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Syntheses and biological evaluation of ring-C modified colchicine analogs

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Abstract

Ring-C modified alkaloids were synthesized from colchicine using iminonitroso Diels–Alder reactions in a highly regio-and stereoselective fashion. Several analogs exhibited cytotoxic activity similar to that of colchicine itself against PC-3 and MCF-7 cancer cell lines, by serving as prodrugs of colchicine through retro Diels–Alder reactions under the assayed conditions. *In vitro* microtubule polymerization assays indicated that these modifications affected their interaction with tubulin.

Keywords

Colchicine; Nitroso Diels–Alder; 2-Nitrosopyridine; Cytotoxicity; Microtubule

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Natural (−)-(a*S*, 7*S*)-colchicine (**1**) (Figure 1), the major alkaloid isolated from *Colchicum autumnale* L. (Liliaceae), is a highly potent antimitotic agent that derives its activity by binding to protein tubulin, the basic subunit component of microtubules.1 Though its use has been limited because of its toxicity, colchicine can still be used as a lead compound for the generation of potential anticancer drugs. Thus, numerous analogs of colchicine have been synthesized in the hope of developing novel, useful drugs with more favorable pharmacological profiles. Structure-activity studies2 reveal that the trimethoxy benzene ring (A) and the methoxy tropone ring (C) of colchicine comprise the minimal structural features of the molecule needed for its high affinity binding to tubulin. One notable example is that isocolchicine (**2**), a colchicine analog differing only in the relative position of the methoxy and carbonyl groups of ring C, is virtually inactive and is unable to inhibit tubulin assembly.3 Ring C is also characterized by two facially differentiated 1,3-diene moieties, most suitable for cycloaddition reactions of the alkaloid.4 While Diels–Alder reactions of colchicine with several symmetrical hetero- and carbo-dienophiles have been reported,5 the biological consequence of this modification has never been disclosed, although these studies should further the understanding of the role of ring C in the development of new colchicine drugs. Our group also reported one example of hetero Diels–Alder reaction of colchicine with a pyridinylnitroso agent.[6] Encouraged by these results, we were interested to further explore the scope of this cycloaddition chemistry of colchicine. Herein we report the synthesis of a series of colchicine analogs modified at the ring C using iminonitroso Diels–Alder (NDA) reactions for modular enhancement of Nature's diversity (MEND). We also describe the results of *in vitro* anticancer activity of the analogs against MCF-7 and PC-3 cell lines as well as their tubulin binding affinity.

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The use of 2.0 equiv of 6-methyl-2-nitrosopyridine **3a** has been demonstrated to react with colchicine to give adduct **6a** as a single isomer[7] in 82% yield (Table 1, entry 1). To further explore the reactivity of alternate nitroso species, nitrosobenzene **4**, as a representative arylnitroso agent, and ayclnitroso agent **5** derived from *in situ* oxidation of benzohydroxamic acid, as a representative transient acylnitroso agent, were also examined in the cycloaddition reactions with colchicine (entries 2–3). However, none were as effective as pyridinylnitroso agent **3a**. Reaction of colchicine with **4** generated the corresponding cycloadduct **6** only in less than 10% yield based on ^{1}H NMR analysis of the crude mixture; however, adduct **6** was not able to be isolated in pure form by chromatography. On the other hand, no NDA reaction occurred upon the treatment with acylnitroso agent **5**. Clearly, 2-pyridinylnitroso **3a**, as a stabilized iminonitroso reagent, constitutes an ideal combination of reactivity and stability for NDA reactions with colchicine.

Next we investigated the scope of iminonitroso agents **3**, in particular, pyridinylnitroso derivatives, in the nitroso Diels–Alder reactions with colchicine. A series of iminonitroso agents **3b–k** was synthesized from the commercially available amine precursors[8] and each was treated with colchicine (**1**) separately. The results are summarized in Table 2. Cycloaddition with 2-nitrosopyridine **3b** gave two regioisomeric adducts **6b** and **7b** in a 3:1 ratio and 78% yield (entry 1). The major isomer **6b** had the same configuration as **6a** by comparison of ^{1}H and ^{13}C NMR profiles, which was formed from the cycloaddition at the 8,12-diene moiety of colchicine and *syn* to the NH-group, with *endo* preference. The structure of the minor adduct, **6b**, was assigned as the regioisomer of **6a**, which also shared the *syn-endo* configuration based on ^{1}H and 2D NMR analyses of related analogs.[9] Interestingly, when 3-methyl-2-nitrosopyridine **3c** was used, no cycloaddition reaction occurred (entry 2). We assumed that this low reactivity resulted from the steric effect of the methyl substituent adjacent to the nitroso functional group. To further confirm this assumption, reaction of **1** with 4-methyl-2-nitrosopyridine **3d** was conducted. As expected, a mixture of adducts **6d** and **7d**, with **6d** predominate, was generated in 61% yield (entry 3). A single isomeric adduct **6e** was obtained in 62% yield from the reaction with 6-ethyl-2-nitrosopyridine **3e** (entry 4). It appeared that halide substituents on the 5-position of the pyridine ring of the nitroso agents did not affect reactivity and selectivity. Thus, adducts **6f–h** and **7f–h** were formed in good yields with **6f–h** as the major products (entries 5–7). Reaction of a more electron deficient nitroso agent, 5-nitro-2-nitrosopyridine **3i**, gave results similar to those previously obtained upon reactions with **3f–h** (entry 8). When quinoline-based nitroso agent **3j** was used, a mixture of adducts **6j** and **7j** was obtained in 72% yield with an 8:1 ratio (entry 9). These results indicated that having a 6-substitutent close to nitrogen is important to induce high regioselectivity in the NDA reactions with colchicine. An alternate iminonitroso agent, 5-methyl-3-nitrosoisoxazole **3k**, was also reacted with colchicine. Compared to the reactions with pyridinylnitroso agents, cyloaddition between **3k** and **1** afforded a mixture of two isomeric adducts **6k** and **7k** in 80% yield with a compromised regioselectivity. The isomers were separated by prep HPLC. These studies indicated that 2-nitrosopyridines **3** are very effective dienophiles with exquisite sensitivity to electronic and steric influences of the diene-containing colchicine alkaloid.

