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Synthesis and Anticancer Activity of New Hydroxamic Acid Containing 1,4-Benzodiazepines

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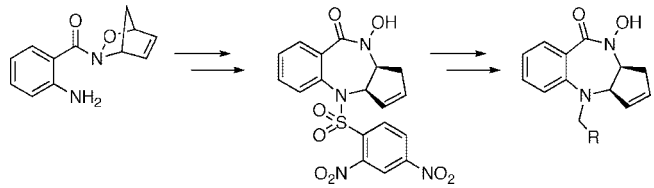
ABSTRACT



By employing an intramolecular Pd(0)-mediated ring opening of an acylnitroso-derived cycloadduct, new hydroxamic acid containing benzodiazepines have been synthesized and have demonstrated biological activity in MCF-7 and PC-3 tumor cell lines. Subsequent N−O bond reduction of the hydroxamate has provided access to amide analogues for SAR studies. During the course of our syntheses, an intermediate oxazoline *N*-oxide was isolated and gave insight into the mechanism of the key Pd(0)-mediated reaction.

1,4-Benzodiazepines are important biomolecules with a wide array of biological activities and therapeutic functions. Benzodiazepines are primarily known for their actions in the central nervous system. In addition to their established anxiolytic activites, 1,4-benzodiazepines also demonstrate activities as antibiotic,[1] antimalarial,[2] and anti-HIV[3] agents. Additionally, there have been several reports of benzodiazepines as anticancer agents,[4] including BMS-214662,[4a] a known farnesyltransferase (FTase) inhibitor, and Bz-423,[4b] which has antiproliferative effects through the regulation of the c-myc protein (Figure 1).

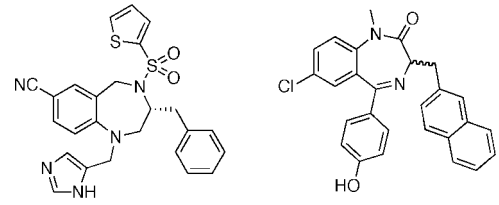


Figure 1. Benzodiazepines with anticancer activity.

Herein we report the synthesis of 1,4-benzodiazepines via a Pd(0)-mediated ring opening of cycloadduct **3a** and subsequent elaboration and evaluation of their anticancer activity in MCF-7 and PC-3 tumor cell lines. In contrast to the benzodiazepines disclosed in the literature, the benzodiazepines synthesized from cycloadduct **3a** contain a hydroxamate embedded within the core structure. This

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(2) Nallan, L.; Bauer, K. D.; Bendale, P.; Rivas, K.; Yokoyama, K.; Hornéy, C. P.; Pendyala, P. R.; Floyd, D.; Lombardo, L. J.; Williams, D. K.; Hamilton, A.; Sebti, S.; Windsor, W. T.; Weber, P. C.; Buckner, F. S.; Chakrabarti, D.; Gelb, M. H.; Van Voorhis, W. C. *J. Med. Chem.* **2005**, *48*, 3704.

(3) Kukla, M. J.; Breslin, H. J.; Diamond, C. J.; Grous, P. P.; Ho, C. Y.; Miranda, M.; Rodgers, J. D.; Sherrill, R. G.; De Clercq, E.; Pauwels, R.; Andries, K.; Moens, L. J.; Janssen, M. A. C.; Janssen, P. A. J. *J. Med. Chem.* **1991**, *34*, 3187.

(4) (a) Hunt, J. T.; Ding, C. Z.; Batorsky, R.; Bednarz, M.; Bhide, R.; Cho, Y.; Chong, S.; Chao, S.; Gullo-Brown, J.; Guo, P.; Kim, S. H.; Lee, F. Y. F.; Leftheris, K.; Miller, A.; Mitt, T.; Patel, M.; Penhallow, B. A.; Ricca, C.; Rose, W. C.; Schmidt, R.; Slusarchyk, W. A.; Vite, G.; Manne, V. *J. Med. Chem.* **2000**, *43*, 3587. (b) Sundberg, T. B.; Ney, G. M.; Subramanian, C.; Opipari, A. W.; Glick, G. D. *Cancer Res.* **2006**, *66*, 1775. (c) Dourlat, J.; Liu, W.; Greash, N.; Garbay, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2527.

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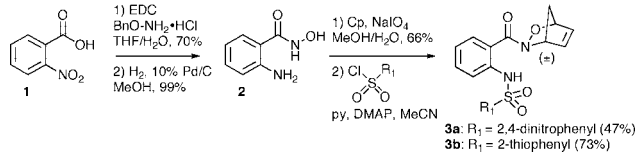
presents the possibility of direct metal binding with the benzodiazepine ring system and makes these compounds potential inhibitors of metalloenzymes such as the aforementioned farnesyltransferase protein.[5] Whereas BMS-214662 contains an imidazole group that coordinates to zinc(II) in the FTase active site,[6] our benzodiazepine derivatives may potentially bind to the active site of FTase through a monoanionic bidentate chelation of the hydroxamate to the zinc(II) ion.

