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Jietacins, azoxy antibiotics with potent nematocidal activity: Design, synthesis, and biological evaluation against parasitic nematodes

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Key words, jietacin, vinyl azoxy, nematocidal, anthelmintic, natural product, late-stage diversification

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A.S., S.Ō., and T.S. designed the research;

A.S., C.W., S.Ō., and T.S. wrote the paper;

A.S., M.K., T.H., K.Y., N.T. and Y.N. synthesized jietacin derivatives and related substrates;

C.W. and C.M. performed the evaluation of anthelmintic activity;

D.M. performed the toxicological analysis;

T.N. and Y.T. isolated natural products;

J.K., S.Ō and T.S. provided scientific direction and established collaboration.

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Highlights

• Jietacin A, an azoxy antibiotic, was shown to have potent anthelmintic activity.

• A simplified jietacin derivative exhibits better anthelmintic activity than that of jietacin A.

• Structure-activity relationships of azoxy antibiotics were revealed.

• A simplified derivative showed anthelmintic activity via oral administration.

• Azoxy motif was found to be a useful platform for drug discovery.

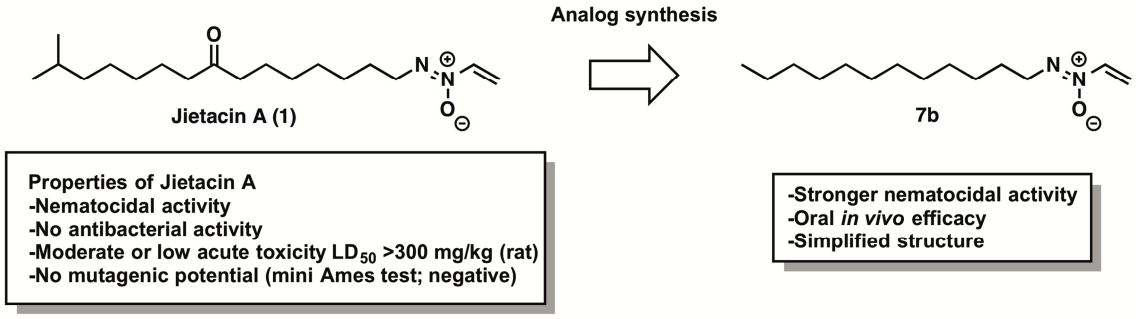
Financial interest

The authors declare no competing financial interest.

Abbreviations

AcOH, acetic acid; Cbz, carboxybenzyl; $(COCl)_2$, oxalyl chloride; DCM, Dichloromethane; DMSO, Dimethyl sulfoxide; DMF, Dimethylformamide; Et_3N, Triethylamine; LD50, lethal dose 50; NaN_3, sodium azide; $NaBH_3CN$, Sodium cyanoborohydride; TBHP, tert-butyl hydroperoxide; TBS, tert-butyldimethylsilyl; TBTA, Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amin)

Graphical abstract



Abstract

Jietacins, an azoxy antibiotic class of chemicals, were isolated from the culture broth of *Streptomyces* sp. KP-197. They have a unique structural motif, including a vinyl azoxy group and a long acyclic aliphatic chain, which is usually branched but non-branched in the case of jietacin C. During a drug discovery program, we found that jietacins display potent anthelmintic activity against parasitic nematodes and that jietacin A has a moderate or low acute toxicity (LD_{50} > 300 mg/kg) and no mutagenic potential in a mini Ames screen. This suggests that jietacins have potential for drug discovery research. In order to create a novel anthelmintic agent, we performed design, synthesis, and biological evaluation of jietacin derivatives against parasitic nematodes. Of these derivatives, we found that a fully synthesized simplified derivative exhibited better anthelmintic activity against three parasitic nematodes than natural jietacins. In addition, it had a better efficacy *in vivo* through oral administration against a mouse nematode. This indicated that the azoxy motif could prove useful as a template for anthelmintic discovery, possibly creating a class of anthelmintic with novel skeletons, a potential new mode of action, and providing further insight for rational drug design.

1. Introduction

1.1 Helminths and anthelmintics

Helminths are parasitic worms that have caused major and widespread disease in both humans and animals for centuries [1]. The two major phyla of helminths include nematodes and platyhelminths. The World Health Organization reported that the number of people infected with soil-transmitted helminths surpasses a billion people worldwide [2]. Such infections are a major cause of long-term illness, leading to productivity loss and poverty, and may be fatal. Likewise, helminth infections in animals are also of grave concern, since they lead to economic losses, animal welfare issues, and impact on human food supplies.

Historically, helminth infections have been treated with several classes of broad spectrum anthelmintics [3], such as benzimidazoles [4], probenzimidazoles [5], salicylanilides and substituted phenols [6], imidazothiazoles [7], tetrahydropyrimidines [8], organophosphates [9], macrocyclic lactones [10-16]. These compounds display variable modes of action and impact, while options for vaccination against helminth parasites remain severely limited. Unfortunately, reports of drug-resistant nematodes have been steadily increasing for currently available classes of anthelmintics [17-19]. Although novel classes of anthelmintic agents, such as octadepsipeptides [20, 21], aminoacetonitriles [22, 23], and spiroindoles [24] have been approved in order to help overcome drug-resistance, anthelmintic agents with a novel skeleton, which may have a new mode of action, are still urgently needed for use in both human and animal health.

1.2 Jietacins, azoxy antibiotics

The Kitasato research team has a long history of searching for anthelmintics from microbial metabolites originating in natural sources. In fact, a collaboration between the Merck, Sharpe and Dohme research laboratories and the Kitasato research group discovered the avermectins, the world's first 'endectocides', the compounds quickly becoming widely used as antiparasitic agents in veterinary medicine [10]. In 1987, during screening for nematocidal agents from natural sources, the azoxy antibiotics

jietacin A (**1**) and B (**2**) were isolated from the culture broth of *Streptomyces sp*. KP-197 (Fig. 2) [25, 26]. More recently, in 2014, we discovered more novel jietacin analogs (jietacin C (**3**) and D (**4**)), together with different components of the aliphatic side chain, from the same culture broth (Fig. 1) [27].

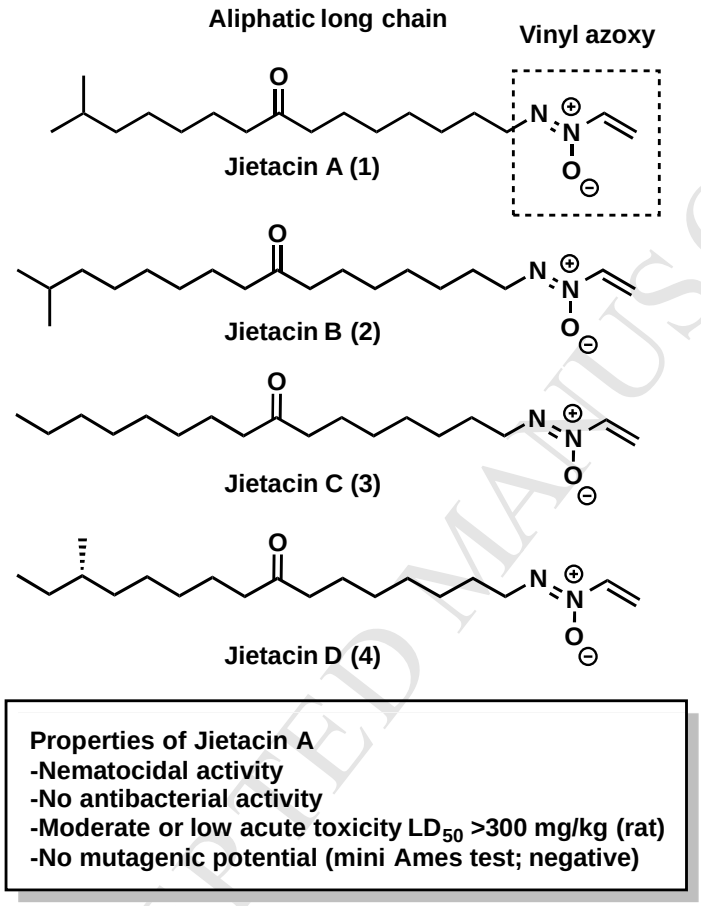


Fig. 1. Structure of azoxy antibiotics (jietacin A-D) and some bioactive properties of jietacin A.

While there are some other natural products bearing an azoxy moiety, such as elaiomycin [28-30], valanimycin [31], maniwamycins [32], LL-BH 872α [33], MH-071, MH-072 [34], and pyrinadine A [35], the jietacins contain a unique structural motif, a vinyl azoxy group and long acyclic aliphatic chains.

In terms of biological activity, we previously reported that **1** and **2** showed 10-times greater nematocidal activity against the pine wood nematode *Bursaphelenchus xylophilus* (LD_{100} = 0.25 ppm) than avermectin B1a (LD_{100} = 2.5 ppm), the main component of the commercially available antiparasitic agent [36]. Given the attractive

nematocidal activity and structural features of jietacins, a drug discovery program was started to identify a potential jietacin-derived candidate as a new anthelmintic. Initially, we further investigated the biological properties of **1** in order to identify a suitable candidate lead compound. As a result, we found that **1** showed potent activity in an assay using *Nippostrongylus braziliensis* (up to 100% inhibition of AChE (acetylcholine esterase) secretion at 10 and 1 ppm dosage) [37]. This is comparable to emodepside, a commercially available antiparasitic agent. The acute oral toxicity of **1** in female rats was absent, with no adverse clinical signs and no lethality at a dosage of 300 mg/kg. The LD_{50} is therefore considered to be >300 mg/kg. No evidence for mutagenic impact was found in a mini-Ames test with three different *Salmonella typhimurium* mutant strains. In contrast to the anthelmintic activity, no antibacterial activity of natural **1** against 27 strains was observed [38].

Given the anthelmintic activity *in vitro* of **1** and the fact that it did not show any significant toxicity in our assays, we decided to utilize this natural product as a lead compound for chemical optimization and clarification of structure-activity relationships (SARs).

Herein, we report the synthesis of novel jietacin derivatives, which showed greater anthelmintic activity against several nematodes than the parent jietacin A, insights into their SARs, and evaluation of biological activity *in vitro* and *in vivo*.

2. Results and discussion

2.1 Initial SAR maps based on natural jietacins

We previously reported on SAR concerned with activity against the pine wood nematode, *Bursaphelenchus xylophilus* [36], and showed that 1) changing a ketone to an acetal or an alcohol did not lead to a significant reduction in activity, suggesting a ketone does not play an important role and that 2) the vinyl azoxy group was necessary for anthelmintic activity at low concentration.

To further investigate SAR, we evaluated *in vitro* activity of four natural products (**1**-**4**), plus one derivative, against gastro-intestinal nematodes and compared the results with those for emodepside and ivermectin (IVM), both commercially available mainstream drugs. Tests were carried out using larvae of *Haemonchus contortus* and

Cooperia curticei, both parasites of sheep, and adults of the rat nematode *Nippostrongylus braziliensis*. Anthelmintic activity for **1**-**4** ((*R*)-**4** is not a natural product [27]) is summarized in table 1. Of significance, **1**-**4** and (*R*)-**4** showed 100% efficacy (inhibition of AChE secretion) in *N. braziliensis* at 1 ppm, the same as emodepside (100% efficacy at 10 and 1 ppm) and ivermectin. Lower concentrations were not tested. Against third-stage larvae of *H. contortus* and *C. curticei*, jietacins showed only low or moderate efficacy with one exception: **3**, showed 100% efficacy at 4 ppm against *H. contortus*, which was again comparable to emodepside and ivermectin. (*R*)-**4** had no efficacy against the larvae.

Overall, we found that, with regard to **2**, **3**, and (*R*/*S*)-**4**, possession of a non-branched side chain (e.g. **3**), caused better anthelmintic activity compared with possession of a branched side chain (e.g. **2** and (*R*/*S*)-**4**), even though they have the same carbon length.

Consequently we found that changing functional groups of the side chains plays a crucial role for generating anthelmintic activity, although the vinyl azoxy group is necessary for the activity based on our previous work [36]. To complete these SARs with respect to side chains, testing of more derivatives will be required.

Table 1

In vitro anthelmintic activity of jietacin A-D, ivermectin, and emodepside.

	H. contortus (efficacy %)[1]			*C. curticei* (efficacy %)[1]			*N. braziliensis* (efficacy %)[2]	
Compound	20 ppm	4 ppm	0.8 ppm	20 ppm	4 ppm	0.8 ppm	10 ppm	1 ppm
Emodepside	80-100	80-100	0	90-100	90-100	0-80	100	100
Ivermectin	80-100	80-100	40-80	90	90	60-80	100	100
Jietacin A (**1**)	0	0	0	60	60	0	100	100
Jietacin B (**2**)	60	0	0	40	0	0	100	100
Jietacin C (**3**)	100	100	0	60	40	0	100	100
Jietacin D (**4**)	40	40	0	40	0	0	100	100
(*R*)-Jietacin D ((*R*)-**4**)	0	0	0	0	0	0	100	100

[1]Efficacy in larval assay: decrease of motility, 100% efficacy means that all larvae were immotile, 0% efficacy means that all larvae were motile. Results for jietacins are presented as the mean of duplicate

tests. In case of the reference compounds emodepside and ivermectin, compounds were tested more often and in at least two batches, each test performed in duplicate. Therefore, a range of the results is indicated. The range observed for emodepside in *C. curticei* at 0.8 ppm is considered to be due to the impact of potential variability in concentration of the highly lipophilic compound at a sensitive point in the dose-response curve. Lower concentrations of emodepside (0.16 ppm, 0.032 ppm) have no efficacy in the respective assay (data not shown).