With these ring-C modified colchicine analogs at hand, *in vitro* cytotoxicity assays against both PC-3 (prostate cancer) and MCF-7 (breast cancer) tumor cell lines were conducted (Table 3). In general, most analogs showed similar activity to colchicine itself. However, relatively low activity was observed with compounds **6i***, **6k** and **7k**. Since in the most cases, the activity was essentially identical, we decided to determine the stability of those adducts to see if they were eventually serving as prodrugs of colchicine itself, perhaps by retro Diels–Alder reaction under the assayed conditions. The experiments were carried out by treating two representative adducts, **6a** and **6k**, in deuterated DMSO at 37 °C, since they exhibited different cytotoxicity. ^{1}H NMR monitoring indicated that indeed 13% of cycloadduct **6a** underwent the retro Diels–Alder reaction to release colchicine within 5 h. After 24 h, half of the starting cycloadduct **6a** was converted to colchicine (Equation 1). The resultant pyridinylnitroso **3a** dimerized and lost oxygen to generate the corresponding azo-oxy compound.[10] In contrast, adduct **6k**, derived from 5-methyl-3-nitrosoisoxazole **3k**, was intact even after 3 days. Obviously, the different thermal stability of colchicine analogs might account for their varied cytotoxic activity.

We also noticed that within the 30 min time frame for the standard microtubule (MT) polymerization assay, colchicine nitroso adducts were stable and no retro Diels–Alder reactions were detected. Therefore, it was feasible to examine the tubulin binding affinities of the adducts. In this regard, several representative spectroscopically pure cycloadducts, **6a–b**, **6k** and **7k**, were subjected to MT polymerization assays. In these experiments, the anti-microtubule activities of selected colchicine analogs were evaluated and compared with reference compounds including colchicine, nocodazole, a microtubule destabilizer, and paclitaxel, a microtubule stabilizer (Figure 2). Interestingly, unlike colchicine, which nearly inhibited the entire MT polymerization at 10 μM, all assayed colchicine analogs showed decreased inhibitory activity towards MT polymerization. Moreover, except for **7k**, colchicine adducts **6a–b** and **6k** appeared to enhance the MT polymerization. Clearly, introduction of a N–O heterocycle at the C8,12 position of ring C of colchicine changed the structural conformation, and thereby affected the colchicine-tubulin interaction.

In conclusion, a series of ring-C modified colchicine analogs using iminonitroso Diels–Alder reactions was synthesized and evaluated for cytotoxic and antimicrotubule activity. The cycloaddition reactions occurred exclusively with the 8,12-diene moiety and often in a highly regio- and stereoselective fashion. Most analogs showed cytotoxic activity against PC-3 and MCF-7 cancer cell lines, by serving as prodrugs of colchicine through retro Diels–Alder reactions under the assayed conditions. *In vitro* microtubule polymerization assays indicated that these analogs changed the interactive properties of colchicine with tubulin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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6. Li FZ, Yang BY, Miller MJ, Zajicek J, Noll BC, Mollmann U, Dahse HM, Miller P. Org Lett 2007;9:2923. [PubMed: 17602642]

7. The stereochemistry of **6a** was conclusively determined by X-ray crystallographic analysis. See ref 6.

8. 2-Nitrosopyridine derivatives were synthesized in a two-step sequence (N, N-dimethyl sulfilimine intermediate formation, followed by oxidation using m-CPBA), see: Taylor EC, Tseng CP, Rampal JB. J Org Chem 1982;47:552.

9. The regioselectivity of the reaction was determined by ^{13}C NMR measurements; for example, in the case of cycloaddition with nitroso **3k** (Table 2, entry 10), the chemical shifts of C-8 of adduct **6k**, and C-12 of adduct **7k** (at 82.8 and 78.7 ppm, respectively) correspond to carbons next to oxygen. The structure of **7k** was further confirmed by 2D NMR studies (gCOSY, gHSQC, gHMBC and gROESY). In general, all the isomers with same configuration share similar ^{1}H and ^{13}C NMR patterns. For example, all the chemical shifts of the C-12 of the major isomers **6a–k** are near 82.0 ppm.

10. For a detailed discussion of retro iminonitroso Diels–Alder reactions, see: Yang BY, Lin WM, Krchnak V, Miller MJ. Tetrahedron Lett 2009;50:5879. [PubMed: 20161032]

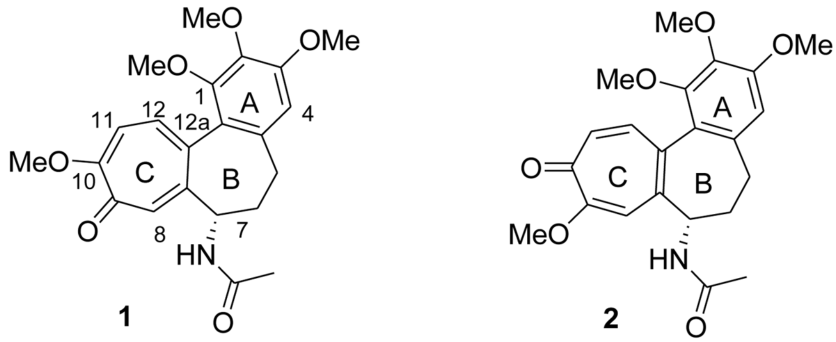

Figure 1.
Colchicine (**1**) and isocolchicine (**2**)

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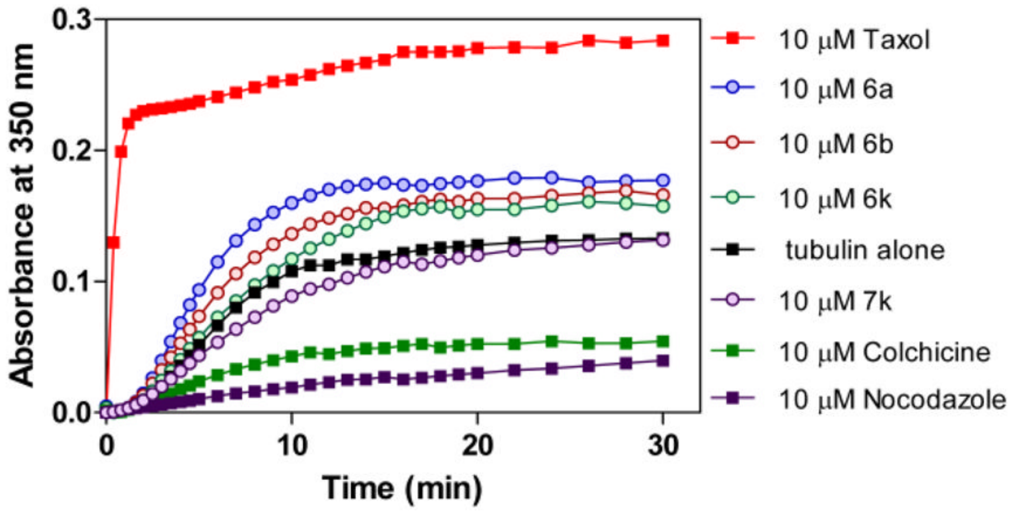
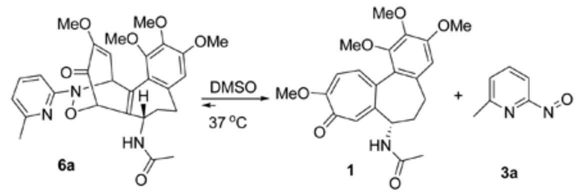