Acylnitroso-derived hetero-Diels−Alder adducts, generated from the reaction of transient acylnitroso species with cyclopentadiene, are synthetically important precursors to a variety of bioactive molecules.[7] Pd(0)-mediated ring openings of acylnitroso-derived cycloadducts have been employed to provide *syn*-1,4-disubstituted cyclopentene derivatives.[8] Construction of the appropriately functionalized cycloadducts **3a** and **3b** began with commercially available 2-nitrobenzoic acid **1** (Scheme 1), which was coupled with *O*-benzyl

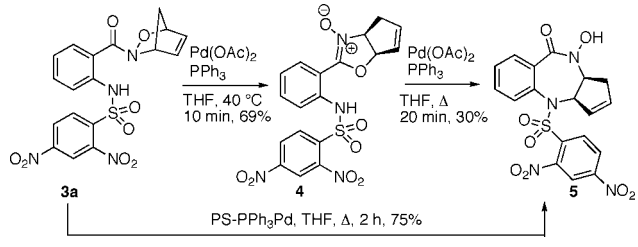
Scheme 1



hydroxylamine and subsequently deprotected and reduced under hydrogenolysis conditions to yield hydroxamic acid **2**. In situ oxidation of hydroxamic acid **2** in the presence of cyclopentadiene afforded the anthranilic acid-based cycloadduct, which was reacted with the appropriate sulfonyl chloride to give cycloadducts **3a** and **3b** in 47% and 73% yield, respectively.

Treatment of cycloadduct **3a** with $Pd(OAc)_2$ and PPh_3 at 40 °C for 10 min generated nitrone **4** (Scheme 2). The

Scheme 2



structure was confirmed by X-ray crystallographic analysis (Figure 2). To our knowledge, this is the first reported example of the formation of an oxazoline *N*-oxide from an acylnitroso-derived hetero-Diels−Alder adduct. Oxazoline *N*-oxides are versatile synthons and have been used in [3+2]

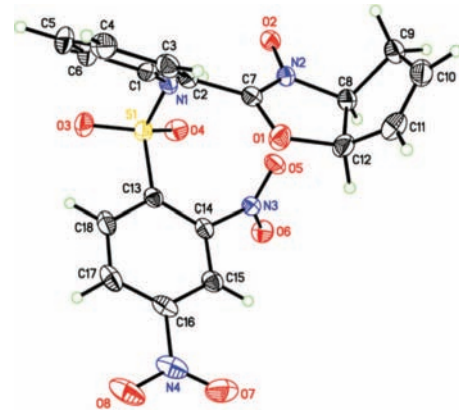


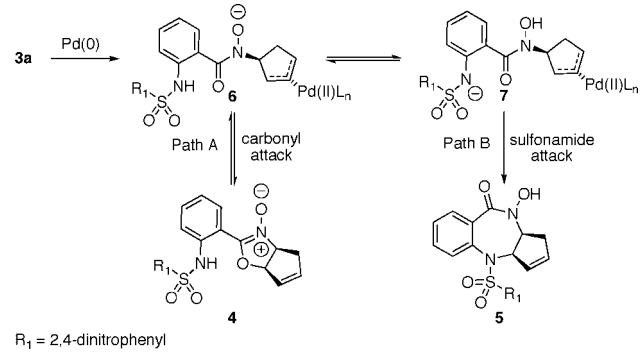
Figure 2. X-ray structure of **4** with thermal ellipsoids drawn at 50% probability.

cycloadditions.[9] After isolation and purification, resubjection of nitrone **4** to $Pd(OAc)_2$ and PPh_3 in refluxing THF gave benzodiazepine **5** in 30% yield.[10]

Treatment of cycloadduct **3a** with 3.5 mol % of polymer-bound triphenylphosphine−Pd(0) in refluxing THF for 2 h facilitated direct conversion to benzodiazepine **5** in an optimized 75% yield.[11] Several palladium reagents were explored and the polymer-bound triphenylphosphine−Pd(0) emerged as the most practical due to the ease of product isolation[12] and increased yield. Direct transformation of adduct **3a** to benzodiazepine **5** was ideal for further elaboration to biologically relevant molecules.

