doi:10.1246/cl.2010.1033
Published on the web August 21, 2010

An Effective Synthesis of a (Pyridin-3-yl)isoxazole via 1,3-Dipolar Cycloaddition Using $ZnCl_2$: Synthesis of a (2-Aminopyridin-3-yl)isoxazole Derivative and Its Antifungal Activity

Keigo Tanaka,* Satoshi Inoue, Norio Murai, Syuji Shirotori, Kazutaka Nakamoto, Shinya Abe, Takaaki Horii, Mamiko Miyazaki, Katsura Hata, Naoaki Watanabe, Makoto Asada, and Masayuki Matsukura
Discovery Research Laboratories, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635

(Received July 5, 2010; CL-100605; E-mail: k6-tanaka@hhc.eisai.co.jp)

We described a rare example of an effective isoxazole synthesis via a 1,3-dipolar cycloaddition using $ZnCl_2$, which allowed us to synthesize novel (2-aminopyridin-3-yl)isoxazole derivatives effectively. In addition, these compounds demonstrated potent antifungal activity in vitro against both *Candida albicans* and *Aspergillus fumigatus*.

Isoxazoles are important compounds in medicinal chemistry because of their pharmacological activity, and are widely used in clinical practice.[1] Numerous approaches to the synthesis of isoxazoles have been reported,[2] including 1,3-dipolar cycloaddition,[3] the cyclization of an ethynyloxime,[4] the reaction of hydroxylamine with an α,β-unsaturated carbonyl compound or a 1,3-dicarbonyl compound,[5] the reaction of *O*-methylhydroxylamine with an α,β-unsaturated carbonyl compound followed by electrophilic cyclization,[6] the reaction of cyclopropyl oximes in the presence of phosphorous oxychloride,[7] the reaction between a halogenated cyclopropane and a nitrosyl cation,[8] and the reaction of an oxime-derived dianion with an ester[9] or an amide.[10] Among these approaches, the 1,3-dipolar-cycloaddition reaction promises to be the most useful for exploratory synthesis in medicinal chemistry because the reaction is compatible with a wide range of functional groups,[11] and because divergent compound libraries can be synthesized in one step by combining a variety of alkynes and nitrile oxides.

In the category of 1,3-dipolar-cycloaddition reactions, there are many reports regarding the improvement in chemical yield by the addition of Lewis acids.[12] On the other hand, in the case of 1,3-dipolar cycloaddition yielding a 3,5-disubstituted isoxazole, there are only a few reports that indicate an improvement in chemical yield,[3] and the addition of a Lewis acid generally has little effect. The decrease in the reactivity of the nitrile oxide due to complexation with the Lewis acid is thought to be the major reason.[13] In the course of our research toward the development of novel antifungal agents, we found a rare example of an effective isoxazole synthesis via a 1,3-dipolar cycloaddition using $ZnCl_2$. In this letter, we describe the details of this reaction and its application to the development of novel antifungal agents.

(2-Aminopyridin-3-yl)isoxazoles **1** were selected as target compounds because they were expected to possess high antifungal potency and to be patentable because of their unique structure (Figure 1).[14]

Because both conventional methods for a 1,3-dipolar-cycloaddition reaction and the improved methods described above[3] did not work well for the synthesis of **1**, we endeavored to develop a more effective method to synthesize **1**.[15] Subsequently, we found that the addition of $ZnCl_2$ resulted in both a decreased amount of by-products and an increased amount of the target compound **1aa** (Table 1). These effects were reflected in a dramatic improvement in the chemical yield of **1aa** based on

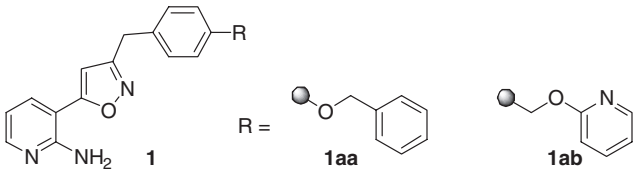
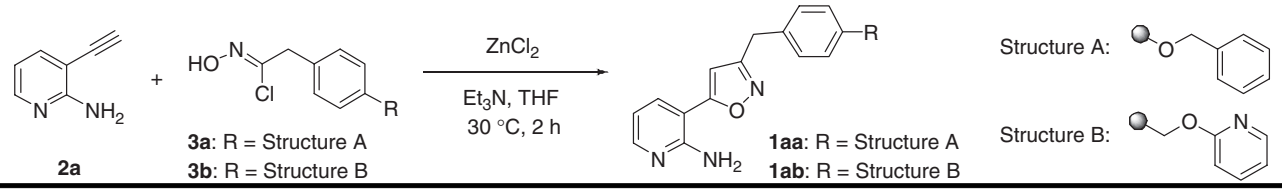