Figure 2.
In vitro microtubule polymerization assay



Equation 1.
Retro Diels–Alder reaction of colchicine nitroso adduct **6a** at 37 °C

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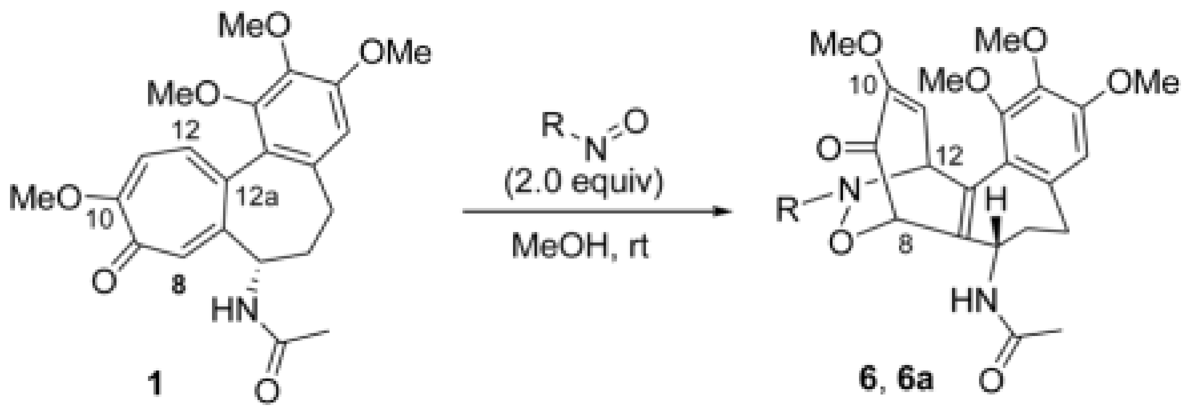
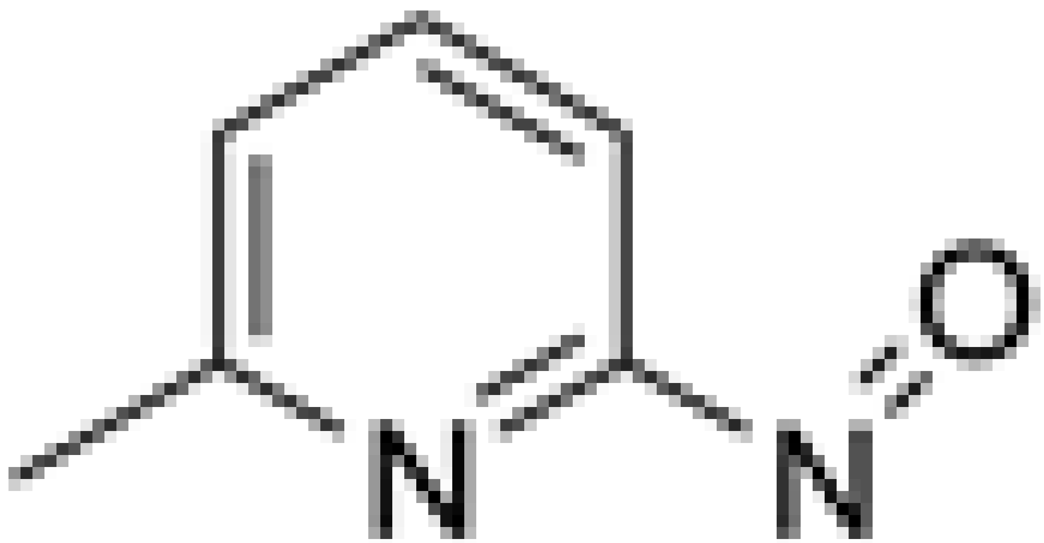
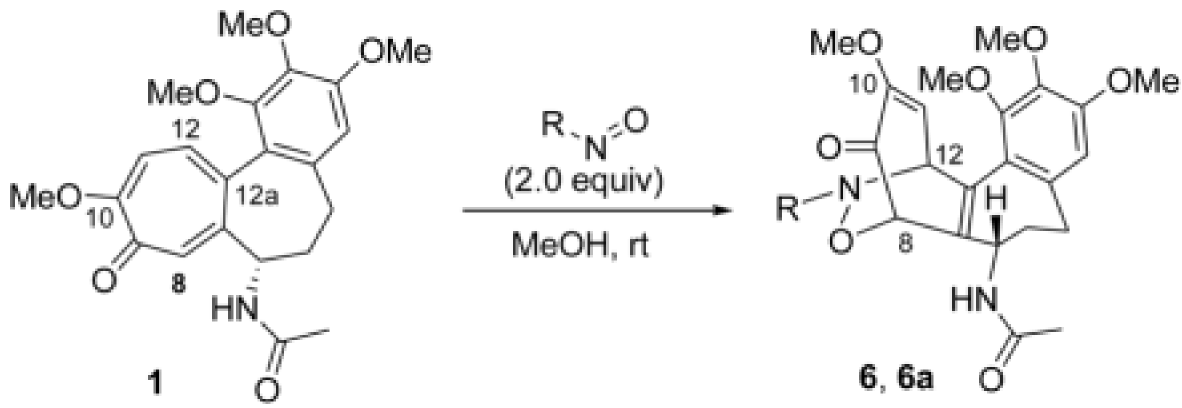
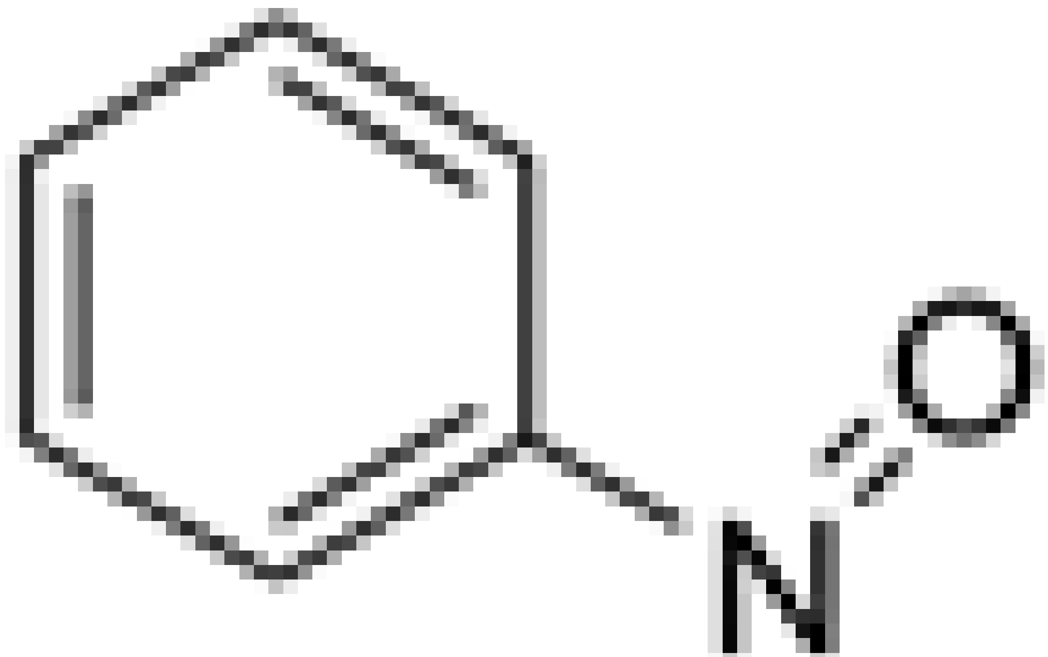
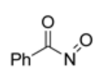
Table 1