A plausible mechanism for the formation of benzodiazepine **5** is proposed in Scheme 3. The hydroxamate carbonyl

Scheme 3



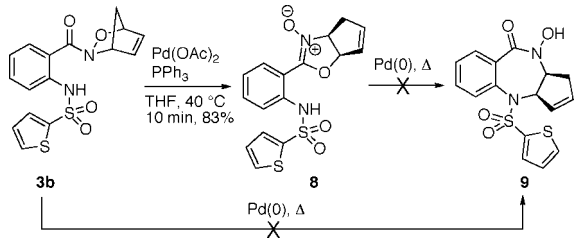
oxygen may act as a nucleophile and allow for reversible attack on π-allyl complex **6** to form nitrone **4**. Cyclization

(5) Bell, I. M. *J. Med. Chem.* **2004**, *47*, 1869.
(6) Reid, T. S.; Beese, L. S. *Biochemistry* **2004**, *43*, 6877.
(7) (a) Jiang, M. X.-W.; Warshakoon, N. C.; Miller, M. J. *J. Org. Chem.* **2005**, *70*, 2824. (b) Li, F.; Brogan, J. B.; Gage, J. L.; Zhang, D.; Miller, M. J. *J. Org. Chem.* **2004**, *69*, 4538.
(8) Mulvihill, M. J.; Surman, M. D.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 4874.
(9) Dirat, O.; Kouklovsky, C.; Langlois, Y. *Org. Lett.* **1999**, *1*, 753.
(10) Low yield due to difficulty with purification. The yield would be expected to be higher with polymer-bound Pd(0).

to the [5,5] system (path A) is faster than formation of the [7,5] system. Prolonged reaction times and/or heat would allow Pd(0) complexation with **4**, which would reintroduce the intermediate π-allyl complex **6**. Intramolecular proton transfer to complex **7** followed by irreversible nucleophilic attack (path B) of the sulfonamide nitrogen provides the thermodynamically preferred benzodiazepine **5**. Further evidence that this pathway could be operative was found when the dinitrobenzenesulfonamide group was replaced with a 2-thiophenesulfonamide.

Treatment of cycloadduct **3b** with $Pd(OAc)_2$ and PPh_3 at 40 °C for 10 min resulted in formation of nitrone **8** in 83% yield (Scheme 4). Prolonged heating of **3b** in the presence of various palladium reagents ($Pd(OAc)_2$, $Pd(dba)_2$, $Pd_2(dba)_3$•$CHCl_3$, PS-PPh_3Pd), phosphine ligands, solvents, and bases resulted in either no reaction or decomposition of the reaction mixture. Isolation and purification of nitrone **8** followed by reexposure to Pd(0) was also unsuccessful. Since the pK_a of the sulfonamide NH is significantly higher in cycloadduct **3b** (compared to **3a**), the essential proton transfer was expected to be less facile and the benzodiazepine would be unable to form. Still, formation of **9** was of particular interest considering its structural similarity to the known anticancer compound BMS-214662.[4a]

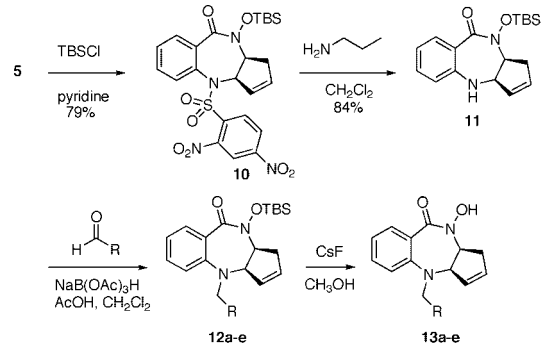
Scheme 4



Unable to convert cycloadduct **3b** directly to the benzodiazepine core, we turned our attention to an alternative multistep route toward functionalized benzodiazepines. Thus, benzodiazepine **5** was treated with TBSCl in pyridine to give protected hydroxamate **10** in good yield (Scheme 5). Upon protection of the hydroxamate, attention was turned to functionalization of the 4-amino position. Treatment of benzodiazepine **10** with excess *n*-propylamine at rt allowed for the mild deprotection of the dinitrobenzenesulfonamide to afford core **11**.[13] The resulting deep yellow dinitro-*N*-propylaniline byproduct was easily separated from **11** by column chromatography. Alternatively, mercaptoacetic acid was evaluated for the deprotection of the dinitrobenzenesulfonamide, but gave inconsistent results.[14]

To test the reactivity of the relatively hindered and electron deficient 4-amino position, benzodiazepine **11** was first

Scheme 5



entry	R	product	isolated yield (two steps)
1		**13a**	34%
2		**13b**	38%
3		**13c**	32%
4		**13d**	33%
5		**13e**	48%

treated with several different sulfonyl chlorides. Reaction with 2-thiophenesulfonyl chloride was first explored in an effort to prepare compound **9**. Unfortunately, compound **11** was completely unreactive with several sulfonyl chlorides, including 2-thiophenesulfonyl chloride, dinitrobenzene sulfonyl chloride, and tosyl chloride.[15] To derivatize **11**, we focused on reductive aminations, as aldehydes are unhindered and strongly electrophilic.

