# Syntheses of 2-Aryliminooxazolidine Derivatives as Trehalase Inhibitors

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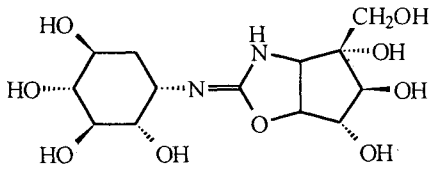
Trehalase (EC 3.2.1.28) is a very specific enzyme that hydrolyses trehalose to two glucose units[1] and is widely distributed in microorganisms, insects, plants and animals[2]. The substrate trehalose is a main source of glucose in insects and fungi. In insects, trehalose is a principal blood sugar and is used to support various energy-requiring functions[3]. In fungi, trehalose is reported to participate in germination of ascospores[4]. Therefore, the development of specific and potent trehalase inhibitors is of great interest for the control of insects and certain fungi.

Some trehalase inhibitors have been isolated from natural sources, such as deoxynojirimycin[5], salbostain[6], validamycins[7], validoxylamines[8] and trehazolin[9]. Among these natural products, trehazolin (**1**) is the most potent one. It exhibits strong antifungal activity toward plant pathogenic fungus, *Rhizoctonia solani*. In the course of screening for novel trehalase inhibitors, we have designed a new group of compounds (**2a**~**2g**) based on the structural model of trehazolin. These compounds have relatively simple structure and can be prepared easily.

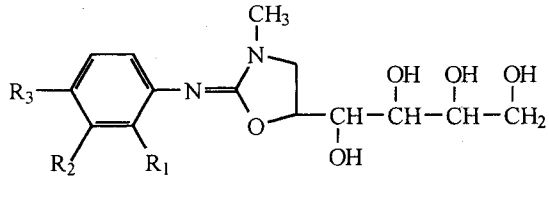
The designed compounds (**2a**~**2g**), 2-aryliminooxazolidine derivatives, were prepared according to the general procedure as shown in Scheme 1. The aryl isothiocyanates (**3a**~**3g**) were prepared according to the reported procedures[10,11]. Reaction of aryl isothiocyanates (**3a**~**3g**) with 1 equiv. of *N*-methyl-D-glucamine in ethanol for 8 hours at room temperature afforded the thioureas (**4a**~**4g**) in 85~95% yields. Treatment of **4a**~**4g** with excess of yellow HgO in acetone - ether (1 : 1) for 24 hours at room temperature resulted in the formation of oxazolidine ring to give 2-aryliminooxazolidine derivatives (**2a**~**2g**) in 70~90% yields (Table 1).

Compounds **2a**~**2g** were subjected to biological assay on inhibitory activity against porcine trehalase *in vitro* by the standard procedure[12] (Table 2). Compound **2a** showed

Fig. 1. Structure of trehazolin and 2-aryliminooxazolidine derivatives.



**1**



**2** **a** $R_1 = H, R_2 = H, R_3 = OH$
**b** $R_1 = F, R_2 = F, R_3 = F$
**c** $R_1 = H, R_2 = Cl, R_3 = F$
**d** $R_1 = H, R_2 = H, R_3 = F$
**e** $R_1 = F, R_2 = H, R_3 = H$
**f** $R_1 = F, R_2 = H, R_3 = F$
**g** $R_1 = H, R_2 = H, R_3 = H$

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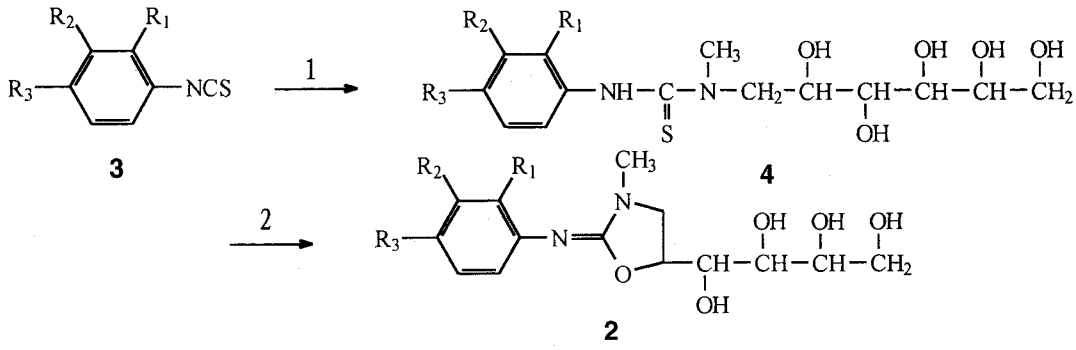
Scheme 1. Syntheses of 2-aryliminooxazolidine derivatives



Reagents and conditions: 1) N-methyl-D-glucamine, $C_2H_5OH$, rt, 8 hours, 85 ~ 95%; 2) HgO, $CH_3COCH_3$ : $C_2H_5OC_2H_5$ = 1 : 1, rt, 24 hours, 70 ~ 90%.