Nitroso Diels–Alder reaction of colchicine (**1**) with different nitroso agents



Entry	Nitroso	Product	Yiel
1	**3a** 	**6a**	8



Entry		Nitroso	Product	Yiel
1	**4**		**6**	<
2[c]	**5**		–[d]	

[a]Isolated yield.

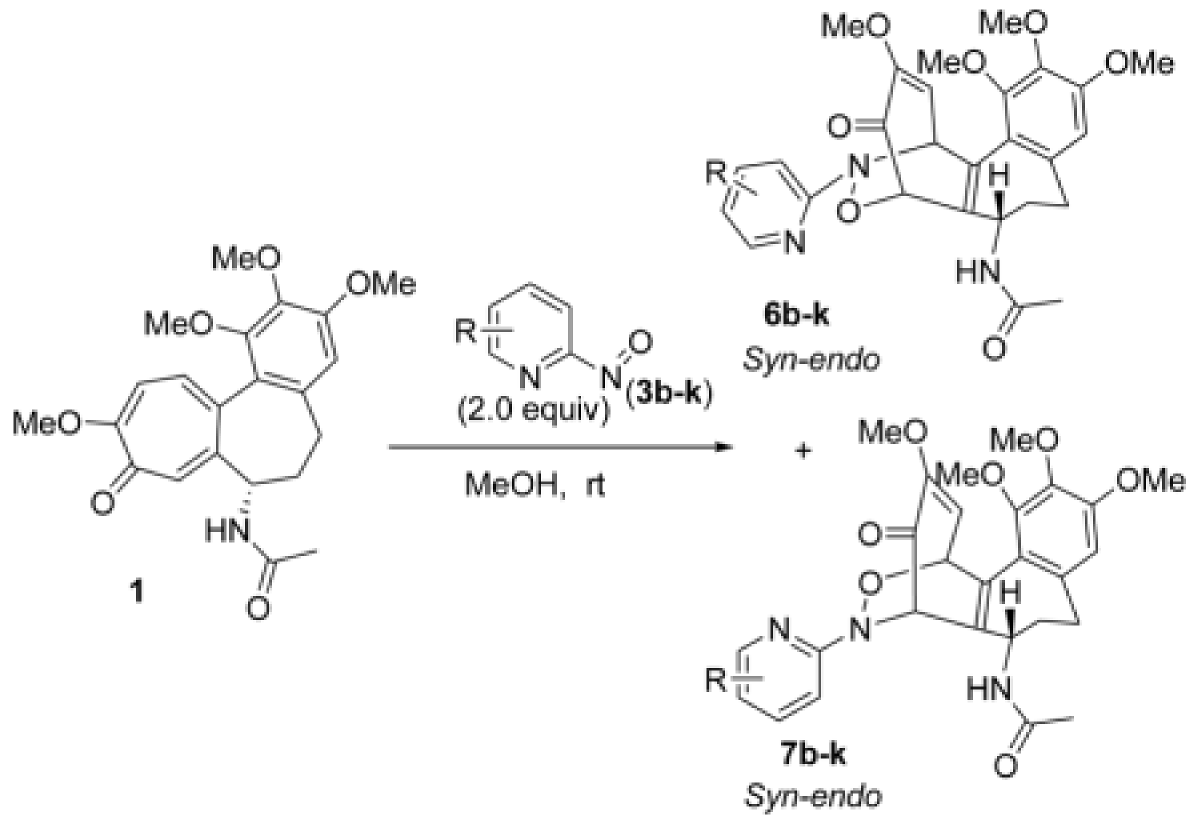
[b]Determined by ^{1}H NMR analysis of crude mixture.

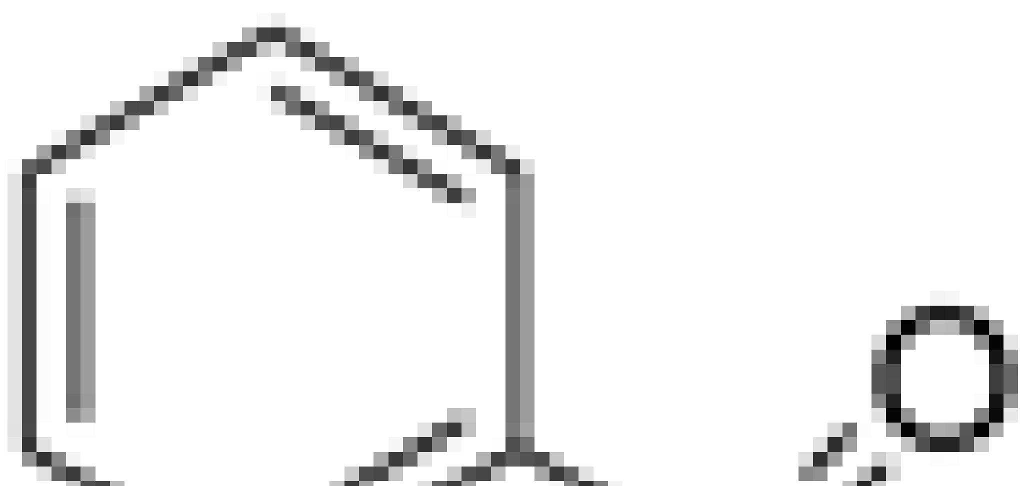
[c]*In situ* oxidation trapping required.