Benzodiazepine **11** was treated with various aldehydes in the presence of acetic acid and molecular sieves to form the corresponding iminium species, which were subsequently reduced with sodium triacetoxyborohydride to give protected benzodiazepines **12a**−**e**. Removal of the TBS group with CsF in MeOH and purification with iron-free silica gel[16] provided hydroxamate-containing benzodiazepines **13a**−**e** in 32−48% yield over two steps (Scheme 5).

An important functionality present in benzodiazepines **13a**−**e** is the hydroxamic acid. This group was anticipated to play a role in the biological activity of these compounds. Therefore, it was important to synthesize several analogues that do not contain the hydroxamate. Benzodiazepine **5** was deprotected with *n*-propylamine in 70% yield (Scheme 6). Benzodiazepine **14** was then subjected to stoichiometric titanocene chloride reduction conditions,[17] which produced

(11) Unoptimized yield with $Pd(OAc)_2/PPh_3/THF/\Delta$ was 56%. See: Surman, M. D.; Mulvihill, M. J.; Miller, M. J. *Org. Lett.* **2002**, *4*, 139.

(12) Simply filtering the resin and concentrating the filtrate afforded a yellow solid, which upon trituration with CH_2Cl_2 gave product **5** as a pure white powder.

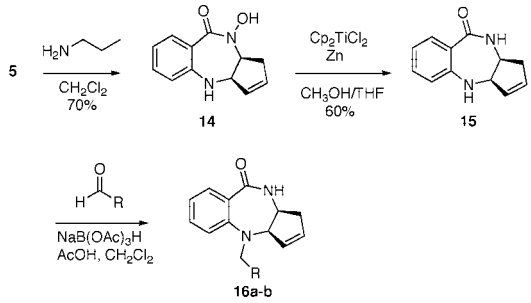
(13) Fukuyama, T.; Cheung, M.; Jow, C.; Hidai, U.; Kant, T. *Tetrahedron Lett.* **1997**, *38*, 5831.

(14) Decomposition of mercaptoacetic acid due to oxidation and occasional deprotection of TBS group proved problematic.

(15) Several bases, solvents, and temperatures were exhaustively explored.

(16) The procedure for the preparation of iron free silica gel may be found in the Supporting Information.

Scheme 6



entry	R	product	isolated yield
1		**16a**	67%
2		**16b**	60%

amide **15** in 60% yield. Benzodiazepine **15** proved more amenable to the same reductive amination conditions (NaBH(OAc)$_3$/AcOH) and analogues **16a**,**b** were synthesized in improved yield.

Several benzodiazepines demonstrated growth-inhibitory activity in MCF-7 (breast cancer) and PC-3 (prostate cancer) tumor cell assays (Table 1). Compounds **5**, **13a**, and **13b** reached the low micromolar range of inhibition against MCF-7 cells. In general, all compounds showed significantly improved inhibitory activity against the MCF-7 cell line. The substituent at the aniline nitrogen of the benzodiazepine seems to play a rather important role in determining activity. On the basis of the results of compounds **16a**,**b**, the hydroxamate has also been demonstrated to be a necessary functionality for activity in this class of benzodiazepines.

In summary, we have reported a new class of benzodiazepines that have shown encouraging biological activity in MCF-7 cell lines. Benzodiazepine **13b** has shown low micromolar activity and is a suitable lead for further SAR studies. The exact mechanism of action for this new class of 1,4-benzodiazepines is currently unknown. Enzyme assays and other means to determine the mechanism of action are under investigation.

(17) Cesario, C.; Tardibono, L.; Miller, M. J. *J. Org. Chem.* **2009**, *74*, 448.

Table 1. Results of Anticancer Screenings[a]

	% inhibition at 20 μM		IC_{50} (μM)	
compd	PC-3	MCF-7	PC-3	MCF-7
5	100	96	4	3
13a	32	92		8
13b	31	90		1.8
13c	<10	15		
13d	16	22		
13e	<10	39		
16a	11	29		
16b	<10	13		

[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: General methods, experimental details, and ^{1}H and ^{13}C NMR spectra for **3b**, **4**, **8**, **10**, **11**, **13a**−**e**, **14**, **15**, and **16a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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