Table 1. Yield, IR and $^1H$ NMR data of 2-aryliminooxazolidine derivatives (**2a**~**2g**).

| Compound | Yield From **4** (%) | IR (KBr, $cm^{-1}$) | | $^1H$ NMR (500 MHz, δ in $D_2O$) | | | |
|---|---|---|---|---|---|---|---|
| | | OH | C=N | ArH | N-$CH_3$ | 5-H | Other H |
| **2a** | 73.3 | 3400 | 1670 | 6.92 (2H, d, J = 8.7 Hz)<br>6.77 (2H, d, J = 8.7 Hz) | 2.86 (3H, s) | 4.70 (1H, m) | 3.40 ~ 3.90 (7H, m) |
| **2b** | 82.7 | 3410 | 1670 | 6.90 (1H, m)<br>6.79 (1H, m) | 2.87 (3H,s) | 4.70 (1H, m) | 3.44 ~ 3.86 (7H, m) |
| **2c** | 87.5 | 3380 | 1680 | 7.16 (1H, dd, J = 6.7, 2.6 Hz)<br>7.10 (1H, t, J = 9.0 Hz)<br>6.93 (1H, m) | 2.91 (3H, s) | 4.74 (1H, m) | 3.51 ~ 3.93 (7H, m) |
| **2d** | 82.1 | 3300 | 1665 | 7.00 (2H, m)<br>6.99 (2H, m) | 2.87 (3H, s) | 4.70 (1H, m) | 3.40 ~ 3.90 (7H, m) |
| **2e** | 88.3 | 3360 | 1670 | 7.05 ~ 7.11 (4H, m) | 2.91 (3H, s) | 4.72 (1H, m) | 3.48 ~ 3.91 (7H, m) |
| **2f** | 91.4 | 3400 | 1690 | 6.96 (1H, td, J = 9.1, 6.3 Hz)<br>6.84 (1H, t, J = 10.7 Hz)<br>6.76 (1H, t, J = 9.1 Hz) | 2.80 (3H, s) | 4.62 (1H, m) | 3.37 ~ 3.81 (7H, m) |
| **2g** | 85.3 | 3330 | 1650 | 7.28 (2H, t, J = 7.8 Hz)<br>7.01 ~ 7.06 (3H, m) | 2.88 (3H, s) | 4.70 (1H, m) | 3.42 ~ 3.90 (7H, m) |

good inhibitory activity against porcine trehalase, compounds **2b** and **2c** showed moderate activity, compounds **2d**~**2f** showed weak activity, and compound **2g** showed no activity. It indicated that the inhibitory activity of the compound depended upon the nature and position of the substituent at the aryl moiety.

Compounds **2a**~**2g** were screened for their fungicidal activity by the spore germination method[13] against four typical pathogenic agricultural fungi, namely, *Rhizoctonia solani*, *Pyricularia oryzae*, *Gibberella zeae* and *Helminthosporium oryzae*. The effect of these compounds on the spore germination of plant pathogenic fungi was carried out at 100 ppm concentration at 25±2°C for 48 hours of incubation. Compound **2a** showed strong inhibitory effect against all the fungi and showed complete inhibitory effect (100%) against *Pyricularia oryzae* at 100 ppm. Compounds **2b** and **2c** also showed obvious activity against all the fungi. The fungicidal activity of

Table 2. Biological activities of 2-aryliminooxazolidine derivatives (**2a~g**).