[d]No cycloaddition observed.

Table 2

Nitroso Diels–Alder reaction of colchicine (**1**) with various iminonitroso agents



Entry	Nitroso	Product	Ratio[c]	Yield (%)[d]
1[a]	**3b** 	**6b** + **7b**	3:1	78

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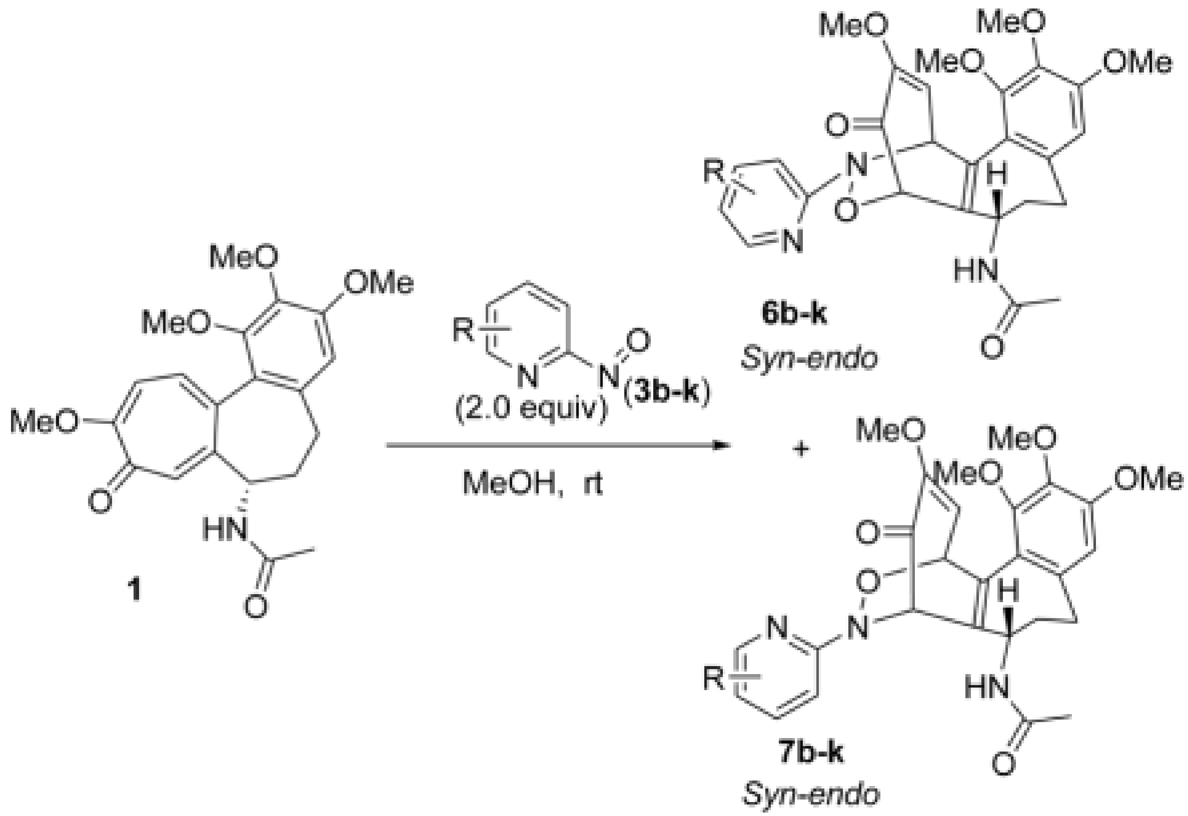
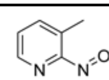
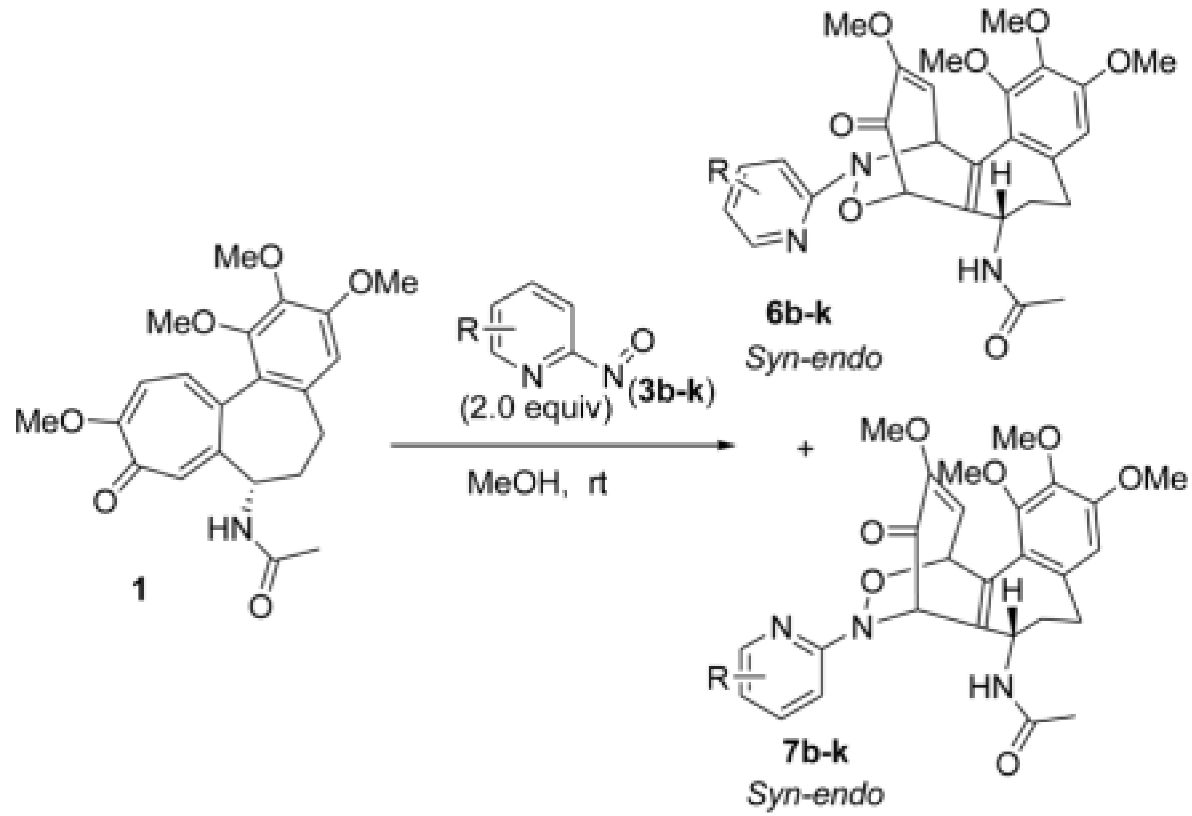
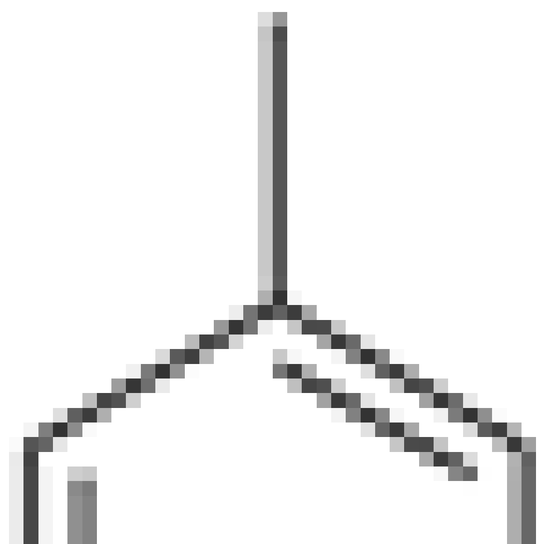
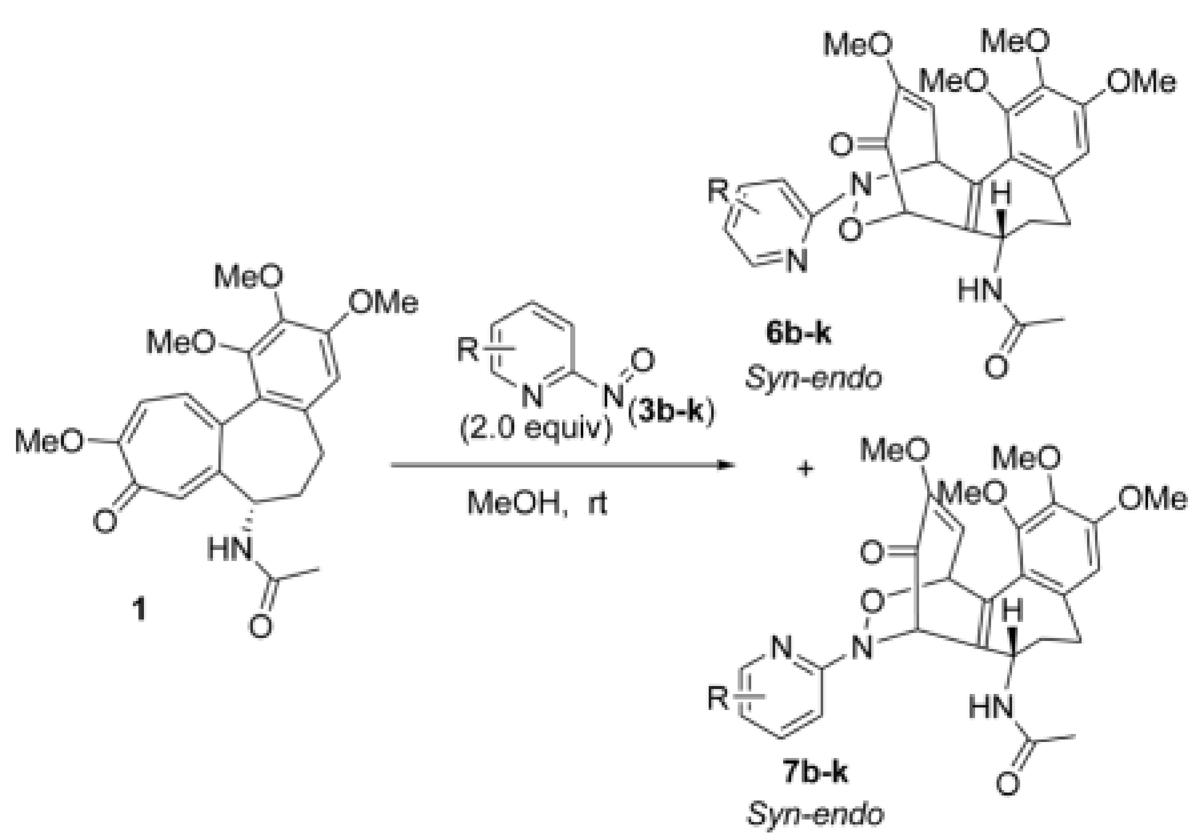
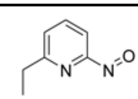
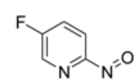
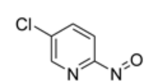