[2]Efficacy in *Nippostrongylus* assay: decrease of acetylcholine esterase activity compared to negative control. Anthelmintic activity was categorized using the following scale: 100: >84%-100% = full activity (complete AChE inhibition compared to negative control); 84: >60%-84% = good activity; 60:>35%-60% = weak activity, and 35: 0%-35% = no activity.

2.2 Strategy for side-chain modification

Based on our initial SAR maps, our new strategy focused on synthesis of various side-chain derivatives, which were 1) alkyl, aryl groups with triazole linkage via click reaction, 2) acyl derivatives with ketone linkage via Grignard reagents, and 3) aliphatic alkyl chains with no linkage (Fig. 2). To accomplish this strategy, we decided to utilize two approaches, late-stage diversification [39-41] and function-oriented synthesis [42, 43].

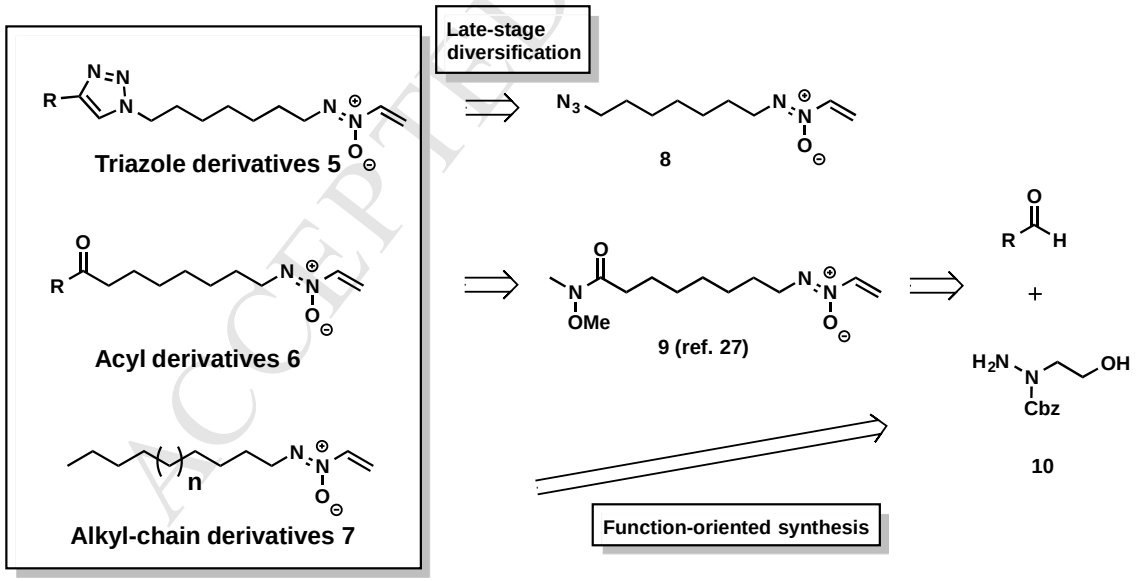
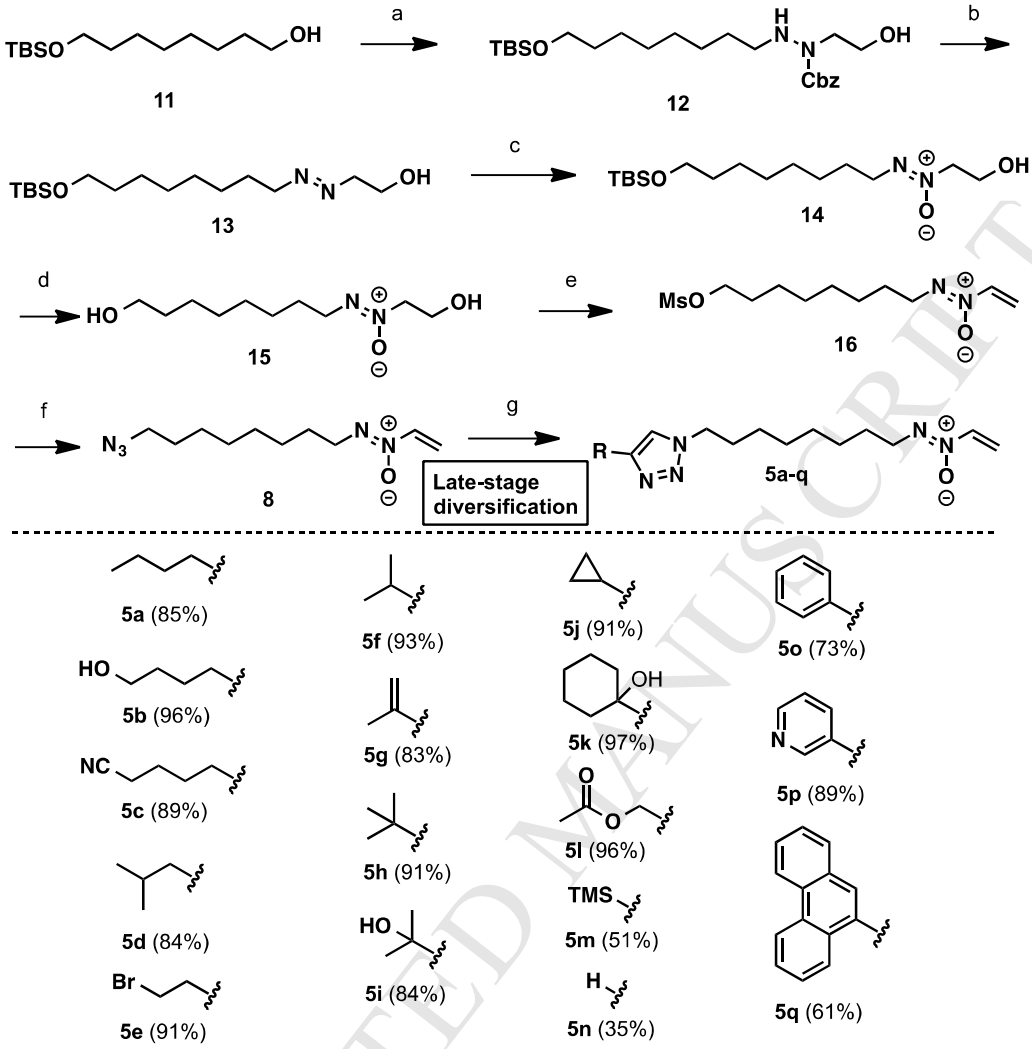


Fig. 2. Strategy; three derivative targets.

2.3 Synthesis

Although the challenging task for this synthesis is to easily construct a vinyl azoxy moiety, we previously developed an effective construction of such a moiety through a 4-step synthesis [27].

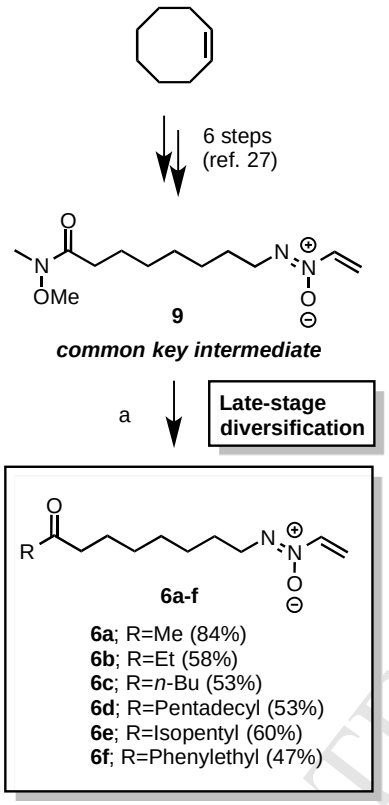
Based on our design, we began with synthesis of triazole derivatives utilizing our synthetic route (Scheme 1). We envisioned that various functional groups can be introduced by late-stage copper-catalyzed triazole reaction with azides and alkynes, in one-step, at the final reaction. Swern oxidation of mono-TBS alcohol **11** [44] gave the corresponding aldehyde (not shown), followed immediately by reductive hydrazination with Cbz hydrazine **10,** to give the corresponding hydrazine **12** in 82% yield over 2 steps. The azo moiety (**13**) was formed in 82% yield by air oxidation of the corresponding hydrazine, after removal of the Cbz group of **12**. Regioselective oxidation [45, 46] of **13** gave azoxy **14** (in 72% yield) followed by removal of TBS group to afford **15** in quantative yield. To construct the vinyl group, bis-mesylation of **15** gave the corresponding mesylated compound, followed by chemoselective β-elimination using the difference of acidity of the two protons to give **16** in 82% yield. Finally, substitution reaction with NaN_3 led to the triazole precursor **8** in 85% yield. With the azide in hand, a late-stage diversification in the presence of a vinyl azoxy moiety utilizing copper catalyzed triazole reaction [47-54] with 16-type acetylenes readily led to the corresponding triazole **5a-q** bearing aromatic, aliphatic, or hydrophilic groups in 35-97% yields [55].



Scheme 1. Synthesis of triazole derivatives **5a-q.** Reagents and conditions;
a) DMSO, $(COCl)_2$, Et_3N, DCM, –78 ˚C, then **10**, AcOH, $NaBH_3CN$, $CHCl_3$, 0 ˚C, 82% over 2 steps, b) H_2, $Pd(OH)_2$, MeOH, then air [O], rt 82%, c) TBHP, $VO(acac)_2$, DCM, 0 ˚C, 72%, d) AcOH, H_2O, THF, rt, quant., e) MsCl, Et_3N, DCM, rt, then DBU, 35 ˚C, 81%, f) NaN_3, DMF, 50 ˚C, 85%, g) R-N_3, $Cu(MeCN)_4PF_6$, TBTA [56], MeOH, rt, (**5a**; 85%, **5b**; 96%, **5c**; 89%, **5d**; 84%, **5e**; 91%, **5f**; 93%, **5g**; 83%, **5h**; 91%, **5i**; 84%, **5j**; 91%, **5k**; 97%, **5l**; 96%, **5m**; 51%, **5n**; 35%, **5o**; 73%, **5p**; 89%, **5q**; 61%).

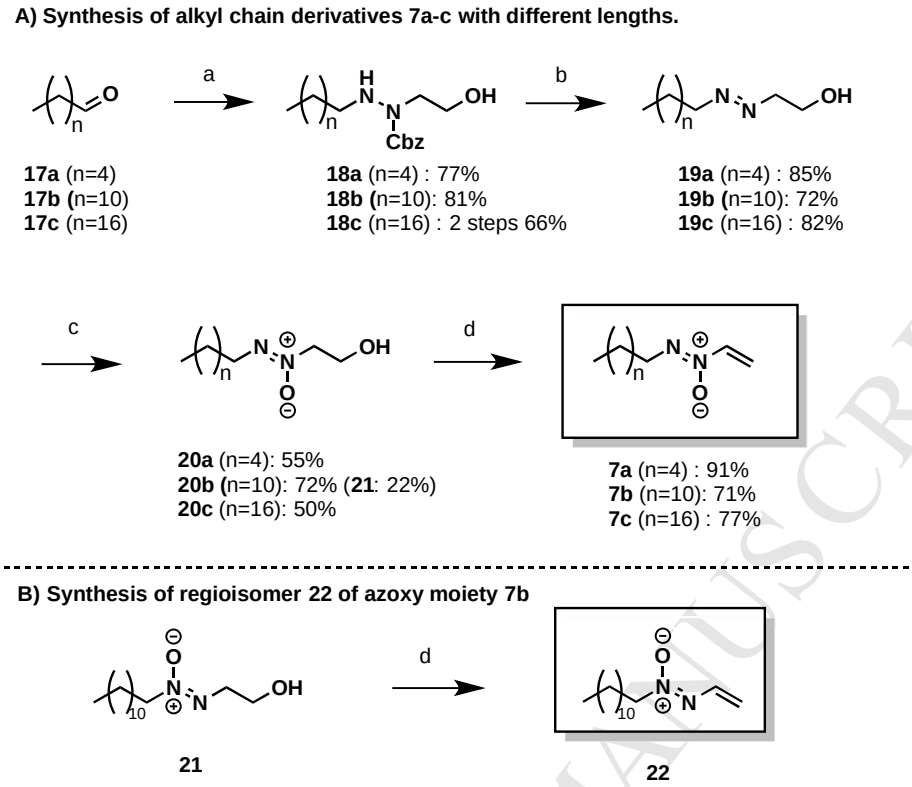
Based on the results with triazole derivatives (vide infra), we focused on making acyl analogs with ketone linkage via Grignard reagents in the presence of $LaCl_3$•2LiCl [57] as an additive, previously developed through our total synthesis process (Scheme 2). We devised a “late-stage diversification” strategy which accelerated easy production of alkyl chain analogs with only one-step from **9**. Since SARs with respect to triazole

analogs indicated that an aliphatic alkyl group showed better activity than others, we introduced some alkyl groups via Grignard reagents in the presence of $LaCl_3$•2LiCl. In fact, treatment of the Weinreb amide **9** [27], which we reported previously, with various Grignard reagents in the presence of $LaCl_3$•2LiCl, gave **6a-g** in 47-84% yields.



