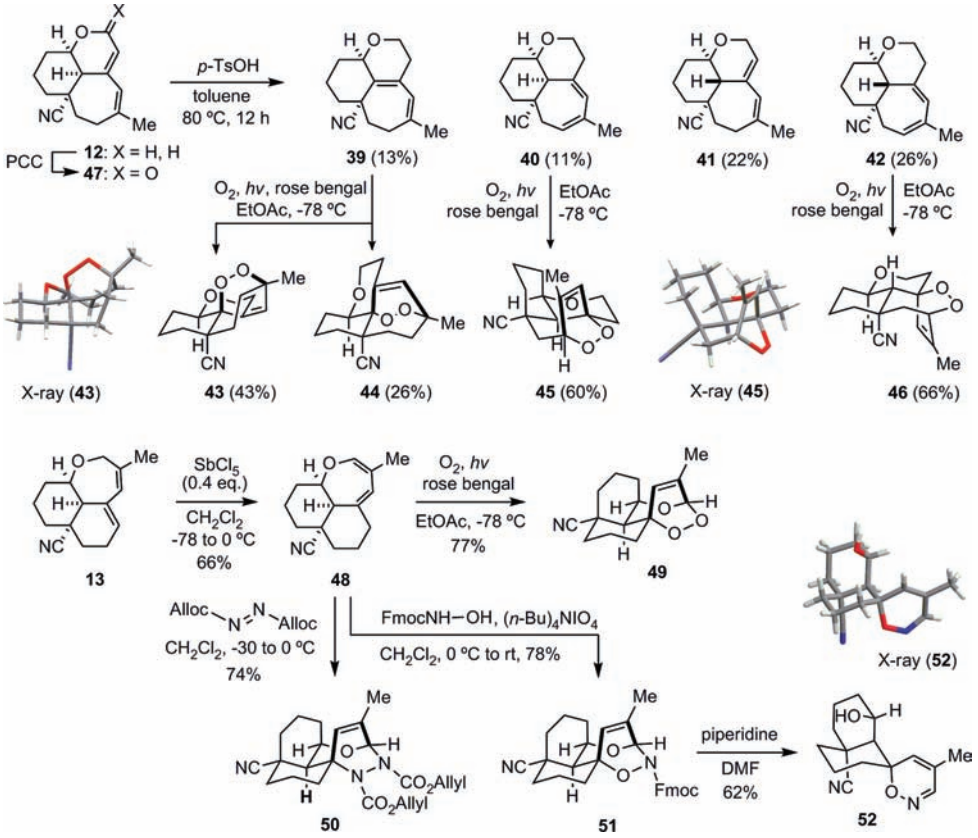
Scheme 3. Synthesis of Endoperoxides and Their Equivalents Starting from Type I and II Dienes



turned our attention to generating regioisomers of **12** and **13** by transpositions of the diene groups incorporated on the *cis-cis*-fused skeletons (Scheme 3). Isomerization of *s-trans* diene **12** upon treatment with *p*-toluenesulfonic acid in toluene at 80 °C produced the *s-cis* dienes **39** (13%) and **40** (11%) with recovery of the starting material **12** (16%). In addition, epimerization of the central sp^3 ring-junction competed to furnish **41** (22%) having the *trans-trans*-fused skeleton, and the regioisomeric *s-cis* diene **42** (26%) was also obtained. Whereas isomerization of **12** provided variations of conjugated dienes under thermodynamically controlled conditions, the conversion of **13** with distinct cyclic arrays proceeded in a regio-controlled manner to form *s-cis* diene **48** in 45% yield as the major product employing the identical Brønsted acid mediator. After screenings of various conditions for the diene transposition, we found that $SbCl_5$ was the best mediator, affording **48** in 66% yield below 0 °C with no more than 0.4 equiv of $SbCl_5$. In comparison, the next best mediators involving $TiCl_4$, $AlCl_3$, and $GaCl_3$ required at least 1.5 equiv to form **48** in moderate yields (14−49%).