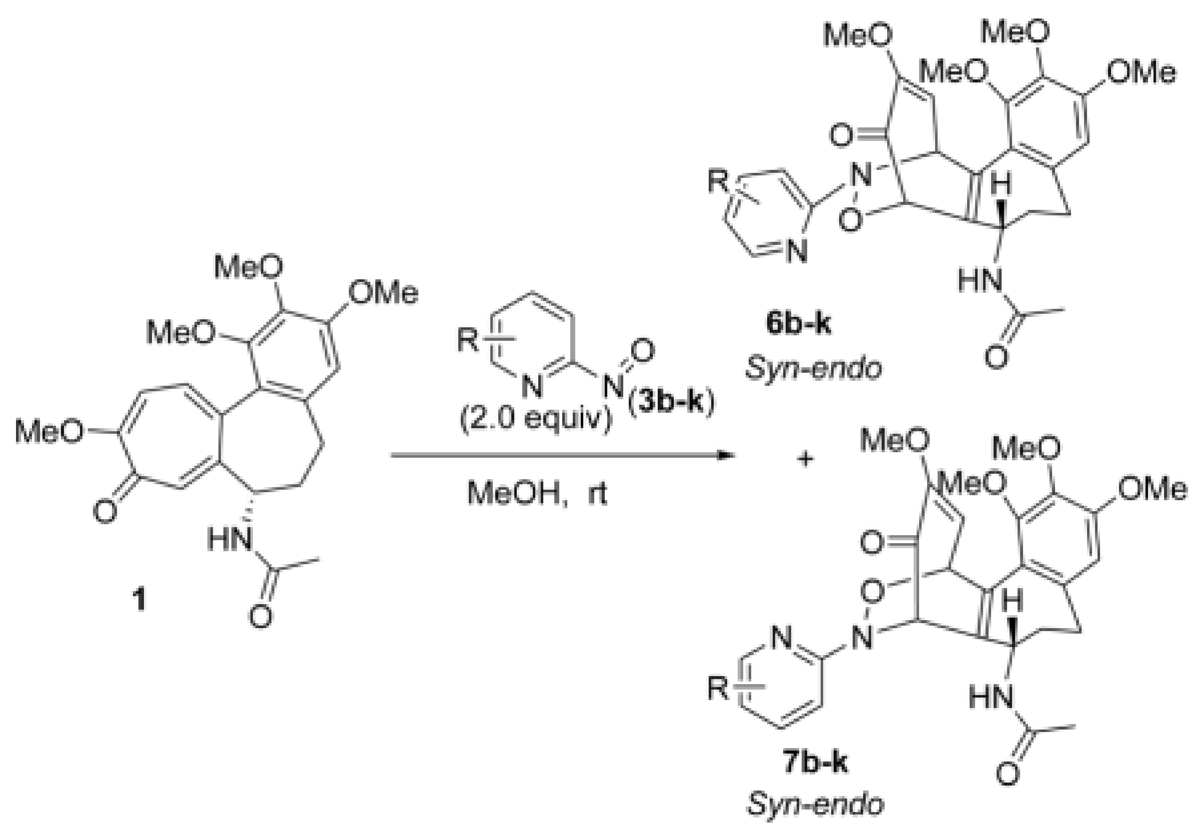
Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
2	**3c**		–	–	0

