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Syntheses and Biological Activity Studies of Novel Sterol Analogs from Nitroso Diels−Alder Reactions of Ergosterol

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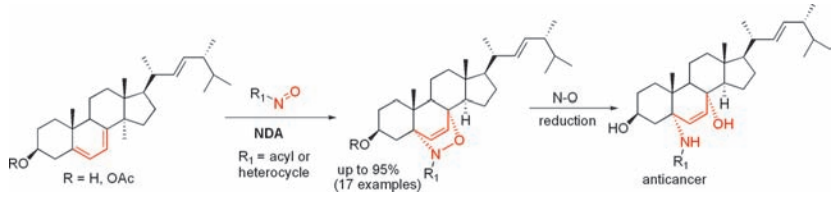
ABSTRACT



A series of novel sterol analogs was prepared using nitroso Diels−Alder reactions with ergosterol. Most cycloaddition reactions proceeded in an excellent regio- and stereoselective fashion. Further N−O bond cleavage of cycloadducts generated compounds with biological activity in PC-3 and MCF-7 cancer cell lines.

Natural products and their derivatives represent the most prolific source of molecular diversity in drug discovery.[1] Functional-group transformation of natural products constitutes one of the main avenues for generating pharmacologically relevant compounds with altered and sometimes improved biological properties. Typical chemical derivatizations of natural products are often limited to standard modification of nucleophilic or electrophilic functional groups. Since many natural products contain multiple functional groups of the same or similar type, derivatization selectivity is often problematic. Hence, new derivatization methods are still needed. Our effort in this area has involved nitroso Diels−Alder (NDA) reactions as efficient methods for derivatization and functionalization of diene-containing natural products. We and others[2] have demonstrated that many complex diene-containing natural products readily undergo nitroso cycloadditions, generating 1-amino-4- hydroxy-2-ene heterocycle scaffolds with high regio- and stereoselectivity. Ergosterol (Figure 1), a natural sterol containing a conjugated diene, was

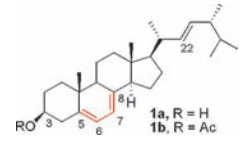


Figure 1. Ergosterol (**1a**) and ergosterol acetate (**1b**).

chosen for further exploration of this method for modular enhancement of Nature's diversity (MEND).[2a] Ergosterol **1a** (ergosta-5,7,22-trien-3β-ol) is biologically relevant as a precursor (a provitamin) to Vitamin D_2[3] and an integral component of fungal cell membranes. The presence of ergosterol in fungal cell membranes coupled with its

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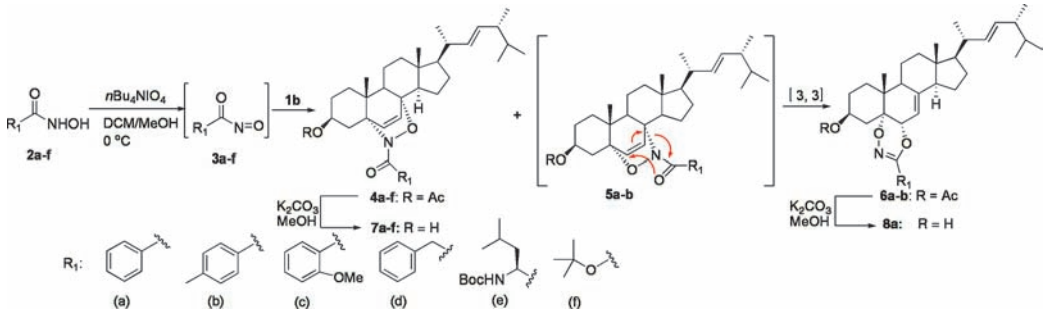
Scheme 1



absence in animal cell membranes makes it a useful target for antifungal drugs.[4] Several groups have shown that sterols with a heteroatom substituent at C-24 or C-25 are effective antifungal compounds.[5] Nitroso Diels−Alder reactions with ergosterol acetate **1b** were reported by Kirby et al.;[2d,e] however, this method was never exploited as a tool for derivatization of sterol compounds and the dienophiles used were often limited to transient aroyl nitroso species. Herein we report the syntheses of novel C5 and C8 disubstituted sterol analogs using nitroso Diels−Alder reactions of ergosterol, and its acetate, with acyl- and iminonitroso agents, and wish to demonstrate the utility of this methodology in natrual product derivatization by evaluation of their biological activity.