Further SAR studies were conducted with these stereo- and regioisomeric dienes (Table 1, entries 9−14). The *trans*−*trans*-fused **41** having a cyclic ether and a nitrile exhibited appreciable activity (IC_{50} = 1.02 μg/mL), comparable to those of **12** and **13** (entries 1 and 2). Interestingly, the racemic *s-trans* dienes **41** and **16a** (entry 5) had equivalent activities and shared similar skeletal and stereochemical properties, possessing oxygen and nitrile functional groups in either 1,3-*anti*- or 1,4-*anti* relationships in the vicinity of the *trans*−*trans*-fused ring-junctions, whereas the activity was abolished with diastereomer **16b** having a 1,4-*syn* diol (entry 6). Anti-trypanosomal activities of the four *s-cis* dienes (**39**, **40**, **42**, and **48**) were decreased compared to those of the corresponding *s-trans* dienes (**12**, **13**, and **41**). In addition, lactone derivative **47** synthesized by oxidation of **12** with PCC[34] also showed diminished activity. Although it is difficult to directly compare the trypanosomal activities of anthecularin (**2**: IC_{50} = 10.1 μg/mL against *T. brucei rhodesiense*; Table 1, entry 18) reported by Karioti and Tasdemir because of inconsistencies of the *T. brucei* strains used, the racemic sesquiterpene analogues bearing an *s-trans* diene moiety (**12**, **13**, and **41**) exhibited either equal or increased anti-trypanosomal activities. Consequently, the SAR study employing tricyclic dienes with systematic variations of cyclic arrays and stereo- and regioisomerism allowed us to reveal novel pharmacophores with 3-D images relevant to the chemical space defined by **12**, **13**, and **41**.

Installation of the Endoperoxide Bridge and Its Equivalents Leading to Artemisinin Analogues. Encouraged by the efficacious activities of *s-trans* dienes (**12**, **13**, and **41**), we next examined the installation of endoperoxides on the corresponding *s-cis* dienes (**39**, **40**, **42**, and **48**) to furnish analogues of artemisinin **1** (**43**−**46** and **49**, Scheme 3). Photo-oxidation of **39** with an sp^2 ring-junction produced two separable diastereomers, **43** and **44**, in 43% and 26% yields, respectively. Diastereocontrolled addition of singlet oxygen to *cis-cis*-fused **40** and **48** occurred at the sterically less-hindered face to afford endoperoxide **45** and trioxane **49** in good yields, whereas photo-oxidation of *trans-trans*-fused **42** proceeded with the opposite face selectivity, leading to **46** in 66% yield. X-ray analyses of the crystalline **43** and **45** as well as NMR analyses including NOE experiments confirmed the structure of the endoperoxides.[35] Among the four *s-cis* dienes, **48** bearing a seven-membered cyclic enol ether

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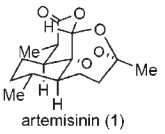
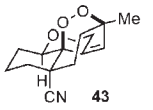
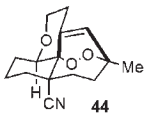
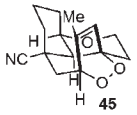
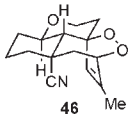
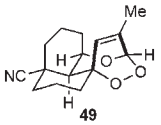
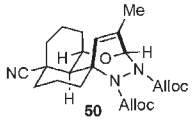
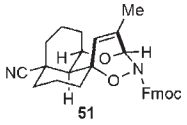
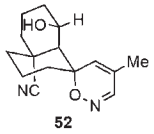
Table 2. *In Vitro* Anti-trypanosomal Activities of Artemisinin 1 and Its Synthetic Analogues against *T. brucei brucei* GUTat 3.1[a]

entry	compound	IC_{50} (μg/mL) anti-trypanosomal activity	IC_{50} (μg/mL) cytotoxicity	selectivity index (SI)
1	 artemisinin (1)	0.94	45.2	48.1
2	 **43**	0.38	59.4	156
3	 **44**	0.16	59.9	374
4	 **45**	1.39	9.1	6.5
5	 **46**	1.18	17.1	14.5
6	 **49**	1.15	23.0	20
7	 **50**	4.88	ND[a]	
8	 **51**	3.68	>100	>27.2
9	 **52**	>12.5	ND[a]	

[a] Culture of trypanosome ($2.0-2.5 \times 10^4$ trypanosomes/mL for GUTat 3.1 strain) was used. The cytotoxicities were evaluated with MRC-5 cells, and the selectivity index (SI) for trypanosomiasis was calculated as (IC_{50} for MRC-5)/(IC_{50} for *T. brucei brucei*). ND means "not determined".