Scheme 2. Synthesis of **6a-f.** Reagents and conditions; a) R-MgBr, $LaCl_3$•2LiCl, THF (**6a**; 84%, **6b**; 58%, **6c**; 53%, **6d**; 53%, **6e**; 60%, **6f**; 47%)

We next envisioned that function-oriented synthesis might be utilized; i.e. total steps will theoretically be reduced from 7 to 4 by deletion of a carbonyl group, because we assumed, based on SARs, that a ketone does not play an important role in anthelmintic activity. In order to demonstrate this, we designed a ketone-deficient alkyl-chain derivative **7b,** based on **6c**. In the same fashion, we designed **7a** and **7c** to reconfirm the impact of side-chain carbon length. In fact, **7a-c** were synthesized from the aldehydes **17a-c** (n=4,10,16) (Scheme 3). Likewise, use of the regioisomer of azoxy moiety **21**, obtained after oxidation of azo, led to the vinyl azoxy **22** in 73 % yield [58-60].



Scheme 3. A) Synthesis of alkyl chain derivatives **7a-c**. B) Synthesis of **22**, regioisomer of azoxy moiety **7b**. Reagents and conditions; a) **10**, AcOH, $NaBH_3CN$, $CHCl_3$, 0 ˚C, (**18a**; 77%, **18b**; 81%, **18c**; 66% (2 steps)), b) H_2, $Pd(OH)_2$, MeOH, then air [O], rt, (**19a**; 85%, **19b**; 72%, **19c**; 82%), c) TBHP, $VO(acac)_2$, DCM, 0 ˚C, (**20a**; 55%, **20b**; 72%, **20c**; 50%), d) MsCl, Et_3N, DCM, rt, then DBU, 35 ˚C, (**7a**; 91%, **7b**; 71%, **7c**; 77%, **22**; 73%)

2.4 Biological evaluation

In vitro anthelmintic activity of triazoles **5a-q** are summarized in Table 2. In order to investigate the impact of the triazole moiety on anthelmintic activity, we designed triazole derivative **5a** to possess the same carbon pendant (15-carbon chain), as **1**. Biological evaluation indicated that both **1** and **5a** had similar properties (100% efficacy at 1 ppm against *N. braziliensis*), suggesting that the triazole moiety might be replaced by a ketone group. We, therefore, focused on making triazole derivatives to introduce various functional groups for better understanding of SARs. Introduction of alcohol (**5b**), nitrile (**5c**), bromo (**5e**), and acetate (**5l**) groups on the alkyl chain led to reduced efficacy in the *N. braziliensis* assay at 1 ppm. Likewise, activity of other alkyl chain

derivatives (**5d**, **5f**, **5g**, **5h**, **5i**, **5j**, and **5k**) and a silyl derivative (**5m**) did not satisfy our criteria (<100% efficacy at 1 ppm against *N. braziliensis*). No n-butyl group (**5n**) on the triazole moiety led to loss of activity (35% efficacy at 1 ppm against *N. braziliensis*). Although a phenyl derivative (**5o**) showed activity (100% at 1 ppm against *N. braziliensis*), it was not effective against either *H. contortus* or *C. curticei*, while other two aromatic derivatives (**5p**, **5q**) had no effect whatsoever. In case of compound **5e**, it should be noted that in the *N. braziliensis* assay it had no activity (35%) at 10 ppm, but weak activity (60%) was found at 1 ppm [61]. As a result, no derivatives with stronger activity than **5a** were obtained. Especially, introduction of a hydrophilic functional group on the triazole moiety clearly decreased activity at 1 ppm against *N. braziliensis.* We thus concluded that efficacy would be affected by changing the aliphatic chain at a side chain.

In terms of biological activities of acyl derivatives, Weinreb amide **9**, a common key intermediate, lost anthelmintic activity (35% efficacy at 1 ppm *N. braziliensis*) (Table 2). Although the alkyl group bearing an appropriate carbon length (methyl **6a**, ethyl **6b**, *n*-butyl **6c**, isopentyl **6e**) showed good anthelmintic activity, pentadexyl **6d** completely lost activity against *N. braziliensis*, presumably due to having a lengthy alkyl chain. Although the phenylethyl **6f** showed good activity against *H. contortus* and *N. braziliensis*, activity of **6f** against *C. curticei* was weak. Consequently, we concluded that anthelmintic activity of the *n*-butyl **6c** demonstrated the best balance (80% efficacy at 4 ppm against *H. contortus*, 100% efficacy at 20 ppm against *C. curticei*, 100% efficacy at 1 ppm against *N. braziliensis*).

Next, we evaluated biological activities of alkyl-chain derivatives (Table 2). Although **7a-c** indicated 100% efficacy at 1 ppm dosage against *N. braziliensis*, almost no activity of **7a** (C6) and **7c** (C16) analogs against *H. contortus* or *C. curticei* was observed. Pleasingly, **7b** (bearing a C12 aliphatic chain) exhibited stronger anthelmintic activity against all three nematodes than the other compounds, indicating that appropriate moderate carbon length is necessary to bestow anthelmintic characteristics. Our attention next turned to regioisomers of the azoxy moiety. Biological evaluation revealed that **22**, a regioisomer of **7b**, had significantly reduced anthelmintic activity, suggesting the direction of the azoxy moiety plays an important role in bestowing anthelmintic activity and that it may be recognized by a target protein.

Table 2

In vitro anthelmintic activity of derivatives **5a-q**, **6a-6f**, **7a-7c**, **9**, and **22**.

	H. contortus (efficacy %)[1]			*C. curticei* (efficacy %)[1]			*N. braziliensis* (efficacy)[2]	
Compound	20 ppm	4 ppm	0.8 ppm	20 ppm	4 ppm	0.8 ppm	10 ppm	1 ppm
5a	100	40	0	100	40	40	100	100
5b	0	0	0	0	0	0	60	60
5c	0	0	0	0	0	0	84	35
5d	60	0	0	90	0	0	100	60
5e	80	0	0	90	40	0	35	60
5f	0	0	0	90	40	0	100	60
5g	100	0	0	100	80	40	100	84
5h	0	0	0	60	0	0	100	60
5i	0	0	0	60	0	0	100	84
5j	60	0	0	100	40	0	100	35
5k	0	0	0	40	40	0	84	35
5l	0	0	0	40	0	0	84	35
5m	0	0	0	60	40	0	100	60
5n	40	0	0	80	0	0	84	35
5o	0	0	0	0	0	0	100	100
5p	0	0	0	80	0	0	100	35
5q	0	0	0	0	0	0	84	60
9	0	0	0	0	0	0	84	35
6a	100	60	40	90	40	0	100	100
6b	100	60	0	100	0	0	100	100
6c	100	80	0	100	0	0	100	100
6d	0	0	0	0	0	0	0	0
6e	100	0	0	100	0	0	100	100
6f	100	60	0	90	0	0	100	100
7a	40	0	0	0	0	0	100	100
7b	100	100	60	100	80	0	100	100
7c	0	0	0	0	0	0	100	100
22	40	0	0	0	0	0	84	35

[1]Efficacy in larval assay: decrease of motility, 100% efficacy means that all larvae were immotile, 0% efficacy means that all larvae were motile

[2]Efficacy in *Nippostrongylus* assay: decrease of acetylcholine esterase activity compared to negative control. Anthelmintic activity was categorized using the following scale: 100: >84%-100% = full activity (complete AChE inhibition compared to negative control); 84: >60%-84% = good activity; 60:>35%-60% = weak activity, and 35: 0%-35% = no activity.

2.5 SAR maps

The SAR of jietacins and their derivatives are summarized; 1) No carbonyl group is needed (**7b** vs **6c**); 2) the vinyl azoxy moiety participates in displaying stronger inhibition (as shown in previous work) [36]; 3) chain length significantly influences anthelmintic activity. Although shorter (**7a**; C6) and longer (**7c**; C16) carbon length decreased activity, moderate chain length (**7b**; C9-14) exhibited good anthelmintic activity against *H. contortus*, and *C. curticei*; 4) non-branched chains tend to correlate with greater potency compared to branched; 5) hydrophobicity in a side chain is preferable, e.g. attachment of hetero atoms in a side chain decreased activity (triazole derivatives **5a-q**); 7) Regioisomer of azoxy moiety, including a vinyl group, plays an important role in nematocidal impact (**7b** vs **22**).

2.6 In vivo assay in mice

Based on the promising *in vitro* results, we subsequently tested selected compounds for their anthelmintic activity *in vivo* using a mouse model. Mice were infected with *Heligmosomoides polygyrus*, a gastrointestinal nematode in mice. Mice were treated orally once or daily on four consecutive days. As shown in Table 3, jietacin A had full efficacy (100%) at 100 mg/kg after 4-day treatment (entry 2). A reduced amount of jietacin A had no efficacy (<50%) at 25 mg/kg on 1 day (entry 3), additionally, no efficacy was observed at 10 mg/kg on 1 day (entry 4). Notably, the derivative **7b** had full efficacy (100%) at 25 mg/kg following 4-day treatment (entry 6). Although this result is not favourably comparable to emodepside (full efficacy, 10 mg/kg at 4-day regimen (entry 1)), this is the first example, to the best of our knowledge, that a compound having an azoxy moiety exhibited 100% anthelmintic efficacy *in vivo*, thereby indicating azoxy compounds have the possibility of becoming good lead candidates in future. While **7b** fully efficacious at a dosage of 25 mg/kg for four days, at a high dosage (100 mg/kg using a 4-day regimen, entry 5) animals showed strong adverse effects and were euthanized for animal welfare reasons after the third treatment. When the dosage was decreased (25 mg/kg on 1 day), efficacy was only 73% (entry 7), and a dosage of 10 mg/kg on a 1-day regimen had no or little effect (<50%, entry 8). Other derivatives bearing a triazole moiety, **5a**, **5g**, **5i**, and **5o** had mostly no efficacy (<50%, entry 9, 10, and 12), or 70% efficacy (entry 11) at 100 mg/kg with a 4-day

regimen, indicating that a triazole moiety may be a significant element for anthelmintic activity.

Table 3

Emodepside and jietacins against *Heligmosomoides polygyru in vivo.*

entry		Dosage (mg/kg)	Treatment days	Formulation	Efficacy
1	Emodepside	10	4	A	100%
2	Jietacin A	100	4	B	100%
3	Jietacin A	25	1	B	<50%
4	Jietacin A	10	1	B	<50%
5	**7b**	100	4	A	toxic
6	**7b**	25	4	B	100%
7	**7b**	25	1	B	73%
8	**7b**	10	1	B	<50%
9	**5a**	100	4	A	<50%
10	**5g**	100	4	A	<50%
11	**5i**	100	4	A	70%
12	**5o**	100	4	A	<50%

Formulation A: 25% Cremophor EL, 75% water.

Formulation B: 10% Transcutol, 10% Cremophor EL, 80% physiol. NaCl solution (0.9%).

3. Conclusion

Our results confirm that jietacins possess potent anthelmintic activity against parasitic nematodes. Jietacin A is known to have moderate or low acute toxicity (LD_{50} > 300 mg/kg) and shows no mutagenic potential in a mini Ames screen, significant indication of the promising properties of this compound for future drug development. In addition, this study revealed not only design, synthesis, and biological evaluation of jietacin derivatives against pathogenic nematodes, but also clarification of their SARs. Of the derivatives evaluated, we found that a simplified derivative, **7b**, exhibited better anthelmintic activity *in vitro* than natural jietacin and had better efficacy *in vivo* following oral administration against a mouse nematode, but lower tolerability. Further

optimization of the compounds is therefore necessary to identify the best drug candidate with a sufficient therapeutic potential. Nonetheless, to the best of our knowledge, this is the first example of compounds with an azoxy moiety exhibiting 100% anthelmintic efficacy *in vivo*. Therefore, our results indicate that an azoxy motif may be a useful template for anthelmintic drug discovery. This has the promise of creating a class of anthelmintics with novel skeletons, a potentially new mode of action, and an insight for rational drug design. Although the purpose of this study was to develop veterinary medicines, we believe that the results provide a useful insight for drug development, with respect to azoxy antibiotics, for both human and animal health.

4. Experimental section

4.1. Chemistry

Pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254, Cat.-No. 1.05744.0001, Merck KGaA, Darmstadt, Germany) were used for analytical and preparative thin layer chromatography. Flash column chromatography was carried out with Kanto Chemical silica gel (Silica gel 60N, spherical neutral, 40-50 µm, Cat.-No. 37563-84, Kanto Chemical Co., Inc., Tokyo Japan) or Merck silica gel 230-400 mesh ASTM (60N, 40-63 µm, Cat.-No. 109385). ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra were recorded at 125 MHz on JEOL ECA-500 (500 MHz, JEOL Ltd., Tokyo, Japan). The chemical shifts are expressed in ppm downfield from internal solvent peaks $CDCl_3$ (7.26 ppm, ^{1}H NMR), $CDCl_3$ (77.16 ppm, ^{13}C NMR), CD_3OD (3.31 ppm, ^{1}H NMR), CD_3OD (49.0 ppm, ^{13}C NMR), and coupling constant (*J* values) are given in Hertz. The coupling patterns are expressed by s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), q (quartet), dq (double quartet), m (multiplet), br (broadened), app (appearance). All infrared spectra were measured using a Horiba FT-210 spectrometer (Horiba Ltd., Kyoto, Japan). High- and low-resolution mass spectra were measured on a JEOL JMS-700 MStation and JEOL JMS-T100LP. Melting points were measured with a Yanaco Micro Melting Point System MP-500P (Anatec Yanaco Corporation, Kyoto, Japan).