A series of hydroxamic acids **2a**−**e** was obtained by reaction of the corresponding methyl esters with hydroxylamine;[6] *N*-hydroxycarbamate **2f** was prepared from direct acylation of hydroxylamine with di-*tert*-butyl dicarbonate. Oxidation of compounds **2a**−**f** to acyl nitroso dienophiles **3a**−**f** with tetrabutylammonium periodate in the presence of ergosterol acetate **1b** gave the 5α-*N*-8α-*O*-adducts **4a**−**f** in various yields (Scheme 1, Table 1). The stereoisomeric *N*-adduct **5a**, a much more sterically congested isomer compared to adduct **4a**.[2d] Reaction with 4-methylbenzohydroxamic acid **2b** gave a similar result, although dioxazine **6b** was only isolated as a mixture with adduct **5b** (entry 2). In contrast, oxidation of 2-methoxybenzohydroxamic acid **2c**, phenylacetohydroxamic acid **2d,** L-leucine-derived hydroxamic acid **2e** and *tert*-butyl *N*-hydroxycarbamate **2f** gave **4c**−**f** as the sole products in good to excellent yields (entry 3−6). Further K_2CO_3 mediated deacetylation of **4a**−**f**, **6a** gave compounds **7a**−**f** and **8a** in 90% average isolated yields.

A nitroso Diels−Alder reaction with ergosterol **1a** was also investigated using nitrosobenzene **8** as a representative aryl nitroso dienophile (entry 1, Scheme 2). The NDA reaction occurred based on TLC and ^{1}H NMR analysis; however, adduct **11** was not able to be isolated in pure form by chromatography. Additionally, cycloadditions with various iminonitroso agents **10,** mainly 2-nitrosopyridine derivatives, were examined (Scheme 2). Those nitroso compounds were prepared from aminoheterocyclic precursors **9** in a two-step sequence (*N*, *N*-dimethyl sulfilimine intermediate formation, followed by oxidation using *m*-CPBA).[7] Most of the

Table 1. Results of Acylnitroso Diels−Alder Reactions

entry	compd	adduct	yield (%)	dioxazine	yield (%)
1	**2a**	**4a**	29	**6a**	54
2	**2b**	**4b**	12	**6b**	63[a]
3	**2c**	**4c**	74	**6c**	0[b]
4	**2d**	**4d**	95	**6d**	0[b]
5	**2e**	**4e**	93	**6e**	0[b]
6	**2f**	**4f**	93	**6f**	0[b]

[a] As a nonseparable mixture of dioxazine **6b** and 5α-*O*-8α-*N*-adduct **5b**. [b] Not detected.

configuration of **4a**−**f** was assigned by ^{1}H NMR studies based on literature analysis of **4a**.[2d] In the case of benzohydroxamic acid **2a** (entry 1, Table 1), similar to Kirby's observation,[2d] dioxazine **6a** was isolated as the major product in 54% yield, with adduct **4a** as the minor product in 29% yield. Compound **6a** was formed as a result of [3, 3] sigmatropic rearrangement of 5α-*O*-8α-

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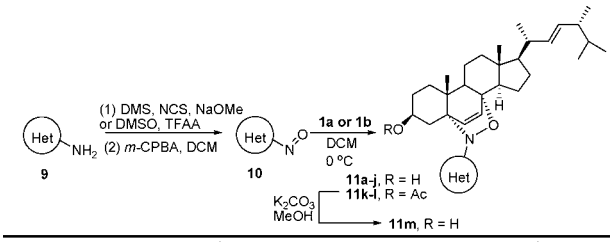
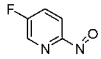
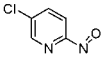
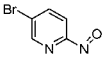
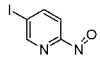
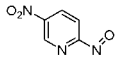
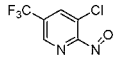
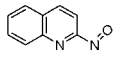
Scheme 2