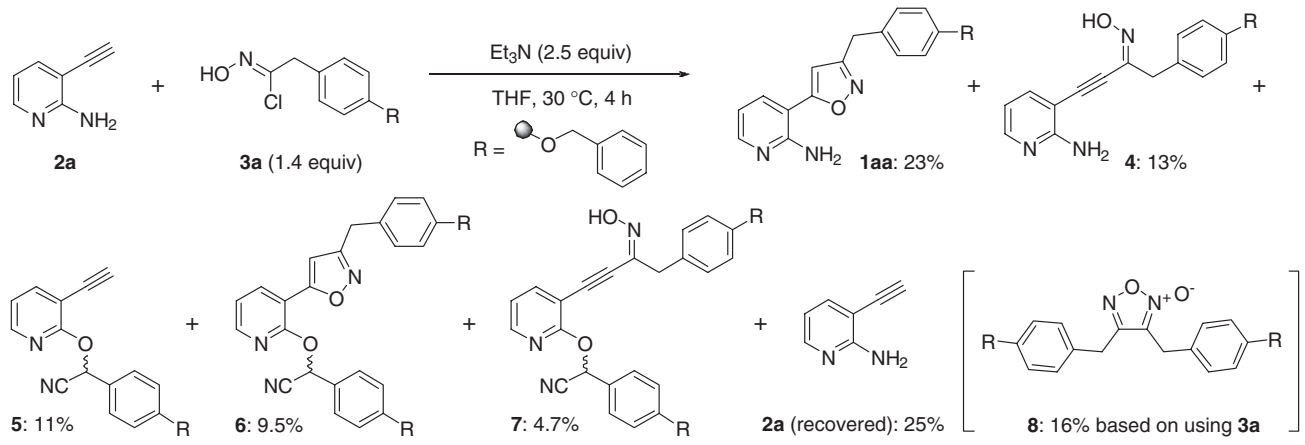
Figure 1. (2-Aminopyridin-3-yl)isoxazole derivatives **1**.

Table 1. Effect of $ZnCl_2$ on isoxazole synthesis using ethynylpyridine **2a** and hydroximoyl chloride **3**[16]



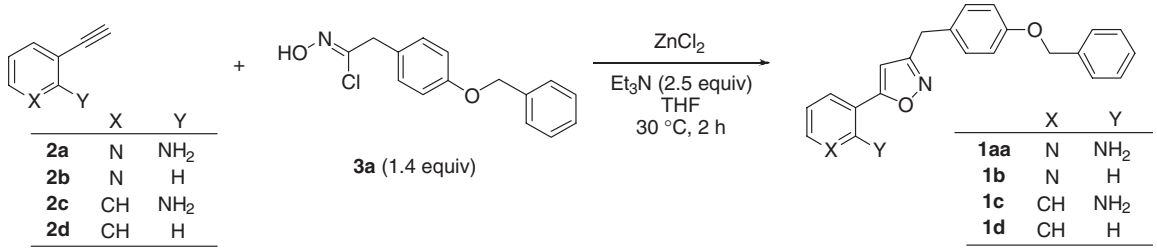
Entry	Hydroximoyl chloride (equiv)	$ZnCl_2$ (equiv)	Et_3N (equiv)	Product	Yield[a]/%		Yield of **1** based on recovered **2**[a]/%
					Product **1**	Recovery **2**	
1	**3a** (1.4)	None	2.5	**1aa**	14	45	25
2	**3a** (3.0)	None	3.5	**1aa**	24 (23[b])	29 (25[b])	34
3	**3a** (1.4)	0.5	2.5	**1aa**	22	46	41
4	**3a** (1.4)	1.4	2.5	**1aa**	36	54	78
5	**3a** (1.4)	2.0	2.5	**1aa**	42	55	93
6	**3a** (3.0)	2.0	3.5	**1aa**	59 (57[b])	33	88
7	**3b** (3.0)	2.0	3.5	**1ab**	66 (69[b,c])	25	88
8	**3b** (3.0)	3.0	3.5	**1ab**	63	28	88

[a]Determined by HPLC versus internal standard. [b]Isolated yield. [c]rt, 18 h.