4.1.1. General procedure for azo formation

10% $Pd(OH)_2$ (2.0 equiv.) was suspended in MeOH (0.1 M) at 0 ˚C under N_2 atmosphere. To the resulting suspension was added a solution of Cbz-hydrazine (1.0 equiv.) in MeOH (0.5 M). The solution was subjected to an atmosphere of hydrogen and stirred at room temperature for 2 h. After the starting material was consumed, the mixture was filtered through a pad of Celite and washed with a mixture of MeOH and $CHCl_3$ (1/10, v/v). The filtrate was concentrated *in vacuo*. The crude product was immediately oxidized at room temperature by exposure to air until the hydrazine was consumed. The crude product was purified by flash column chromatography to give the azo compound.

4.1.2 General procedure for azoxy formation

To a solution of azo compound (1.0 equiv.) and $VO(acac)_2$ (0.1 equiv.) in DCM (0.1 M) was added TBHP (5.5 M in decane, 1.2 equiv.) at 0 ˚C under N_2 atmosphere. After stirring at the same temperature until the starting material was consumed, the resulting mixture was quenched with saturated aqueous $Na_2S_2O_3$, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography to give the azoxy compound and its regioisomer.

4.1.3. General procedure for triazole reaction

To a solution of azide **8** in MeOH (0.2 M) at room temperature were added acetylenes (1.2 equiv.), tetrakis(acetonitrile)copper(I) hexafluorophosphate ($Cu(MeCN)_4PF_6$, 0.05 equiv.), and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 0.05 equiv.). The reaction mixture was stirred at room temperature until the starting material was consumed. After the reaction mixture was concentrated *in vacuo*, the crude product was purified by silica gel column chromatography (hexane/EtOAc=5/1 to $CHCl_3$/MeOH=10/1) to afford the desired triazole compounds **5a-q**.

4.1.4. General procedure for Grignard reaction

To a solution of $LaCl_3$·2LiCl (4.0 equiv.) in THF (2.0 M) was added drop-wise Grignard reagent (4.0 equiv.). After stirring for 30 min at 0 ℃, a mixture of Grignard

reagent and $LaCl_3 \cdot 2LiCl$ was added to a solution of Weinreb amide (1.0 equiv.) in THF (0.5M) at –40 ℃ in another flask and the mixture was stirred for 10 min. The reaction mixture was then quenched with saturated aqueous NH_4Cl, extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by preparative PLC on silica gel to afford a ketone analog.

4.1.5. General procedure for reductive hydrazination

To a solution of **10** (1.0 equiv.) in MeOH (0.1 M) was added drop-wise aldehyde (1.0 equiv.) at 0 ˚C. After stirring at room temperature for 30 min, to the mixture was added AcOH (2.0 equiv.) and $NaBH_3CN$ (6.0 equiv.) at 0 ˚C. The mixture was allowed to warm to room temperature and stirred until all starting material was consumed. The resulting mixture was diluted with $CHCl_3$ and washed with pH 7.2 phosphate buffer, and H_2O, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography to give a corresponding Cbz-hydrazine.

4.1.6. General procedure for construction of vinyl azoxy group

To a solution of azoxy ethylalcohol (1.0 equiv.) in DCM (0.1 M) were added Et_3N (2.0 equiv.) and MsCl (1.2 equiv.) at room temperature. After stirring for 10 min, the mixture was added to DBU (2.0 equiv.) and stirred at 35 ˚C for 3 min. The resulting mixture was diluted with EtOAc and washed with saturated aqueous NH_4Cl and H_2O, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative TLC on silica gel to give the vinyl azoxy compound.

4.1.7. 8-[(tert-Butyldimethylsilyl)oxy]octan-1-ol **(11)**

To a solution of 1,8-octadiol (10.0 g, 68.4 mmol) in MeOH (340 mL) were added Et_3N (47.8 mL, 342 mmol), TBSCl (9.79 g, 65.0 mmol) and DMAP (0.418 g, 3.42 mmol) and the mixture was stirred at 0 ˚C under nitrogen atmosphere. After stirring for 1 h, the reaction mixture was added to $CHCl_3$ (600 mL) washed with saturated aqueous $NaHCO_3$ (900 mL), saturated aqueous NH_4Cl (900 mL, x2) and brine (900 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc=15/1 to 5/1) to afford **11** (9.06 g, 51%) as a colorless oil.

IR (Diamond Prism) ν (cm^{-1}): 3349, 2931, 2858, 1471, 1387, 1362, 1255, 1099, 1059,

837, 775, 665; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 3.63 (t, *J* = 6.6 Hz, 2H), 3.59 (t, *J* = 6.6 Hz, 2H), 1.58-1.46 (complex m, 4H), 1.35-1.30 (complex m, 8H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 63.3, 62.9, 32.8, 32.7, 29.4 (2C), 25.9 (3C), 25.69, 25.66, 18.3, -5.3 (2C); HRMS (FAB, NBA) *m/z* : 261.2250 $[M+H]^+$, calcd for $C_{14}H_{33}O_2Si$: 261.2242.

4.1.8. Benzyl 2-{8-[(tert-butyldimethylsilyl)oxy]octyl}-1-(2-hydroxyethyl)-hydrazine-1-carboxylate (**12**)

To a solution of DMSO (4.46 mL, 62.8 mmol) in DCM (96.0 mL) was added oxalyl chloride (2.65 mL, 100 mmol) drop-wise using a syringe at –78 ˚C under nitrogen atmosphere. After stirring for 30 min, to the reaction mixture was added **11** (5.03 g, 19.3 mmol) drop-wise using a syringe, the mixture being stirred for an additional 30 min at –78 ˚C, before Et_3N (16.1 mL, 377 mmol) was added. After stirring for 10 min at –78 ˚C, the reaction mixture was warmed to 0 ˚C and stirred for 1 h, then washed with saturated aqueous NH_4Cl (90 mL), 10% aqueous $NaHSO_3$ (90 mL, x2), saturated aqueous $NaHCO_3$ (90 mL), brine (90 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was taken on to the next step without purification. To a stirred solution of **10** (4.87 g, 23.2 mmol) in $CHCl_3$ (190 mL) at 0 ˚C was added the crude aldehyde, the reaction mixture subsequently being warmed to room temperature and stirred. After stirring for 45 min, the reaction mixture was cooled to 0 ˚C, and AcOH (2.4 mL, 38.6 mmol) was added. After stirring for 10 min, the mixture was added to $NaBH_3CN$ (7.28 g, 377 mmol) at 0 ˚C and stirred for 10 min at room temperature. The reaction mixture was washed with phosphate buffer solution in water (pH 7.2, 190 mL), brine (190 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc=5/1 to 3/1) to afford **12** (7.19 g, 82%) as a colorless oil.

IR (Diamond Prism) ν (cm^{-1}) : 3995, 2931, 2854, 1697, 1458, 1404, 1350, 1250, 1095, 833, 771, 694; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.36 (m, 5H), 5.16 (s, 2H), 3.80 (m, 2H), 3.62 (t, *J* = 4.9 Hz, 2H), 3.59 (t, *J* = 6.9 Hz, 2H), 2.88 (m, 2H), 1.51-1.45 (complex m, 4H), 1.28 (complex m, 8H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 156.5, 135.9, 128.2 (2C), 127.9, 127.7 (2C), 67.3, 62.9, 61.3, 49.9, 32.5, 29.2, 29.0, 28.9 27.5, 26.8, 25.7 (3C), 25.4, 18.1, –5.5 (2C); HRMS (FAB, NBA) *m/z* : 451.3144 $[M+H]^+$, calcd for $C_{24}H_{45}N_2O_4Si$: 453.3149.

4.1.9. (E)-2-({8-[(tert-Butyldimethylsilyl)oxy]octyl}diazenyl)ethan-1-ol (**13**)

12 (8.80 g, 19.4 mmol) was converted to **13** (5.04 g, 82%) which appeared as a yellow oil, according to the general procedure for azo formation.

IR (Diamond Prism) ν (cm^{-1}) : 3316, 3224, 2931, 2854, 1511, 1462, 1427, 1334, 1257, 1203, 1057, 972, 872, 833, 764,717, 617; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.04 (m, 2H), 3.99 (m, 2H), 3.81 (t, *J* = 6.8 Hz, 2H), 3.59 (t, *J* = 6.9 Hz, 2H), 1.79 (dt, *J* = 14.4, 6.9 Hz, 2H), 1.50 (m, 2H), 1.36-1.31 (complex m, 8H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 69.9, 69.6, 63.3, 60.3, 32.8, 29.3, 29.2, 27.5, 27.2, 26.0 (3C), 25.7, 18.3, –5.3 (2C); HRMS (FAB, NBA) *m/z* : 317.2617 $[M+H]^+$, calcd for $C_{16}H_{37}N_2O_2Si$: 317.2624

4.1.10. (Z)-2-{8-[(tert-Butyldimethylsilyl)oxy]octyl}-1-(2-hydroxyethyl)diazene 1-oxide (**14**)

13 (1.00 g, 3.16 mmol) was converted to **14** (751 mg, 72%), appearing as a yellow oil, according to the general procedure for azoxy formation. IR (Diamond Prism) ν (cm^{-1}) : 3396, 2931, 2854, 1504, 1466, 1419, 1311, 1250, 1195, 1095, 833, 771, 663; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.31 (m, 2H), 4.04 (m, 2H), 3.59 (t, *J* = 6.9 Hz, 2H), 3.45 (t, *J* = 6.9 Hz, 2H), 1.71 (dt, *J* = 14.4, 6.9 Hz, 2H), 1.50 (m, 2H), 1.41-1.31 (complex m, 8H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 70.4, 63.3, 59.5, 52.1, 32.8, 29.3 (2C), 27.7, 27.0, 26.0 (3C), 25.7, 18.4, –5.3 (2C); HRMS (FAB, NBA) *m/z* : 333.2569 $[M+H]^+$, calcd for $C_{16}H_{37}N_2O_3Si$: 333.2573.

4.1.11. (Z)-1-(2-Hydroxyethyl)-2-(8-hydroxyoctyl)diazene 1-oxide (**15**)

To a stirred solution of **14** (5.88 g, 17.7 mmol) in THF (51 mL) at 0 ˚C were added water (51 mL) and AcOH (76 mL), the mixture then being warmed to room temperature. After stirring for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (180 mL) and extracted with EtOAc (150 mL, x2). The combined organic layers were washed with brine (300 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc=1/2) to afford **15** (3.86 g, quant) as a colorless solid.

mp 47.5 ˚C; IR (Diamond Prism) ν (cm^{-1}): 3314, 3224, 2931, 2854, 1512, 1469, 1427, 1336, 1257, 1203, 1057, 972, 872, 833, 764, 717, 617; ^{1}H NMR (500 MHz, $CDCl_3$) δ

(ppm) : 4.31 (m, 2H), 4.05 (m, 2H), 3.64 (dt, *J* = 5.4, 1.2 Hz, 2H), 3.45 (t, *J* = 6.9 Hz, 2H), 1.71 (dt, *J* = 14.4, 6.9 Hz, 2H), 1.55 (dt, *J* = 13.8, 6.9 Hz, 2H) 1.41-1.32 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 70.5, 62.8, 59.3, 52.1, 32.6, 29.2, 29.1, 27.6, 26.9, 25.6; HRMS (FAB, NBA) *m/z* : 219.1713 $[M+H]^+$, calcd for $C_{10}H_{23}N_2O_3$: 219.1709.

4.1.12. (Z)-2-{8-[(Methylsulfonyl)oxy]octyl}-1-vinyldiazene 1-oxide (**16**)

To a solution of **15** (0.502 g, 2.30 mmol) in DCM (23 mL) were added Et_3N (0.950 mL, 6.89 mmol) and MsCl (0.430 mL, 5.51 mmol). After stirring for 5 min, the corresponding bis-mesylate was observed on TLC. DBU (1.03 mL, 6.89 mmol) was added and the mixture was stirred at 35 ˚C for 3 min. The reaction mixture was washed with saturated aqueous NH_4Cl (20 mL x2), brine (30 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/EtOAc=2/1) to provide product **16** (0.447 g, 70%) as a colorless solid.

mp 52.1 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3124, 3039, 2931, 2854, 2190, 1466, 1419, 1327, 1265, 1184, 1041, 941, 825, 733, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.10 (dd, *J* = 15.0, 8.0 Hz, 1H), 6.42 (d, *J* = 15.0 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.22 (t, *J* = 6.9 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 3.00 (s, 3H), 1.76-1.73 (complex m, 4H), 1.42-1.35 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 143.4, 115.3, 70.1, 52.5, 37.3, 29.1 (2C), 28.9, 27.6, 27.0, 25.3; HRMS (FAB, NBA) *m/z* : 279.1383 $[M+H]^+$, calcd for $C_{11}H_{23}N_2O_4S$: 279.1379.