entry	nitroso		product	yield (%)
1[a]	**8**		**11**	-
2[b]	**10a**		**11a**	92
3[b]	**10b**		**11b**	95
4[b]	**10c**		**11c**	92
5[b]	**10d**		**11d**	87
6[b]	**10e**		**11e**	90
7[b]	**10f**		**11f**	88
8[b]	**10g**		**11g**	82
9[b]	**10h**		**11h**	82
10[b]	**10i**		**11i**	84
11[b]	**10j**		**11j**	81
12[c]	**10k**		**11k** + **11l**	95

[a] **11** failed to be isolated through silica gel chromatography. [b] 1.2 equiv of nitroso was used. [c] Ergosterol acetate **1b** was used.

iminonitroso compounds could be isolated and stored in pure form, except for **10k**. The NDA reactions between ergosterol **1a** and iminonitroso species **10a**−**j** usually were complete within 30 min at 0 °C. In each case, the cycloadducts **11a**−**j** were obtained as single 5α*O*-8α*N*-adducts in good to excellent yields after column chromatography (Scheme 2). The regio- and stereochemistry of the adducts was assigned based on our previous report and NMR studies.[2a] The instability of nitroso agent **10k** required its immediate use, so it was trapped *in situ* with ergosterol acetate **1b**. Interestingly, both regioisomers **11k** and **11l** were obtained in a 8.5:1 ratio in 95% combined yield (entry 12, Scheme 2). The major adduct **11k** has the same stereochemistry as cycloadducts **11a**−**j**, based on X-ray crystallography (Figure 2). Further deacetylation of **11k** generated compound **11m**. Clearly, 2-nitrosopyridines **10**, as stabilized forms of iminonitroso reagents, constitute an ideal combination of reactivity and stability for NDA reactions with ergosterol, relative to benzenenitroso **8** and acylnitroso moieties **3**, respectively

We next set out to demonstrate the synthetic utility of ergosterol nitroso cycloadducts through N−O bond cleavage, a method for generating 1,4-amino alcohols suitable for

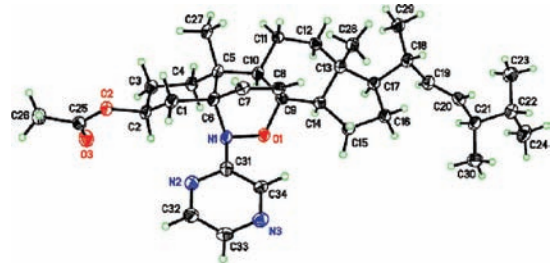
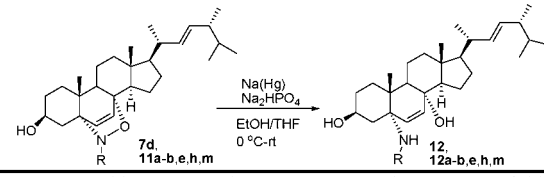
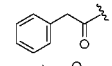
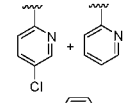
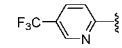


Figure 2. X-ray of cycloadduct **11k** with ergosterol acetate **1b**.

further diversification. Adduct **11a** was chosen as model for optimizing reaction conditions. Reactions with SmI_2/THF,[8] Zn/CH_3COOH, $Mo(CO)_6$,[9] etc. were attempted; however, all of these conditions failed to yield desired product **12a** (Supporting Information). Detailed screenings showed that Na amalgam[10] successfully reduced the N−O bond of **11a** to give **12a** in 82% yield (entry 1, Scheme 3). Then, a series

Scheme 3



entry	adduct	product	R (product)	yield (%)
1	**11a**	**12a**		82
2	**7d**	**12**		26
3	**7f**	-		trace
4	**11b**	**12b**		61
5[a]	**11e**	**12e** + **12b**		60 (1:3)
6[b]	**11h**	**12h**		59
7	**11m**	**12m**		63

[a] −Cl of R was partially reduced. [b] −Cl of R was fully reduced.