Scheme 1. Isolated products in the isoxazole synthesis without $ZnCl_2$.

Table 2. Isoxazole synthesis using alkyne **2** and hydroximoyl chloride **3a** with or without $ZnCl_2$



	X	Y
2a	N	NH_2
2b	N	H
2c	CH	NH_2
2d	CH	H

	X	Y
1aa	N	NH_2
1b	N	H
1c	CH	NH_2
1d	CH	H

Entry	Alkyne	$ZnCl_2$ (equiv)	Product	Yield[a]/%		Yield of **1** based on recovered **2**[a]/%
				Product **1**	Recovery **2**	
1	**2a**	None	**1aa**	14	45	25
2	**2a**	2.0	**1aa**	42	55	93
3	**2b**	None	**1b**	45	33	67
4	**2b**	2.0	**1b**	62	29	87
5	**2c**	None	**1c**	30[b]	14[b]	35[b]
6	**2c**	2.0	**1c**	24[b]	36[b]	38[b]
7	**2d**	None	**1d**	30	29	42
8	**2d**	2.0	**1d**	28	33	42

[a]Determined by HPLC versus internal standard (Entries 1 and 2). Determined by NMR versus internal standard (Entries 3–8). [b]Isolated yield.

recovered starting material from 25% to 93% (Entries 1 and 5). The optimal amount of $ZnCl_2$ utilized was 2.0 equivalents (Entries 3–5, 7, and 8). Utilizing 3.0 equivalents of hydroximoyl chloride **3a** gave a better yield of **1aa** than 1.4 equivalents (Entry 6). The optimized reaction method also worked well for hydroximoyl chloride **3b** (Entry 7).

These reactions were analyzed in detail as shown below. In the case of the reaction without $ZnCl_2$, several products were obtained (Scheme 1).[17] Along with isoxazole **1aa** (23%) and recoverd **2a** (25%), ethynyloxime **4** (13%), cyanohydrin derivatives **5** (11%), **6** (9.5%), and **7** (4.7%) were isolated.[18] In addition, furazan **8** was obtained in 16% yield based on using **3a**.

Then ^1H NMR monitoring of these reactions in THF-d_8 with or without $ZnCl_2$ was conducted. Several bits of information were obtained as follows: 1) in the case of reaction with $ZnCl_2$ (2 equiv), only isoxazole **1aa** and furazan **8** could be detected as products and other products such as compounds **4**–**7** were not detected throughout the reaction; 2) immediately after Et_3N was added to the reaction mixture containing **2a**, **3a**, and $ZnCl_2$ (both 1 and 2 equiv) at 0 °C, **3a** was converted to the corresponding nitrile oxide which gradually disappeared at 30 °C concurrent with the production of **1aa** and **8**; 3) the proton on the terminal alkyne in **2a** was detected throughout the reaction with a reasonable mass balance, which indicated that Zn–acetylide was not formed in this reaction; 4) protons on the pyridine ring, the terminal alkyne, and the isoxazole ring in **1aa** and **2a** led to a significant low-field shift in the ^1H NMR by the addition of $ZnCl_2$ (both 1 and 2 equiv) compared with the $ZnCl_2$-free conditions; and 5) protons on the pyridine ring and the isoxazole ring in **1aa** caused a shift to a lower field by using 2 equivalents of $ZnCl_2$ compared with 1 equivalent of $ZnCl_2$, whereas the chemical shift of all protons on **2a** was almost the same in both cases.