4.1.13. (Z)-2-(8-Azidooctyl)-1-vinyldiazene 1-oxide (**8**)

To a solution of **16** (1.52 g, 5.46 mmol) in DMF (56 mL) was added NaN_3 (0.526 g, 8.19 mmol) at 50 ˚C. After stirring for 1.5 h, water (50 mL) was added to the reaction mixture which was then extracted with a mixture of hexane/EtOAc = 1/1 (25 mL/25 mL, v/v). The organic layer was separated and the aqueous layer was extracted with a mixture of hexane/EtOAc=1/1 (25 mL/25 mL, v/v). The combined organic layer were washed with brine (150 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc=2/1) to afford **8** (1.05 g, 85%) as a yellow oil.

IR (Diamond Prism) ν (cm^{-1}) : 3112, 3089, 2931, 2858, 2096, 1732, 1506, 1473, 1419,

1373, 1348, 1311, 1265, 1052, 953, 773, 723, 654; ^{1}H NMR (500 MHz, CD_3OD) δ (ppm) : 7.21 (dd, *J* = 15.0, 7.5 Hz, 1H), 6.35 (d, *J* = 15.0 Hz, 1H), 5.57 (d, *J* = 7.5 Hz, 1H), 3.52 (t, *J* = 6.9 Hz, 2H), 3.28 (t, *J*= 6.9 Hz, 2H), 1.76 (m, 2H), 1.59 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.45-1.38 (complex m, 8H); ^{13}C NMR (125 MHz, CD_3OD) δ (ppm) : 144.9, 116.0, 53.6, 52.5, 30.4 (2C), 30.0, 29.0, 28.3, 27.9; HRMS (FAB, NBA) *m/z* : 226.1671 $[M+H]^+$, calcd for $C_{10}H_{20}N_5O$: 226.1668.

4.1.14. (Z)-2-[8-(4-Butyl-1H-1,2,3-triazol-1-yl)octyl]-1-vinyldiazene 1-oxide (**5a**)

According to the general procedure for synthesis of triazole analogs, **8** (80.9 mg, 0.359 mmol) with 1-hexyne (50 μL, 0.431 mmol) was converted to **5a** (93.7 mg, 85%) as a colorless solid.

mp 62.2 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3214, 3078, 2924, 2854, 1643, 1550, 1466,1411, 1342, 1265, 1157,1057, 957, 749, 779, 725, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.24 (s, 1H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.30 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 7.5 Hz, 2H), 2.71 (d, *J* = 7.5 Hz, 2H), 1.88 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.74 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.65 (m, 2H), 1.43-1.29 (complex m, 10H), 0.93 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 148.3, 143.4, 120.3, 115.2, 52.4, 50.1, 31.5, 30.3, 29.0, 28.8, 27.6, 26.9, 26.4, 25.3, 22.3, 13.8; HRMS (ESI) *m/z* : 330.2269 $[M+Na]^+$, calcd for $C_{16}H_{29}N_5NaO$: 330.2270

4.1.15. (Z)-2-{8-[4-(4-Hydroxybutyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5b**)

According to the general procedure for synthesis of triazole analogs, **8** (80.2 mg, 0.356 mmol) with 5-hexyne-1-ol (50 μL, 0.427 mmol) was converted to **5b** (93.1 mg, 81%) as a colorless solid.

mp 69.9 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3448, 3402, 3116, 3062, 2924, 2854, 1550, 1465, 1342, 1255, 1211, 1165, 1049, 997, 949, 864, 787, 648; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.28 (s, 1H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.31 (t, *J* = 7.5 Hz, 2H), 3.68 (t, *J* = 6.9 Hz, 2H), 3.52 (t, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 1.98 (brs, 1H), 1.88 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.81-1.73 (complex m 4H), 1.64 (m, 2H), 1.43-1.34 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 147.8, 143.2, 120.4, 115.2, 61.9, 52.3, 50.0, 32.0, 30.1, 28.9,

28.7, 27.4, 26.8, 26.2, 25.5, 25.1; HRMS (ESI) *m/z* : 346.2221 $[M+Na]^+$, calcd for $C_{16}H_{29}N_5NaO_2$: 346.2219

4.1.16. (Z)-2-{8-[4-(4-Cyanobutyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5c**)

According to the general procedure for synthesis of triazole analogs, **8** (80.9 mg, 0.359 mmol) with 6-heptynenitrile (50 μL, 0.431 mmol) was converted to **5c** (106.7 mg, 89%) as a colorless solid.

mp 50.5 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3109, 3070, 2924, 2854, 2244, 2209, 2185, 2013, 1643, 1550, 1465, 1408, 1319, 1273, 1219, 1157, 1049, 964, 841, 771, 656; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.29 (s, 1H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.31 (t, *J* = 7.2 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 2.77 (t, *J* = 7.2Hz, 2H), 2.38 (t, *J* = 6.9 Hz, 2H), 1.87 (m, 4H), 1.74 (m, 4H), 1.42-1.29 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 146.6, 143.2, 120.4, 119.3, 115.0, 52.2, 49.9 , 30.0, 28.7, 28.6, 28.1, 27.4, 26.7, 26.1, 24.49, 24.45, 16,6; HRMS (ESI) *m/z* : 355.2230 $[M+Na]^+$, calcd for $C_{17}H_{28}N_6NaO$: 355.2222.

4.1.17. (Z)-2-[8-(4-Isobutyl-1H-1,2,3-triazol-1-yl)octyl]-1-vinyldiazene 1-oxide (**5d**)

According to the general procedure for synthesis of triazole analogs, **8** (80.5 mg, 0.355 mmol) with 4-methyl-1-pentyne (50 μL, 0.426 mmol) was converted to **5d** (92.0 mg, 84%) as a colorless solid.

mp 49.9 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3116, 3062, 2924, 2854, 1550, 1466, 1365, 1327, 1273, 1219, 1157, 1049, 957, 856, 771, 717, 663; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.29 (s, 1H), 7.11 (dd, *J* = 14.9, 8.0 Hz, 1H), 6.44 (d, *J* = 14.9 Hz, 1H), 5.51 (d, *J* = 8.0 Hz, 1H), 4.33 (t, *J* = 7.5 Hz, 2H), 3.56 (t, *J* = 7.5 Hz, 2H), 2.61 (d, *J* = 6.9 Hz, 2H), 1.98 (m, 1H), 1.91 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.76 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.45-1.35 (complex m, 8H), 0.96 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 147.1, 143.5, 120.9, 115.3, 52.5, 50.1, 34.8, 30.3, 29.1, 28.9, 28.7, 27.6, 27.0, 26.4, 22.3 (2C); HRMS (ESI) *m/z* : 330.2275 $[M+Na]^+$, calcd for $C_{16}H_{29}N_5NaO$: 330.2270

4.1.18. (Z)-2-{8-[4-(2-Bromoethyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5e**)

According to the general procedure for synthesis of triazole analogs, **8** (80.8 mg, 0.358 mmol) with 1-hexyne (40 μL, 0.433 mmol) was converted to **5e** (116.8 mg, 91%) as a colorless solid.

mp 55.3 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3162, 3124, 2924, 2854, 1550, 1466, 1419, 1319, 1257, 1219, 1142, 1057, 957, 883, 764, 725, 671; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.43 (s, 1H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 4.33 (t, *J* = 7.5 Hz, 2H), 3.65 (t, *J*= 6.9 Hz, 2H), 3.53 (t, *J* = 7.5 Hz, 2H), 3.30 (t, *J* = 6.9 Hz, 2H), 1.90 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.74 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.43-1.31 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 144.5, 143.3, 121.4, 115.2, 52.3, 50.1, 31.6, 30.1, 29.3, 28.9, 28.7, 27.5, 26.8, 26.2; HRMS (ESI) *m/z* : 380.1059 $[M+Na]^+$, calcd for $C_{14}H_{24}N_5BrNaO$: 380.1062

4.1.19. (Z)-2-[8-(4-Isopropyl-1H-1,2,3-triazol-1-yl)octyl]-1-vinyldiazene 1-oxide (**5f**)

According to the general procedure for synthesis of triazole analogs, **8** (80.8 mg, 0.359 mmol) with 3-methyl-1-butyne (40 μL, 0.431 mmol) was converted to **5f** (97.8 mg, 93%) as a colorless solid.

mp 51.3 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3109, 3062, 2924, 2854, 1550, 1466, 1365, 1327, 1265, 1211, 1173, 1103, 1049, 949, 849, 764, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.22 (s, 1H), 7.09 (dd, *J* = 14.9, 8.0 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H), 5.48 (d, *J* = 8.0 Hz, 1H), 4.29 (t, *J* = 7.5 Hz, 2H), 3.52 (t, *J* = 6.9 Hz, 2H), 3.08 (m, 1H), 1.88 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.74 (dt, *J* = 14.6, 7.3 Hz, 2H), 1.43-1.29 (complex m, 8H), 1.30 (d, *J* = 7.0 Hz, 6H),; ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 154.3, 143.3, 118.8, 115.1, 52.3, 49.9, 30.1, 28.9, 28.7, 27.5, 26.8, 26.3, 25.7, 22.4 (2C); HRMS (ESI) *m/z* : 316.2123 $[M+Na]^+$, calcd for $C_{15}H_{27}N_5NaO$: 316.2113

4.1.20. (Z)-2-{8-[4-(Prop-1-en-2-yl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide **(5g)**

According to the general procedure for synthesis of triazole analogs, **8** (80.0 mg, 0.355 mmol) with 2-methyl-1-butene-1-yne (86.2 mg, 0.426 mmol) was converted to **5g** (85.0 mg, 83%) as a colorless amorphous.

IR (Diamond Prism) ν (cm^{-1}) : 3008, 2931, 2854, 1643, 1466, 1373, 1319, 1219, 1111, 1049, 949, 895, 748, 663; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.46 (s, 1H), 7.03 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.34 (d, *J* = 14.9 Hz, 1H), 5.63 (s, 1H), 5.43 (d, *J* = 7.5 Hz, 1H),

5.01 (m, 1H), 4.26 (t, *J* = 7.5 Hz, 2H), 3.46 (t, *J*= 6.9 Hz, 2H), 2.07 (s, 3H), 1.83 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.66 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.35-1.25 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 148.4, 143.2, 133.5, 119.3, 115.1, 112.0, 52.3, 50.0, 30.1, 28.9, 28.7, 27.4, 26.8, 26.2, 20.5; HRMS (ESI) *m/z* : 314.1962 $[M+Na]^+$, calcd for $C_{15}H_{25}N_5NaO$: 314.1957

4.1.21. (Z)-2-{8-[4-(tert-Butyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5h**)

According to the general procedure for synthesis of triazole analogs, **8** (80.6 mg, 0.358 mmol) with 3,3-dimethyl-1-butyne (50 μL, 0.430 mmol) was converted to **5h** (99.5 mg, 91%) as a colorless amorphous.

IR (Diamond Prism) ν (cm^{-1}) : 3124, 3070, 2931, 2854, 1643, 1542, 1466, 1365, 1275, 1218, 1126, 1049, 949, 841, 764, 717, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.22 (s, 1H), 7.10 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.29 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 1.88 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.75 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.44-1.32 (complex m, 8H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 157.3, 143.3, 118.1, 115.1, 52.2, 49.9, 30.5, 30.2 (3C), 30.1, 28.8, 28.7, 27.4, 26.8, 26.3; HRMS (ESI) *m/z* : 330.2271 $[M+Na]^+$, calcd for $C_{16}H_{29}N_5NaO$: 330.2270

4.1.22. (Z)-2-{8-[4-(2-Hydroxypropan-2-yl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyl-diazene 1-oxide (**5i**)

According to the general procedure for synthesis of triazole analogs, **8** (82.8 mg, 0.368 mmol) with 2-methy-3-butyne-2-ol (40 μL, 0.441 mmol) was converted to **5i** (103.6 mg, 95%) as a colorless solid.

mp 71.0 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3309, 3124, 2970, 2924, 2854, 1543, 1466, 1365, 1265, 1211, 1192, 1134, 1049, 964, 489, 764, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.42 (s, 1H), 7.08 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.39 (d, *J* = 14.9 Hz, 1H), 5.48 (d, *J* = 7.5 Hz, 1H), 4.30 (t, *J* = 7.5 Hz, 2H), 3.51 (t, *J*= 6.9 Hz, 2H), 2.80 (s, 1H), 1.87 (dt, *J* = 13.8, 6.9 Hz, 2H), 1.72 (dt, *J* = 15.0, 7.5 Hz, 2H), 1.61 (s, 6H), 1.41-1.30 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 155.5, 143.3, 118.9, 115.3, 68.3, 52.3, 50.1, 30.3 (2C), 30.1, 28.9, 28.7, 27.5, 26.8, 26.3; HRMS (ESI) *m/z* : 332.2064 $[M+Na]^+$, calcd for $C_{15}H_{27}N_5NaO_2$: 332.2062