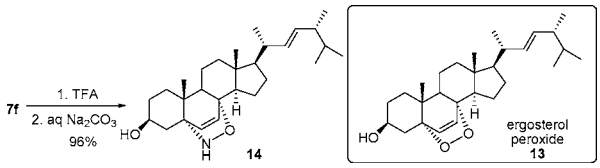
of ergosterol nitroso adducts was subjected to the optimized Na(Hg) conditions. While low transformation was found with acylnitroso adducts **7d** and **7f** (entries 2−3), moderate yields were achieved for pyridinylnitroso adducts. Not surprisingly, these conditions also reduced the −Cl substituent (entries 5−6).

Ergosterol peroxide **13**[11] is a natural sterol derivative that possesses a variety of biological properties including immunosuppressive and antitumor activity. Simply removing the *N*-Boc group of cycloadduct **7f** afforded a quick access

(8) Revuelta, J.; Cicchi, S.; Brandi, A. *Tetrahedron. Lett.* **2004**, *45*, 8375.

to compound **14** as an isoelectronic analog of ergosterol peroxide **13** (Scheme 4).

Scheme 4



Broad antibacterial testing of these novel sterol analogs using the argar diffusion method revealed no significant activity against Gram-positive or Gram-negative organisms. However, N−O cleaved analogs had their largest zone of inhibition against the fungal strain, *Sporobolomyces salmonicolor*, whereas ergosterol, **1a**, and adducts did not (Supporting Information). More interestingly, N−O reduced compounds **12**, **12a**−**b**,**e**,**h**,**j**, along with ergosterol acylnitroso adducts **7a**−**f**, showed growth inhibitory activity in MCF-7 (breast cancer) and PC-3 (prostate cancer) tumor cell assays, while parent erogsterol, **1a**, and most pyridinylnitroso adducts were relatively inactive (entries 1, 9, 10, 12, 14−16, Table 2). All N−O reduced analogs reached the low micromolar range of inhibition against both PC-3 and MCF-7 cells (entries 17−22). In general, all active compounds showed significantly improved inhibitory activity against the MCF-7 cell line. These biological assays clearly suggest that the nitroso heterocycle changed the biological activity profile of its parent natural product.

In summary, we have reported that nitroso Diels−Alder reactions between ergosterol (or ergostrol acetate) and 2-nitrosopyrdines as well as acylnitroso agents proceeded with excellent regio- and stereoselectivity. A new class of sterol analogs that show encouraging anticancer activity was generated through N−O bond cleavage of the cycloadducts. The method presented herein provides a novel and efficient way to derivatize diene-containing natural products. Efforts to determine the exact mechanism of anticancer action and further diversification of ergosterol adducts are in progress.

Table 2. Results of Anticancer Screenings[a]

		% inhibition at 20 μM		IC_{50} (μM)	
entry	compd	PC-3	MCF-7	PC-3	MCF-7
1	**1a**	10	15	–	–
2	**7a**	64	97	–	14
3	**7b**	40	97	16	14
4	**7c**	94	97	11	6
5	**7d**	76	96	12	14
6	**7e**	57	95	12	8
7	**7f**	73	89	17	20
8	**14**	48	82	>20	>20
9	**11a**	<10	15	–	–
10	**11b**	15	29	–	–
11	**11c**	76	100	–	15
12	**11d-e, g-h**	<10	<10	–	–
13	**11i**	22	90	–	17
14	**11j**	15	<10	–	–
15	**11k**	15	29	–	–
16	**11m**	8	20	–	–
17	**12**	94	94	9	6.5
18	**12a**	100	100	6	2
19	**12b**	100	100	6	3.5
20	**12e**	100	100	7	3
21	**12h**	100	100	10	2.5
22	**12j**	100	100	4	2.5

[a] Trichostatin A was used as the positive control (MCF-7 IC_{50} = 16 nM, PC-3 IC_{50} = 160 nM).

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Supporting Information Available: Full experimental procedures, characterization data and copies of ^{1}H NMR and ^{13}C NMR spectra, protocols of antibacterial and anticancer assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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