exhibited the most proficient reactivity toward the cycloaddition. This reactivity prompted us to perform hetero-Diels–Alder reactions with azo and nitroso compounds to install "–N–N–" and "–N–O–" bridges in place of peroxides.[36] Diastereoselective cycloaddition of **48** with diallyl azodicarboxylate proceeded below room temperature to produce **50** in 74% yield, although removal of the carbamate ester group to produce the diaza-analogue corresponding to **49** proved to be difficult. Similarly, upon treatment of **48** with an acyl nitroso dienophile generated *in situ* via oxidation of the corresponding hydroxycarbamate, the hetero-Diels–Alder reaction occurred in a regio- and stereocontrolled manner to afford **51** in 78% yield. Treatment with piperidine to remove the Fmoc group, however, caused cleavage of the *N,O*-acetal, giving rise to crystalline **52** in 62% yield. X-ray analysis of **52** revealed a unique structure composed of a spiro-fused oxazine ring.[35]

Anti-trypanosomal Activities of Artemisinin Analogues. On the basis of the considerable anti-trypanosomal activity of artemisinin **1** against *T. brucei brucei* GUTat 3.1 strain (IC_{50} = 0.94 μg/mL), which we confirmed (Table 2, entry 1), collections of artemisinin analogues possessing regio- and stereoisomeric variations of peroxide bridges were evaluated (entries 2–9). The peroxide **44** synthesized from the diene **12** (type I) exhibited the most significant activity (IC_{50} = 0.16 μg/mL), which was superior to that of artemisinin **1**. The diastereomeric peroxide **43** also exhibited potent activity (IC_{50} = 0.38 μg/mL), and **45** bearing a regioisomeric peroxide showed diminished but appreciable activity (IC_{50} = 1.39 μg/mL). The other peroxides **46** and **49** also displayed anti-trypanosomal activities with IC_{50} values of 1.18 and 1.15 μg/mL, respectively, which are almost equivalent to the efficacies of **1** and the corresponding *s-trans* dienes **13** and **41**. Compounds **50** and **51** bearing –N–N– and –N–O– bonds share an identical framework with peroxide **49** and retained moderate activities, whereas spirocyclic **52** exhibited a loss of activity. Thus, all of the artemisinin analogues bearing peroxide bridges showed appreciable anti-trypanosomal activities (IC_{50} < 1.4 μg/mL; Figure 3).[37] Notably, the peroxides **43** and **44** showed higher SIs compared to those for suramin and eflornithine, and **44** satisfies the hit criteria for trypanosomiasis (IC_{50} < 0.2 μg/mL, SI >100).[38] Thus, we demonstrated that SAR studies of tetracyclic sesquiterpene analogues with increased sp^3 character and 3-D structural diversity allowed us to define novel pharmacophores for the development of anti-trypanosomal lead candidates.

CONCLUSIONS

Our approach to produce collections of fused molecules with structural novelty and complexity features concise access and diversification of privileged structural motifs generated from a proper selection of natural products with structural and functional homologies. The high hit rate and the discovery of new anti-trypanosomal agents demonstrated the effectiveness of this approach and the potential to generate novel lead compounds composed of natural product-like elaborated cores with densely integrated sp^3-hybridized stereocenters and heteroatom-containing substituents.[39] Although SAR studies of fused molecules have often been limited to alterations of substituents and functionalities attached to an identical core, we performed SAR studies exploiting canonical sets of tricyclic skeletons with systematic variations of the sp^3 ring-junctions and cyclic arrays, which provided insight into the stereoscopic view of pharmacophores. Accordingly, the

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synthetic approach exploring chemical space relevant to natural products is a complementary means for screenings of small molecules from natural sources and will play an increasingly important and indispensable role in the development of biologically active agents without prior structural information about the biological targets.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and ^{1}H and ^{13}C NMR spectra of compounds **12**−**18**, **22**, **23**, **26**, **27**, **31**−**52**; complete refs 13c and 29m; CIF files for **43** and **52**. This material is available free of charge via the Internet at http://pubs.acs.org.

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