4.1.23. (Z)-2-[8-(4-Cyclopropyl-1H-1,2,3-triazol-1-yl)octyl]-1-vinyldiazene 1-oxide (**5j**)

According to the general procedure for synthesis of triazole analogs, **8** (82.2 mg, 0.365 mmol) with cyclopropylacetylene (40 μL, 0.438 mmol) was converted to **5j** (97 mg, 91%) as a colorless solid.

mp 66.1 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3132, 3100, 2924, 2854, 2021, 1558, 1466, 1412, 1327, 1265, 1211, 1157, 1049, 957, 895, 802, 764, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.20 (s, 1H), 7.09 (dd, *J* = 14.9, 8.0 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H,), 5.50 (d, *J* = 8.0 Hz, 1H), 4.27 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 7.5 Hz, 2H), 1.94 (m, 1H), 1.86 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.74 (dt, *J* = 7.5, 14.9, 2H), 1.43-1.30 (complex m, 8H), 0.94 (m, 2H), 0.83 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 150.1, 143.4, 119.4, 115.3, 52.5, 50.2, 30.3, 29.0, 28.9, 27.6, 27.0, 26.4, 7.7 (2C), 6.7; HRMS (ESI) *m/z* : 314.1956 $[M+Na]^+$, calcd for $C_{15}H_{25}N_5NaO$: 314.1957

4.1.24. (Z)-2-{8-[4-(1-Hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5k**)

According to the general procedure for synthesis of triazole analogs, **8** (80.0 mg, 0.355 mmol) with 1-Ethynyl-1-cyclohexanol (53.2 mg, 0.427 mmol) was converted to **5k** (124.0 mg, 97%) as a colorless solid.

mp 88.4 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3224, 3101, 3055, 2931, 2854, 1466, 1415, 1358, 1319, 1257, 1173, 1049, 949, 902, 849, 764, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.42 (s, 1H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 15.5 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 4.32 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 6.8 Hz, 2H), 2.13 (brs, 1H), 2.00-1.86 (complex m, 7H), 1.79-1.70 (complex m, 4H), 1.65-1.52 (complex m, 3H), 1.43-1.32 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 155.5, 143.3, 119.3, 115.3, 69.4, 52.4, 50.1, 38.0 (2C), 30.1, 28.9, 28.7, 27.5, 26.8, 26.3, 25.3, 21.9 (2C); HRMS (ESI) *m/z* : 372.2377 $[M+Na]^+$, calcd for $C_{18}H_{31}N_5NaO_2$: 372.2375.

4.1.25. (Z)-2-{8-[4-(Acetoxymethyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5l**)

According to the general procedure for synthesis of triazole analogs, **8** (82.4 mg, 0.366 mmol) with propargyl acetate (40 μL, 0.439 mmol) was converted to **5l** (114.1 mg, 97%) as a yellow solid.

mp 56.0 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3124, 2924, 2013, 1736, 1466, 1365, 1227,

1149, 1034, 957, 849, 787, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.58 (s, 1H), 7.10 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 5.21 (s, 2H), 4.34 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 2.07 (s, 3H), 1.90 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.75 (dt, *J* = 14.9, 7.5 Hz, 2 H), 1.42-1.31 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 170.6, 143.2, 142.5, 123.3, 115.1, 57.5, 52.2, 50.1, 30.0, 28.8, 28.6, 27.4, 26.7, 26.2, 20.6; HRMS (ESI) *m/z* : 346.1860 $[M+Na]^+$, calcd for $C_{15}H_{25}N_5NaO_3$: 346.1855

4.1.26. (Z)-2-{8-[4-(Trimethylsilyl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5m**) and *(Z)-2-[8-(1H-1,2,3-triazol-1-yl)octyl]-1-vinyldiazene 1-oxide* (**5n**)

According to the general procedure for synthesis of triazole analogs, **8** (83.1 mg, 0.368 mmol) with trimethylsilylacethylene (60 μL, 0.442 mmol) was converted to **5m** (30.9 mg, 51%) and **5n** (32.0 mg, 35%) as a colorless solid.

5m; mp 55.6 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3093, 2931, 2854, 1643, 1466, 1365, 1327, 1250, 1196, 1126, 1049, 949, 841, 756, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.48 (s, 1H), 7.08 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H), 5.48 (d, *J* = 7.5 Hz, 1H), 4.35 (t, *J* = 7.4 Hz, 2H), 3.51 (t, *J* = 6.9 Hz, 2H), 1.89 (dt, *J* = 13.7, 6.9 Hz, 2H), 1.73 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.42-1.33 (complex m, 8H), 0.30 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 146.3, 143.3, 128.6, 115.2, 52.4, 49.6, 30.3, 28.9, 28.8, 27.6, 26.9, 26.4, –1.2 (3C); HRMS (ESI) *m/z* : 346.2042 $[M+Na]^+$, calcd for $C_{15}H_{29}N_5NaOSi$: 346.2039

5n; mp 54.2 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3111, 2952, 2927, 2856, 2102, 1722, 1487, 1468, 1325, 1271, 1217, 1122, 1084, 1049, 960, 949, 827, 761, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.71 (s, 1H), 7.53 (s, 1H), 7.10 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.42 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 4.39 (t, *J* = 7.5 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 1.92 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.75 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.44-1.35 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 143.3, 133.7, 123.1, 115.3, 52.4, 50.1, 30.2, 29.0, 28.8, 27.6, 26.9, 26.3; HRMS (ESI) *m/z* : 274.1647 $[M+Na]^+$calcd for $C_{12}H_{21}N_5NaO$: 274.1644.

4.1.27. (Z)-2-(8-(4-Phenyl-1H-1,2,3-triazol-1-yl)octyl)-1-vinyldiazene 1-oxide (**5o**)

According to the general procedure for synthesis of triazole analogs, **8** (82.8 mg, 0.368 mmol) with ethynyl benzene (50 μL, 0.442 mmol) was converted to **5o** (88.0 mg,

73%) as a colorless solid.

mp 95.2 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3116, 2923, 2854, 1466, 1345, 1305, 1265, 1093, 1049, 949, 841, 764, 701, 654; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.83 (d, *J* = 6.9 Hz, 2H), 7.74 (s, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 8.6 Hz, 1H), 7.08 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H,), 5.48 (d, *J* = 8.0 Hz, 1H), 4.39 (t, *J* = 6.9 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 1.95 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.74 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.44-1.33 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 147.7, 143.4, 130.7, 128.8 (2C), 128.0, 125.7 (2C), 119.3, 115.3, 52.4, 50.4, 30.3, 29.0, 28.9, 27.6, 26.9, 26.4; HRMS (ESI) *m/z* : 350.1959 $[M+Na]^+$, calcd for $C_{18}H_{25}N_5NaO$: 350.1957.

4.1.28. (Z)-2-{8-[4-(Pyridin-3-yl)-1H-1,2,3-triazol-1-yl]octyl}-1-vinyldiazene 1-oxide (**5p**)

According to the general procedure for synthesis of triazole analogs, **8** (81.0 mg, 0.360 mmol) with 1-hexyne (43.9 mg, 0.427 mmol) was converted to **5p** (105 mg, 89%) as a colorless solid.

mp 73.3 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3116, 2924, 2854, 1574, 1466, 1342, 1219, 1049, 957, 810, 710, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 9.01 (s, 1H), 8.58 (d, *J* = 4.0 Hz, 1H), 8.26 (m, 1H), 7.86 (d, *J* = 1.7 Hz, 1H), 7.41 (m, 1H), 7.09 (dd, *J* = 14.9, 7.4 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.4 Hz, 1H), 4.43 (t, *J* = 7.4 Hz, 2H), 3.53 (t, *J* = 6.9 Hz, 2H), 1.97 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.75 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.45-1.34 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 148.9, 146.8, 144.4, 143.2, 132.7, 126.7, 123.5, 119.8, 115.1, 52.2, 50.3, 30.1, 28.8, 28.6, 27.4, 26.7, 26.2; HRMS (ESI) *m/z* : 351.1909 $[M+Na]^+$, calcd for $C_{17}H_{24}N_6NaO$: 351.1909.

4.1.29. (Z)-2-(8-(4-(Phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)octyl)-1-vinyldiazene 1-oxide (**5q**)

According to the general procedure for synthesis of triazole analogs, **8** (81.0 mg, 0.360 mmol) with 9-ethynyl-phenanthrene (86.2 mg, 0.427 mmol) was converted to **5q** (94.0 mg, 61%) as a colorless solid.

mp 97.4 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3147, 2931, 2854, 1466, 1373, 1273, 1219, 1049, 957, 903, 825, 764, 725, 663; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 8.78 (d, *J* = 8.1 Hz, 1H), 8.71 (d, *J* = 8.1 Hz, 1H), 8.39 (d, *J* = 7.5 Hz, 1H), 8.00 (s, 1H), 7.92 (d, *J* =

6.9 Hz, 1H), 7.85 (s, 1H), 7.71-7.60 (complex m, 4H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H), 5.48 (d, *J* = 7.5 Hz, 1H), 4.50 (t, *J* = 7.5 Hz, 2H), 3.55 (t, *J* = 6.9 Hz, 2H), 2.04 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.77 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.47-1.35 (complex m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 146.5, 143.3, 131.2, 130.6, 130.2, 130.0, 128.7, 128.1, 126.9, 126.79, 126.76, 126.7, 126. 6, 126.1, 122.8, 122.6, 122.4, 115.2, 52.3, 50.3, 30.2, 28.9, 28.8, 27.5, 26.8, 26.4; HRMS (ESI) *m/z* : 450.2262 $[M+Na]^+$, calcd for $C_{26}H_{29}N_5NaO$: 450.2270.

4.1.30. (Z)-2-(8-Oxononyl)-1-vinyldiazene 1-oxide (**6a**)

According to the general procedure for synthesis of ketone analogs using Grignard reagents, **9** (42 mg, 0.162 mmol) was converted to **6a** (29 mg, 84%) as a yellow oil.
IR (Diamond Prism) ν (cm^{-1}) : 2931, 2858, 1662, 1466, 1415, 1381, 1315, 1265, 1176, 1115, 1053, 995, 953, 764, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.53 (t, *J* = 6.9 Hz, 2H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.13 (s, 3H), 1.75 (tt, 7.5, 6.9 Hz, 2H), 1.58 (m, 2H), 1.42 (m, 2H), 1.38-1.27 (complex m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 209.1, 143.3, 115.2, 52.4, 43.5, 29.7, 28.95, 28.88, 27.5, 26.8, 23.6; HRMS (ESI) *m/z* : 213.1604 $[M+H]^+$, calcd for $C_{11}H_{21}N_2O_2$: 213.1603.

4.1.31. (Z)-2-(8-Oxodecyl)-1-vinyldiazene 1-oxide (**6b**)

According to the general procedure for synthesis of ketone analogs using Grignard reagents, **9** (50 mg, 0.182 mmol) was converted to **6b** (16 mg, 58%) as a yellow oil.
IR (Diamond Prism) ν (cm^{-1}) : 2931, 2854, 1712, 1466, 1373, 1219, 949, 761, 656 ; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.52 (t, *J* = 7.2 Hz, 2H), 2.40 (complex m, 4H), 1.74 (m, 2H), 1.57 (m, 2H), 1.43-1.29 (complex m, 6H), 1.04 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 211.9, 143.4, 115.3, 52.5, 42.3, 35.8, 29.11, 29.09, 27.6, 27.0, 23.8, 7.82; HRMS (FAB, NBA) *m/z* : 227.1759 $[M+H]^+$, calcd for $C_{12}H_{23}N_2O_2$: 227.1760.

4.1.32. (Z)-2-(8-Oxododecyl)-1-vinyldiazene 1-oxide (**6c**)

According to the general procedure for synthesis of ketone analogs using Grignard reagents, **9** (50 mg, 0.182 mmol) was converted to **6c** (26 mg, 53%) as a yellow oil.

IR (Diamond Prism) ν (cm^{-1}) : 2931, 2870, 1704, 1458, 1373, 1211, 962, 778, 648; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.52 (t, *J* = 7.5 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 1.74 (m, 2H), 1.57-1.51 (complex m, 4H), 1.43-1.26 (complex m, 8H), 0.89 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 211.6, 143.4, 115.3, 52.5, 42.7, 42.5, 29.1 (2C), 27.6, 27.0, 26.0, 23.7, 22.3, 13.8; HRMS (FAB, NBA) : *m/z* 255.2072 $[M+H]^+$, calcd for $C_{14}H_{27}N_2O_2$: 255.2073.

4.1.33. (Z)-2-(8-Oxotricosyl)-1-vinyldiazene 1-oxide (**6d**)

According to the general procedure for synthesis of ketone analogs using Grignard reagents, **9** (50 mg, 0.182 mmol) was converted to **6d** (41 mg, 53%) as a colorless solid. mp 67.7 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 2916, 2846, 1704, 1473, 1380, 1265, 1419, 1381, 1331, 1265, 1053, 937, 714, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.53 (t, *J* = 7.5 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.75 (m, 2H), 1.56 (complex m, 4H), 1.43-1.20 (complex m, 30H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 211.6, 143.4, 115.3, 52.5, 42.8, 42.7, 31.9, 29.7 (6C), 29.5 (2C), 29.4, 29.35, 29.26, 29.1, 27.6, 27.0, 23.9, 23.7, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 409.3794 $[M+H]^+$, calcd for $C_{25}H_{49}N_2O_2$: 409.3794.