| Compound | Trehalase inhibitory activity ($IC_{50}$, M) | Fungicidal activity (100 ppm, %) | | | |
|---|---|---|---|---|---|
| | | *Rhizoctonia solani* | *Pyricularia oryzae* | *Gibberella zeae* | *Helminthosporium oryzae* |
| **2a** | $4.29 \times 10^{-6}$ | 89.5 | 100 | 73.1 | 92.9 |
| **2b** | $6.67 \times 10^{-5}$ | 88.2 | 95.5 | 68.2 | 84.9 |
| **2c** | $8.15 \times 10^{-5}$ | 64.3 | 69.2 | 65.1 | 78.6 |
| **2d** | $1.74 \times 10^{-4}$ | 64.3 | 38.5 | 44.2 | 50.0 |
| **2e** | $2.07 \times 10^{-4}$ | 38.6 | 30.8 | 30.2 | 42.9 |
| **2f** | $8.54 \times 10^{-4}$ | 0 | 0 | 0 | 0 |
| **2g** | $> 10^{-3}$ | 7.9 | 0 | 0 | 0 |

compounds **2a~2g** was in accordance with their trehalase inhibitory activity.

In conclusion, the 2-aryliminooxazolidine derivatives (**2a~2g**) were synthesized from aryl isothiocyanates and showed obvious trehalase inhibitory activity *in vitro* and obvious fungicidal activity toward *Rhizoctonia solani*, *Pyricularia oryzae*, *Gibberella zeae* and *Helminthosporium oryzae*. Among these compounds, **2a** was proved to be the most potent one.

## References


1) CLIFFORD, K. H.: Stereochemistry of the hydrolysis of trehalose by the enzyme trehalase prepared from the flesh fly Sarcophaga barbarta. Eur. J. Biochem. 106: 337~340, 1980
2) ELBGIN, A. D.: The metabolism of $\alpha,\alpha$-trehalose. Adv. Carbohydr. Chem. Biochem. 30: 227~256, 1974
3) SACKTOR, B. S. & E. WORMSER-SHAVIT: Regulation of metabolism in working muscle *in vivo*. I. Concs. Of some glycolytic, tricarboxylic acid cycle, and amino acid intermediates in insect flight muscle during flight. J. Biol. Chem. 241: 634~638, 1966
4) THEVELEIN, J. M. & K. A. JONS: Reversibility characteristics of glucose-induced trehalase activation associated with the breaking of dormancy in yeast ascospores. Eur. J. Biochem. 136: 583~588, 1983
5) MURAO, S. & S. MIYATA: Isolation and characterization of a new trehalase inhibitors, S-GI. Agric. Biol. Chem. 44: 219~222, 1980
6) LASZLO, V.; F. HANS-WOLFRAM & S. ARNO: Salbostatin, a novel trehalase inhibiting metabolite from *Streptomyces albus* ATCC 21838. Angew. Chem. 106: 1936~1937, 1994
7) ASANO, N.; T. YAMAGUCHI, Y. KAMEDA & K. MATSUI: Effect of validamycins on glycohtdrolases of *Rhizoctonia solani*. J. Antibiotics 40: 526~529, 1987
8) KAMEDA, Y.; N. ASANO, T. YAMAGUCHI & K. MATSUI: Validoxylamine as trehalase inhibitors. J. Antibiotics 40: 563~565, 1987
9) ANDO, O.; H. SATAKE, K. ITOI, A. SATO, M. NAKAJIMA, S. TAKAHASHI, H. HARUYAMA, Y. OHKUMA, T. KINOSHITA & R. ENOKITA: Trehazolin, a new trehalase inhibitor. J. Antibiotics 44: 1165~1168, 1991
10) ZHIBIN, L.; Q. XUHONG, L. ZHI, L. ZHONG & S. GONGHUA: A facile synthesis of aryl isothiocyanates from arylamines. Org. Prep. Proced. Int. 32: 571~573, 2000
11) GE, L.; T. HIROKUNI & O. TAKAHITO: An improved procedure for the preparation of isothiocyanates from primary amines by using hydrogen peroxide as the dehydrosulfurization reagent. J. Org. Chem. 62: 4539~4540, 1997
12) KANZAKI, H.; T. NIIHAMA, A. KOBAYASHI & K. KAWAZU: An effective method of screening glucose-rich microbial culture filtrates for insect trehalase inhibitors. Biosci. Biotech. Biochem. 59: 398~400, 1995
13) CHOUBEY, A. K.; A. K. TRIPATHI & R. SINGH: Synthesis, antifungal and antibacterial activities of some new 2-benzylideneamino-5-arylimino-3-oxo-1,2,4-thiadiazolidines. Indian J. Chem. 37: 145~150, 1998