Judging from these results, in the case of reaction without $ZnCl_2$, not only did 1,3-dipolar cycloaddition, but also nucleophilic attack of the terminal alkyne and primary amine on the nitrile oxide occurred. On the other hand, in the case of reaction

Table 3. Antifungal activities of isoxazoles **1**

Compound	MIC/μg mL^{-1} [a]	
	C. albicans[b]	*A. fumigatus*[c]
1aa	0.20	0.20
1ab	0.050	0.20
Fluconazole	0.39	>25
Amphotericin B	1.6	0.78

[a]Minimum inhibitory concentrations (MIC) were defined as the lowest concentrations at which a prominent decrease in turbidity could be observed visually compared to that in the control well. [b]CAF2-1 strain. [c]Tsukuba strain.

with $ZnCl_2$, the 1,3-dipolar cycloaddition was found to be preferable. One might conclude that the electron density of the 2-aminopyridine moiety and the terminal alkyne in **2a** became lowered by the chelation of the 2-aminopyridine with $ZnCl_2$, and which prevented the undesired nucleophilic addition reaction.

Then we studied the effects of substrates (Table 2). The addition of $ZnCl_2$ also worked well in the reaction using ethynylpyridine **2b** (Entries 3 and 4). On the other hand, in the case of ethynylaniline **2c** and ethynylbenzene **2d**, the addition of $ZnCl_2$ showed less impact on the reaction (Entries 5–8). These results indicate that the nitrogen atom on the pyridine ring in 2-aminopyridine **2a** might play an important role in the effective 1,3-dipolar cycloaddition using $ZnCl_2$.

We next evaluated the in vitro antifungal activity of (2-aminopyridin-3-yl)isoxazole derivatives **1** (Table 3). Isoxazoles **1aa** and **1ab** demonstrated more potent antifungal activity against both *Candida albicans* and *Aspergillus fumigatus* compared with fluconazole and amphotericin B which are commonly used antifungal agents in clinical practice. Studies on the development of (2-aminopyridin-3-yl)isoxazole derivatives **1** as antifungal agents are underway.

In summary, we synthesized novel (2-aminopyridin-3-yl)isoxazole derivatives **1** effectively via 1,3-dipolar cycloaddition using $ZnCl_2$. In addition, we demonstrated potent in vitro antifungal activity of **1** against both *Candida albicans* and *Aspergillus fumigatus*.

We thank Mr. Masaki Kato and Ms. Yumi Asai in the Analytical Research, Eisai Co., Ltd., for the confirmation of the chemical structure of by-products.

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15 Variables changed in optimization included: Lewis acids [Preferable; $ZnCl_2$ and $ZnBr_2$. Less effective; $CeCl_3$, ZnI_2, $MgBr_2$, $CuBr_2$, $BF_3{\cdot}OEt_2$, $Ti(O\textit{i}\text{-}Pr)_4$, $Yb(OTf)_3$, Et_2AlCl, $Cu(OTf)_2$, $Sc(OTf)_3$, $AlCl_3$, and $ZnMe_2$]; solvents [Preferable; THF and DME. Less effective; CH_3CN, *t*-BuOMe, *i*-PrOH, toluene, and CH_2Cl_2]; bases [Most preferable; Et_3N. Preferable; *i*-Pr_2NEt and *N*-methylmorpholine. Undesirable; *n*-BuLi. No addition of the base results in lower yield of product **1**].

16 Typical procedure of the $ZnCl_2$-mediated isoxazole synthesis using 2-amino-3-ethynylpyridine and a hydroximoyl chloride (Table 1): To a stirred solution of 2-amino-3-ethynylpyridine (0.17 mmol) in tetrahydrofuran (2 mL) were successively added hydroximoyl chloride **3a** (0.51 mmol), $ZnCl_2$ (0.34 mmol), and triethylamine (0.59 mmol) at 0 °C, then the reaction mixture was stirred for 2 h at 30 °C. To the reaction mixture was added aqueous ammonium chloride at room temperature, which was then extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate, followed by brine, and the solvent was evaporated under a reduced pressure. The residue was purified by NH silica gel column chromatography (ethyl acetate:heptane = 1:1) to obtain (2-aminopyridin-3-yl)isoxazole (**1a**).

17 The reaction mixture was purified by silica gel column chromatography directly without aqueous workup.

18 For a plausible reaction mechanism for the formation of cyanohydrin derivatives, see Supporting Information. Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