4.1.34. (Z)-2-(11-Methyl-8-oxododecyl)-1-vinyldiazene 1-oxide (**6e**)

According to the general procedure for synthesis of ketone analogs using Grignard reagents, **9** (10 mg, 0.040 mmol) was converted to **6e** (6.7 mg, 60%) as a yellow oil.
IR (Diamond Prism) ν (cm^{-1}): 2931, 2862, 1709, 1469, 1369, 1319, 1265, 1053, 953, 771, 725, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.53 (t, *J* = 6.9 Hz, 2H), 2.39 (t, *J* = 6.9 Hz, 2H), 2.38 (t, *J* = 6.9 Hz, 2H), 1.75 (m, 2H), 1.60-1.28 (complex m, 11H), 0.88 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 211.6, 143.4, 115.2, 52.4, 42.6, 40.8, 32.6, 29.1 (2C), 27.65, 27.56, 26.9, 23.7, 22.3 (2C); HRMS (FAB, NBA) *m/z* : 269.2229 $[M+H]^+$, calcd for $C_{15}H_{29}N_2O_2$: 269.2229.

4.1.35. (Z)-2-(8-Oxo-10-phenyldecyl)-1-vinyldiazene 1-oxide (**6f**)

According to the general procedure for synthesis of ketone analogs using Grignard

reagents, **9** (90 mg, 0.350 mmol) was converted to **6g** (49.7 mg, 47%) as a yellow oil. IR (Diamond Prism) ν (cm^{-1}) : 3031, 2927, 2854, 2333, 2198, 1967, 1709, 1604, 1469, 1412, 1369, 1327, 1265, 1057, 949, 744, 652; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.29-7.25 (m, 2H), 7.20-7.16 (m, 3H), 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.48 (d, *J* = 7.5 Hz, 1H), 3.53 (t, *J* = 7.5 Hz, 2H), 2.89 (t, *J* = 8.1 Hz, 2H), 2.72 (t, *J* = 8.1 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.74 (tt, *J* = 7.5, 6.9 Hz, 2H), 1.56 (tt, *J* = 7.5, 6.9 Hz, 2H), 1.41 (m, 2H), 1.36-1.23 (complex m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 210.3, 143.4, 141.1, 128.4 (2C), 128.3 (2C), 126.0, 115.3, 52.5, 44.2, 43.0, 29.8, 29.1, 29.0, 27.6, 27.0, 23.7; HRMS (FAB, NBA) *m/z* : 303.2067 $[M+H]^+$, calcd for $C_{18}H_{27}N_2O_2$: 303.2073.

4.1.36. Benzyl 2-hexyl-1-(2-hydroxyethyl)hydrazine-1-carboxylate (**18a**)

10 (7.85 g, 37.4 mmol) and **17a** (4.15 mL, 34.0 mmol) were converted to **18a** (7.65 g, 77%) as a colorless oil. according to the general procedure for reductive hydrazination.

IR (Diamond Prism) ν (cm^{-1}) : 2927, 2858, 2337, 1971, 1689, 1450, 1404, 1350, 1215, 1119, 1057, 980, 864, 752, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.39-7.31 (complex m, 5H), 5.16 (s, 2H), 3.81 (brs, 2H), 3.62 (t, *J* = 4.6 Hz, 2H), 2.88 (brs, 2H), 1.45 (m, 2H), 1.36-1.18 (complex m, 6H), 0.87 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 156.5, 136.0, 128.4 (2C), 128.1 (2C), 127.8, 67.6, 61.9, 49.8 (2C), 31.5, 27.6, 26.6, 22.4, 13.9; HRMS (FAB, NBA) *m/z* : 295.2021 $[M+H]^+$, calcd for $C_{16}H_{27}N_2O_3$: 295.2022.

4.1.37. Benzyl 2-dodecyl-1-(2-hydroxyethyl)hydrazine-1-carboxylate (**18b**)

10 (100 mg, 0.476 mmol) and **17b** (112 μL, 0.476 mmol) were converted to **18b** (147 mg, 81%) as a colorless oil, according to the general procedure for reductive hydrazination.

IR (Diamond Prism) ν (cm^{-1}) : 3410, 2924, 2854, 1697, 1450, 1350, 1119; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.38-7.32 (complex m, 5H), 5.16 (s, 2H), 3.80 (brt, 2H), 3.60 (t, *J* = 4.6 Hz, 2H), 2.86 (t, *J* = 6.9 Hz, 2H), 1.43 (m, 2H), 1.31-1.25 (complex m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 156.6, 136.0, 128.5 (2C), 128.2 (2C), 128.0, 67.7, 62.5, 49.8 (2C), 31.9, 29.6 (2C), 29.54, 29.46, 29.4, 29.3, 27.7, 27.0, 22.6, 14.1; HRMS (FAB, NBA) *m/z* 379.2963 $[M+H]^+$, calcd for

$C_{22}H_{39}N_2O_3$: 379.2961.

4.1.38. Benzyl 1-(2-hydroxyethyl)-2-octadecylhydrazine-1-carboxylate (**18c**)

To a solution of DMSO (427 µL, 6.01 mmol) in DCM (2 mL) was added oxalyl chloride (254 µL, 2.96 mmol). drop-wise by syringe, at –78 ˚C under nitrogen atmosphere. After stirring for 30 min, to the reaction mixture was added a solution of 1-octadecanol (500 mg, 1.85 mmol) in DCM (2 mL), drop-wise by syringe, and the mixture was stirred for an additional 30 min at –78 ˚C, and Et_3N (1.54 ml, 11.1 mmol) was then added. After stirring at –78 ˚C for 30 min, the reaction mixture was warmed to 0 ˚C and quenched with saturated aqueous NH_4Cl (5 mL) and extracted with $CHCl_3$ (5 mL, x2). The combined organic layers were washed with 10% aqueous $NaHSO_3$ (10 mL, x 2), H_2O (15 mL), saturated aqueous $NaHCO_3$ (15 mL) and brine (15 ml), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product **17c** was taken on to the next step without purification. To a stirred solution of **10** (466 mg, 2.22 mmol) in $CHCl_3$ (15 ml) at 0 ˚C was added a solution of the crude product **17c** in $CHCl_3$ (4 mL), then the reaction mixture was warmed to room temperature and stirred. After stirring for 30 min, the mixture was cooled to 0 ˚C and to the mixture were added AcOH (210 µL, 3.70 mmol) and $NaBH_3CN$ (580 mg, 9.24mmol). After stirring for 30 min at room temperature, the reaction mixture was washed with phosphate buffer in solution (pH 7.2, 25mL) and brine (25mL), dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc=2/1) to afford the hydrazine compound **18c** as a colorless solid (563 mg, 66%).

mp 45.7 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 2927, 2858, 2337, 1971, 1689, 1450, 1404, 1350, 1215, 1119, 1057, 980, 864, 752, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.39-7.31 (complex m, 5H), 5.16 (s, 2H), 3.80 (brs, 2H), 3.61 (t, *J* = 4.6 Hz, 2H), 2.87 (t, *J* = 6.9 Hz, 2H), 1.44 (m, 2H), 1.37-1.18 (complex m, 30H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 156.6, 136.1, 128.6 (2C), 128.3 (2C), 128.0, 67.8, 62.6, 49.9 (2C), 31.9, 29.7 (7C), 29.63, 29.56, 29.49, 29.45, 29.3, 27.7, 27.0, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 463.3895 $[M+H]^+$, calcd for $C_{28}H_{51}N_2O_3$: 463.3900.

4.1.39. (E)-2-(Hexyldiazenyl)ethan-1-ol (**19a**)

18a (2.00 g, 6.79 mmol) was converted to **19a** (918 mg, 85%) as a yellow oil, according to the general procedure for azo formation.

IR (Diamond Prism) ν (cm^{-1}) : 3417, 2927, 2862, 1508, 1435, 1415, 1319, 1192, 1061, 856, 756, 729; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.03 (t, *J* = 4.6 Hz, 2H), 3.98 (t, *J* = 4.6 Hz, 2H), 3.81 (t, *J* = 7.5 Hz, 2H), 2.12 (brs, 1H), 1.79 (dt, *J* = 7.5, 6.9 Hz, 2H), 1.39-1.26 (complex m, 6H), 0.88 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 69.9, 69.6, 60.2, 31.5, 27.5, 27.0, 22.5, 14.0; HRMS (FAB, NBA) *m/z* : 159.1502 $[M+H]^+$, calcd for $C_8H_{19}N_2O$: 159.1497.

4.1.40. (E)-2-(Dodecyldiazenyl)ethan-1-ol (**19b**)

18b (1.00 g, 2.64 mmol) was converted to **19b** (467 mg, 73%) as a yellow oil, according to the general procedure for azo formation.

IR (Diamond Prism) ν (cm^{-1}) : 3456, 2924, 2854, 1743, 1458, 1373, 1219, 1057; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.03 (t, *J* = 4.0 Hz, 2H), 3.98 (t, *J* = 4.0 Hz, 2H) 3.81 (t, *J* = 7.5 Hz, 2H), 2.21 (br, 1H) 1.79 (dt, *J* = 7.5, 6.9 Hz, 2H), 1.33-1.25 (complex m, 18H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 69.9, 69.6, 60.3, 31.9, 29.60 (2C), 29.57, 29.5, 29.4, 29.3, 27.6, 27.3, 22.7, 14.1; HRMS (FAB, NBA) *m/z* 243.2439 $[M+H]^+$, calcd for $C_{14}H_{31}N_2O$: 243.2436

4.1.41. (E)-2-(Octadecyldiazenyl)ethan-1-ol (**19c**)

18c (560 mg, 1.21 mmol) was converted to **19c** (326 mg, 82%) as a yellow solid, according to the general procedure for azo formation.

mp 51.8 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3390, 2916, 2846, 1462, 1057, 845, 725; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.04-3.99 (complex m, 4H), 3.81 (t, *J* = 6.9 Hz, 2H), 1.80 (dt, *J* = 7.5, 6.9 Hz, 2H), 1.38-1.20 (complex m, 30H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 69.8, 69.7, 60.4, 31.9, 29.69 (6C), 29.65 (2C), 29.6, 29.5, 29.38, 29.36, 27.6, 27.3, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 327.3372 $[M+H]^+$, calcd for $C_{20}H_{43}N_2O$: 327.3375.

4.1.42. (Z)-2-Hexyl-1-(2-hydroxyethyl)diazene 1-oxide (**20a**)

19a (348 mg, 2.20 mmol) was converted to **20a** (210 mg, 55%) as a yellow oil, according to the general procedure for azoxy formation.

IR (Diamond Prism) ν (cm^{-1}) : 3356, 2916, 2846, 2249, 2148, 1982, 1655, 1504, 1462, 1412, 1319, 1200, 1115, 1065, 1022, 876, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.31 (t, *J* = 4.6 Hz, 2H), 4.04 (t, *J* = 4.6 Hz, 2H), 3.45 (t, *J* = 7.5 Hz, 2H), 1.71 (dt, *J* =

7.5, 6.9 Hz, 2H), 1.39 (m, 2H), 1.34-1.28 (complex m, 4H), 0.89 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 70.5, 59.1, 52.0, 31.3, 27.2, 26.8, 22.4, 13.8; HRMS (FAB, NBA) *m/z* : 175.1447 $[M+H]^+$, calcd for $C_8H_{19}N_2O_2$: 175.1447.

4.1.43. (Z)-2-Dodecyl-1-(2-hydroxyethyl)diazene 1-oxide (**20b**) and *(Z)-1-dodecyl-2-(2-hydroxyethyl)diazene 1-oxide* (**21**)

19b (100 mg, 0.413 mmol) was converted to **20b** as a colorless solid (76.8 mg, 72%) and regioisomer **21** (23.5 mg, 22%) as a yellow oil, according to the general procedure for azoxy formation.

20b; IR (Diamond Prism) ν (cm^{-1}) : 3371, 2916, 2846, 1743, 1540, 1373, 1196; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.30 (t, *J* = 4.6 Hz, 2H), 4.03 (m, 2H) 3.43 (t, *J* = 7.5 Hz, 2H), 3.19 (t, *J* = 6.3Hz, 1H), 1.70 (dt, 7.5 Hz, 6.9 Hz, 2H), 1.33-1.20 (complex m, 18H), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 70.5, 59.6, 52.3, 32.0, 29.74 (3C), 29.65, 29.5 (2C), 27.9, 27.1, 22.8, 14.2; HRMS (FAB, NBA) m/z 259.2387$[M+H]^+$, calcd for $C_{14}H_{31}N_2O_2$: 259.2386

21; IR (Diamond Prism) ν (cm^{-1}) : 3386, 2954, 2915, 2854, 1511, 1466, 1311, 1049; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.20 (t, *J* = 7.0 Hz, 2H), 3.95 (m, 2H) 3.58 (t, *J* = 5.3 Hz, 2H), 1.96 (m, 2H), 1.89 (s, 1H), 1.3 3-1.25 (complex m, 18H), 0.88 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 69.7, 56.0, 54.2, 31.8, 29.5 (2C), 29.4, 29.3, 29.2, 28.9, 27.7, 26.2, 22.6, 14.0; HRMS (FAB, NBA) *m/z* 259.2386 $[M+H]^+$, calcd for $C_{14}H_{31}N_2O_2$: 259.2386.