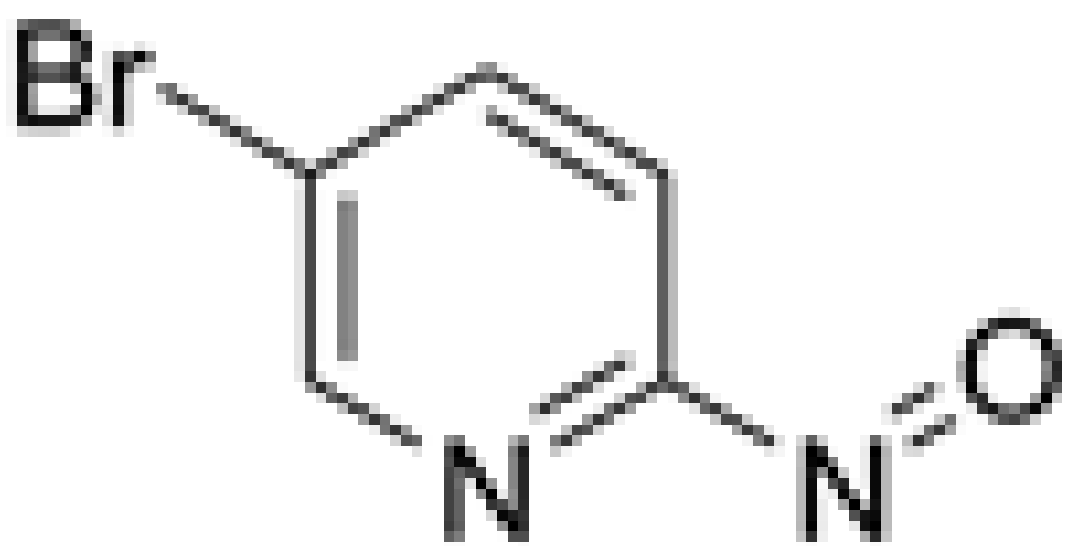


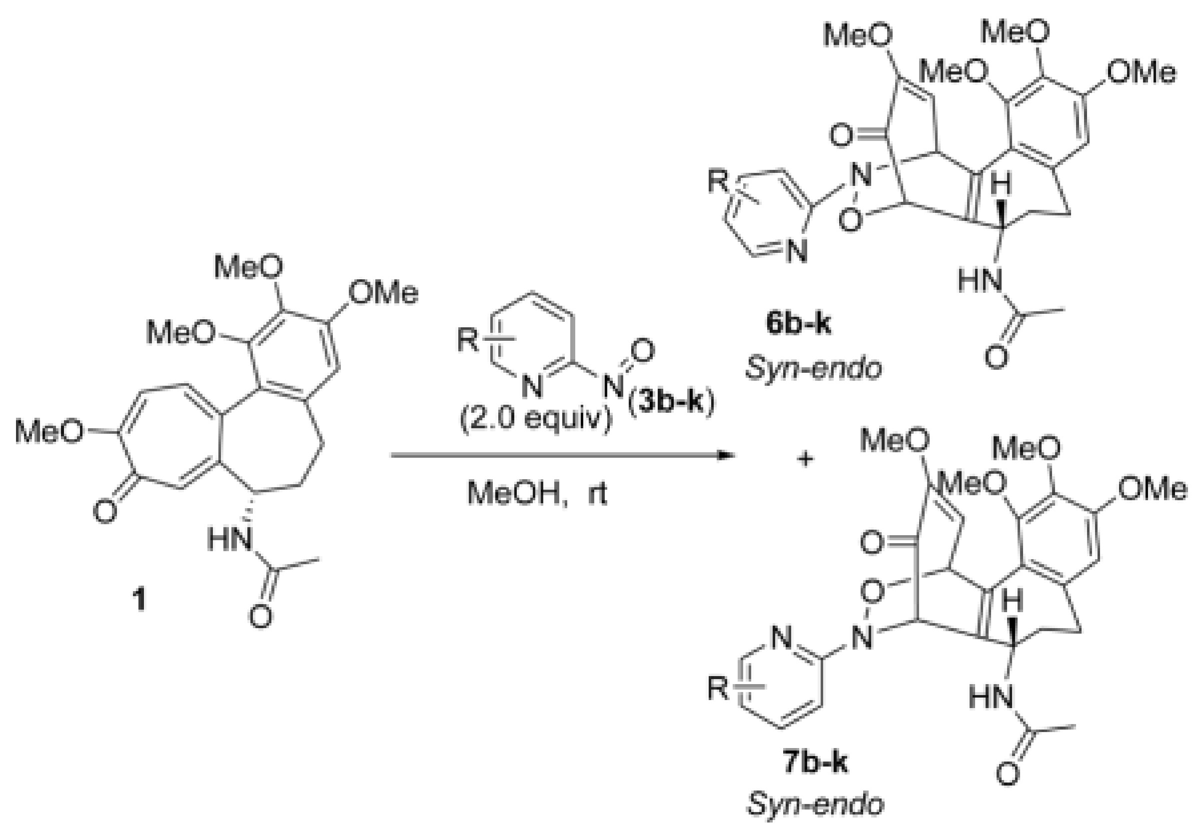
Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
3[a]	**3d**		**6d** + **7d**	6:1	61