4.1.44. (Z)-1-(2-Hydroxyethyl)-2-octadecyldiazene 1-oxide (**20c**)

19c (140 mg, 0.429 mmol) was converted to **20c** (75 mg, 50%) as a colorless solid, according to the general procedure for azoxy formation.

mp 62.3 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 3356, 2916, 2846, 2249, 2148, 1982, 1655, 1504, 1462, 1412, 1319, 1200, 1115, 1065, 1022, 876, 656; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 4.32 (t, *J* = 4.6 Hz, 2H), 4.05 (t, *J* = 4.6 Hz, 2H), 3.45 (t, *J* = 7.5 Hz, 2H), 1.72 (dt, *J* = 7.5 Hz, 6.9 Hz, 2H), 1.39 (m, 2H), 1.38-1.20 (complex m, 28H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 70.3, 59.5, 52.2, 31.9, 29.70 (6C), 29.65 (2C), 29.6, 29.5, 29.4, 29.3, 27.8, 27.0, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 343.3319 $[M+H]^+$, calcd for $C_{20}H_{43}N_2O_2$: 343.3325.

4.1.45. (Z)-2-Hexyl-1-vinyldiazene 1-oxide (**7a**)

20a (200 mg, 1.15 mmol) was converted to **7a** (163 mg, 91%) as a yellow oil, according to the general procedure for construction of a vinyl azoxy group.

IR (Diamond Prism) ν (cm^{-1}) : 2954, 2927, 2858, 1469, 1419, 1377, 1323, 1265, 1053, 953, 771, 729, 652; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.09 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.41 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 7.5 Hz, 1H), 3.54 (t, *J* = 6.9 Hz, 2H), 1.76 (dt, *J* = 7.5, 6.9 Hz, 2H), 1.42 (m, 2H), 1.35-1.30 (complex m, 4H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 143.4, 115.3, 52.6, 31.5, 27.5, 27.0, 22.5, 14.0; HRMS (FAB, NBA) *m/z* : 157.1336 $[M+H]^+$, calcd for $C_8H_{17}N_2O$: 157.1341.

4.1.46. (Z)-2-Dodecyl-1-vinyldiazene 1-oxide (**7b**)

20b (30.0 mg, 0.116 mmol) was converted to **7b** (19.8 mg, 71%) as a colorless oil, according to the general procedure for construction of a vinyl azoxy group.

IR (Diamond Prism) ν (cm^{-1}) : 2924, 2854, 1745, 1466, 1365, 1219; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.08 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.40 (d, *J* = 14.9 Hz, 1H) 5.47 (d, *J* = 7.5 Hz, 1H), 3.52 (t, *J* = 6.9 Hz, 2H), 1.74 (m, 2H), 1.40 (m, 2H) 1.35-1.16 (complex m, 16H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 143.6, 115.3, 52.7, 32.0, 29.8, 29.75 (2C), 29.67, 29.5 (2C), 28.0, 27.2, 22.8, 14.2; HRMS (FAB, NBA) *m/z* 241.2280 $[M+H]^+$, calcd for $C_{14}H_{29}N_2O$: 241.2280.

4.1.47. (Z)-2-Octadecyl-1-vinyldiazene 1-oxide (**7c**)

20c (70 mg, 0.204 mmol) was converted to **7c** (51 mg, 77%) as a yellow solid, according to the general procedure for construction of a vinyl azoxy group.

mp 46.3 ˚C; IR (Diamond Prism) ν (cm^{-1}) : 2954, 2927, 1469, 953, 792; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.10 (dd, *J* = 14.9, 7.5 Hz, 1H), 6.42 (d, *J* = 14.9 Hz, 1H), 5.50 (d, *J* = 7.5 Hz, 1H), 3.54 (t, *J* = 6.9 Hz, 2H), 1.76 (dt, *J* = 7.5, 6.9 Hz, 2H), 1.42 (m, 2H), 1.37-1.17 (complex m, 28H), 0.88 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 143.4, 115.3, 52.6, 31.9, 29.69 (6C), 29.65 (2C), 29.6, 29.5, 29.4 (2C), 27.8, 27.1, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 325.3223 $[M+H]^+$, calcd for $C_{20}H_{41}N_2O$: 325.3219.

4.1.48. (Z)-1-Dodecyl-2-vinyldiazene 1-oxide (**22**)

21 (10 mg, 0.0387 mmol) was converted to **22** (6.7 mg, 73%) as a yellow oil,

according to the general procedure for construction of a vinyl azoxy group. IR (NaCl) ν (cm^{-1}) : 2931, 2870, 1732, 1466, 1346, 1269, 1161, 976, 906, 725; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.71 (dd, *J* = 15.5, 8.0 Hz, 1H), 5.71 (d, *J* = 15.5 Hz, 1H), 5.36 (d, *J* = 8.0 Hz, 1H), 4.16 (t, *J* = 6.9 Hz, 2H), 1.97 (m, 2H), 1.42 (m, 2H), 1.39-1.25 (complex m, 20H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) : 137.7, 117.5, 69.6, 31.9, 29.6 (2C), 29.5, 29.34, 29.32, 29.0, 27.9, 26.4, 22.7, 14.1; HRMS (FAB, NBA) *m/z* : 241.2270 $[M+H]^+$, calcd for $C_{14}H_{29}N_2O$: 241.2280.

4.2. Biological study

4.2.1. Anthelmintic activity against nematode larvae

Infective larvae of *Haemonchus contortus* and *Cooperia curticei* were isolated from faecal cultures of mono-infected sheep. Larvae were ex-sheathed using sodium hypochlorite (NaClO), washed with water and subsequently with Ringer's solution. Test compounds were dissolved in DMSO at a concentration of 20 mg/mL, and this stock solution was further diluted as necessary. Each compound was tested in duplicate at different concentrations, starting with 100 ppm as the highest concentration. Approximately 40 larvae of *H. contortus* or *C. curticei* were transferred into a test tube containing the test compound. Each tube contained the same amount of DMSO. Larvae were then incubated at 37 ˚C. Negative controls were incubated without compound but with the same proportion of solvent (DMSO). After 5 days, larval motility was automatically recorded and compared to that observed in the negative controls. Motility was recorded in the in the following categories of efficacy: 100%, 90%, 80%, 60%, 40%, 0%; 100% efficacy meaning that all larvae were immotile, 0% efficacy meaning that all larvae were motile.

4.2.2. Anthelmintic activity against adult Nippostrongylus braziliensis

Adult *Nippostrongylus brasiliensis* worms were isolated from the small intestine of infected female Wistar rats and washed thrice in sterile Ringer's solution (supplemented with clotrimazole and enrofloxacin, final concentration 0.1 μg/mL and 10 μg/mL, respectively) and subsequently thrice with incubation medium (final concentration of enrofloxacin and clotrimazole 0.15 μg/mL and 15 μg/mL, respectively). Compounds were dissolved in DMSO to a concentration of 20,000 ppm, and diluted 1:10 and 1:100, respectively. Five μL of these solutions were added to 1 mL of incubation medium to

reach final concentrations of 10 and 1 ppm (μg/mL). Each compound and concentration was tested in duplicate. A negative control with DMSO only without test compound was run for comparison. Emodepside was used as positive control in concentrations of 10 and 1 ppm.

Five worms (mixed sexes) were incubated in 1 ml of that medium for five days at 37 °C. After incubation, activity of acetylcholine esterase (AChE) secreted by the worms was determined. Anthelmintic activity was ranked after incubation using the following scale: 100: >84%-100% = full activity (complete AChE inhibition compared to negative control); 84: >60%-84% = good activity; 60:>35%-60% = weak activity, and 35: 0%-35% = no activity.

4.2.3. Anthelmintic activity in vivo against Heligmosomoides polygyrus in mice

NMRI female mice were orally infected with 60 infective *Heligmosomoides polygyrus* larvae. On day 11 post infection, mice were allocated to groups and treated with either the test item (suspension or solution) or a placebo. The formulation was either 25% Cremophor EL / 75% water (formulation A) or 10% Transcutol / 10% Cremophor EL / 80% physiol. NaCl solution (0.9%) (formulation B). Each treatment group consisted of two mice, the untreated control group consisting of five mice. Animals were treated orally once, or daily for four consecutive days, starting on day 11 post infection. At 7-8 days after the first treatment, animals were necropsied, *N. polygyrus* worms counted, and efficacy calculated in comparison to the average worm burden in the placebo-treated control group. Calculated efficacy of <50% was considered to be no efficacy.

4.2.4. Toxicological results

In separate preliminary studies the acute oral toxicity and the mutagenic potential of jietacin A were tested.

4.2.4.1. Acute oral toxicity

To determine acute oral toxicity in rats, a method based on Organization of Economic Cooperation and Development (OECD) guideline 423 was used. Three fasted female Wistar rats were treated after an acclimatization of five days. Jietacin A was administered once orally as a freshly prepared suspension of 30 mg/mL in water /

cremophor EL (2% v/v), at a volume of 10 mL/kg body weight, resulting in a dosage of 300 mg/kg body weight. Subsequently, animals were assessed daily for a period of seven days. No clinical signs and no lethality were observed. From this preliminary result the LD_{50} of jietacin A is considered to be > 300 mg/kg.

4.2.4.2. Mini-Ames screening assay

A mini-Ames screening assay (pre-incubation method) was performed to test for point mutagenic effects in *Salmonella typhimurium* mutant strains TA 98, TA 100 and TA 102, with and without metabolic activation. In this method, bacteria were pre-incubated in a total volume of 175 µL (125 µL buffer, 25 µL bacterial suspension, 25 µL stock solution of compound dissolved in vehicle (DMSO), or DMSO only for negative control) for 20 min at 37 ˚C, before they were transferred to a soft agar plate. For metabolic activation, the 125 µL buffer in the sample was replaced by 125 µL liver extract (S9 mix). Tested concentrations of the test compound jietacin A were 5-2000 µg per plate. Plates were incubated at 37 ˚C for 48 in the dark. Assessment was performed by colony counting and comparison with the negative control. No relevant increase in incidence of revertants was observed, up to and including 2000 µg per tube. Therefore, no evidence for mutagenic potential of jietacin A was observed.

4.2.5. Antibacterial activity measurement

Antibacterial activity (Table S1) of jietacin derivatives against 27 strains, e.g. gram-positive and –negative bacteria, including drug–susceptible and drug–resistant strains, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterobacter aerogen*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* were investigated using the National Committee for Clinical Laboratory Standards method [62].

Table S1

Antibacterial activity for Jietacin A, and **7b**.

Strain/cpds	Jietacin A (**1**)	**7b**

		MIC (μg/mL)	
1	*Staphylococcus aureus* FDA209P[a]	>128	>128
2	*S. aureus* Smith[a]	>128	>128
3	MRSA KUB853[b]	>128	>128
4	MRSA KUB854[b]	>128	>128
5	MRSA 70[b]	>128	>128
6	MRSA 92-1191[b]	>128	>128
7	*S.aureus* KUB857[c]	>128	>128
8	*S.aureus* KUB858[d]	>128	>128
9	*S.aureus* KUB859[e]	>128	>128
10	*S.aureus* KUB860[f]	>128	>128
11	*S. epidermidis* KUB795[g]	>128	>128
12	*Micrococcus luteus* ATCC9341[h]	>128	>128
13	*Enterococcus faecalis* ATCC29212	>128	>128
14	*E.faecalis* NCTC12201[i]	>128	>128
15	*E.faecium* NCTC12204[i]	>128	>128
16	*Escherichia coli* NIHJ JC-2[h]	>128	>128
17	*Citrobacter freundii* ATCC8090[h]	>128	>128
18	*Klebsiella pneumoniae* NCTC9632[h]	>128	>128
19	*Proteus mirabilis* IFO3849[h]	>128	>64
20	*P. vulgaris* OX-19[h]	>128	>64
21	*Morganella morganii* IID Kono[h]	>128	>64
22	*Serratia marcescens* IFO12648[h]	>128	>64
23	*Enterobacter cloacae* IFO13535[h]	>128	>64
24	*E. aerogenes* NCTC10006[h]	>128	>64
25	*Pseudomonas aeruginosa* 46001[h]	>128	>64
26	*P. aeruginosa* E-2[h]	>128	>64
27	*Acinetobacter calcoaceticus* IFO12552[h]	>128	>64

[a]Staphylococcus aureus FDA209P and Smith: susceptible strains. [b]MRSA KUB853, MRSA KUB854, MRSA 70, and MRSA 92-1191: MRSA strains isolated from clinical patients. [c]S. aureus KUB857: macrolide resistant strain, encoded by erm gene. [d]S. aureus KUB858: macrolide resistant strain, encoded by erm gene. [e]S. aureus KUB859: encoded by erm gene. [f]S. aureus KUB860: encoded by erm and mef gene. [g]S. epidermidis KUB795: strains isolated from clinical patients , [h]Standard strain, [i]Enterococcus faecalis NCTC12201: encoded by van A gene. [j]E. faecium NCTC12204: encoded by van A gene.

Financial interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at…

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