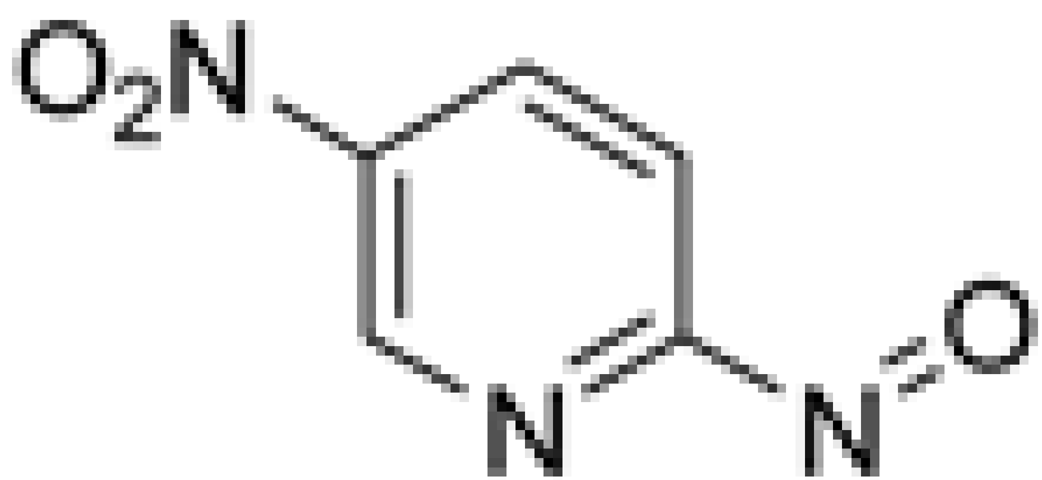


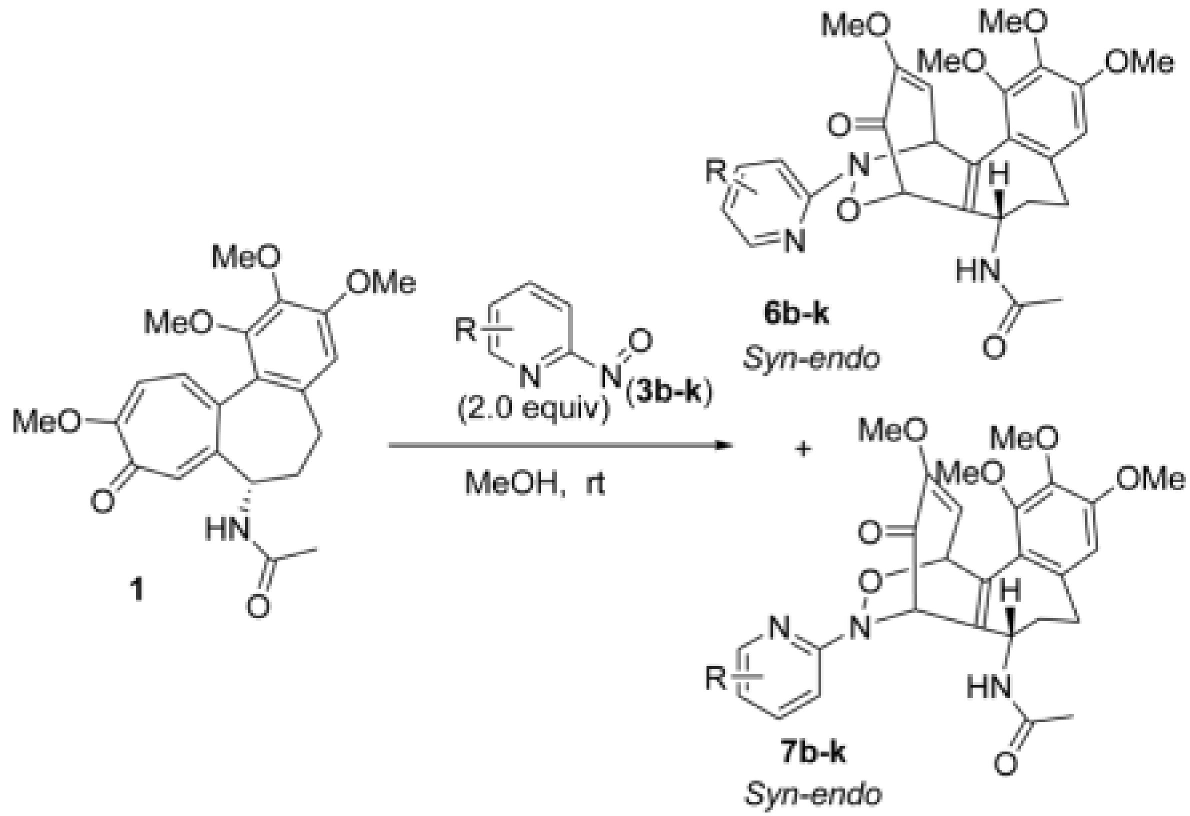
Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
4	**3e**		**6e**	–	62
5[b]	**3f**		**6f** + **7f**	7:1	75
6[b]	**3g**		**6g** + **7g**	7:1	78

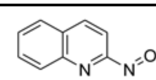
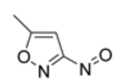


Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
7[b]	**3h**		**6h** + **7h**	6:1	74



Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
8[b]	**3i**		**6i** + **7i**	7:1	73



Entry		Nitroso	Product	Ratio[c]	Yield (%)[d]
9[b]	**3j**		**6j** + **7j**	8:1	72
10	**3k**		**6k** + **7k**	2:1	80

[a]Cycloadducts **6b** and **6d** were obtained pure after column chromatography, along with a mixure of two isomeric adducts (**6b** and **7b**, **6d** and **7d**), respectively.

[b]Cycloadducts were obtained as a mixture of two regioisomers after column chromatography.

[c]Determined by ^{1}H NMR of the crude reaction mixture.

[d] Isolated yield.

Table 3

Cytotoxic activity against PC-3 and MCF-7 cell line for colchicine nitroso adducts

Compds	IC_{50}, PC-3 (nM)	IC_{50}, MCF-7 (nM)
colchicine (1)	20	12
6a	25	20
6b	28	17
6d	14	10
6e	23	22
6f*	15.6	15
6g*	10	15.6
6h*	24	20
6i*	250	230
6k	250	190
7k	245	250

*Adducts **6f–i** were tested with a small amount of regio-isomeric adducts **7f–i** present (for ratios, see Table 2).