Synthesis and Quantitative Structure−Activity Relationships of Fluorine-Containing 4,4-Dihydroxylmethyl-2-aryliminooxazo(thiazo)lidines as Trehalase Inhibitors

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Five fluorine-containing 4,4-dihydroxylmethyl-2-aryliminooxazolidines and five 4,4-dihydroxylmethyl-2-aryliminothiazolidines were synthesized and evaluated for their inhibitory activity against trehalase in vitro. All these compounds were very readily synthesized compared with the natural trehalase inhibitors. They had moderate inhibitory activity toward trehalase, and showed larvicidal activity and inhibition action to insect flight. The steric parameters and semiempirical quantum parameters of these compounds were acquired by using the molecular modeling method and the PM3-SCF-MO method, respectively. A quantitative structure−activity relationship between half-inhibitory concentrations toward trehalase and the above parameters was established.

Keywords: *Fluorine-containing 4,4-dihydroxylmethyl-2-anilinooxazo(thiazo)lidines; trehalase inhibitors; insect flight; synthesis; QSAR*

INTRODUCTION

Trehalase (EC 3.2.1.28) is a very specific enzyme that hydrolyzes 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 insect flight and some 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 is the most potent one; it has strong inhibitory activity against silkworm trehalase in vitro (IC_{50} 4.9×10^{-8} M) and exhibits strong antifungal activity toward the plant pathogenic fungus *Rhizoctonia solani*. A notable feature of this inhibition is the formation of trehazolin−trehalase complex through the bridgehead nitrogen in trehazolin and the carbonyl group in trehalase (*10*, *11*), and the hydroxyl groups in trehazolin were topologically essential for the tight binding of trehazolin to the active sites of trehalase through hydrogen bonds (*12*, *13*).

Although trehazolin and its analogues have specific and strong inhibitory activity against trehalase in vitro, they do not show any insecticidal activity toward insects in vivo. The reason is that they cannot penetrate insect skin and reach the trehalase target because of too many hydrophilic hydroxyl groups in its structure. In addition, the structures of trehazolin and its analogues are complicated and their synthesis is very difficult. Although many minor modifications on the structure of trehazolin have been approached, so far there have been no reports of any artificial synthetic mimics of trehalase inhibitor, which have relatively simple structures and can be prepared easily. As we are interested in the insect-behavior regulator, the artificial synthetic trehalase inhibitor becomes our target. It is known that the nonsteroid and aromatic RH-5849 with hydrophobicity, which is used as an insect-growth regulator, mimics the action of steroid 20-HE with polyhydroxy group and hydrophilicity (*14*). Inspired by this discovery, we designed and synthesized a new group of compounds (**1**−**10**) on the basis of the action mechanism of trehazolin (*10*−*13*) and its structural model (having a N=C−NH unit, heterocyclic moiety, and polyhydroxyl groups). We have tried to increase the hydrophobicity and penetrating ability by introducing a benzene ring and fluorine atoms. Of course, the fluorine atoms also have the possibility of forming hydrogen bonds with the active site of trehalase. It was expected that these small molecules might show some insect-flight inhibition and larvicidal activities in vivo.

The semiempirical quantum chemical method and molecular modeling were also used to study the structure−activity relationship of the synthesized compounds against trehalase in vitro. The formulas, value of IC_{50}, and relevant variants of all synthesized compounds are listed in Table 1. Scheme 1 shows the structures of trehazolin and compounds **1**−**10**.

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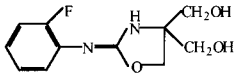
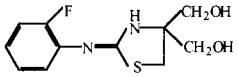
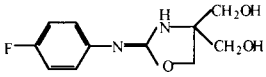
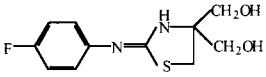
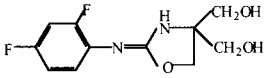
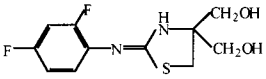
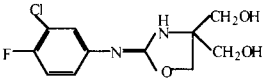
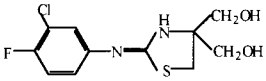
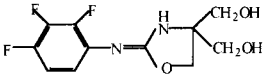
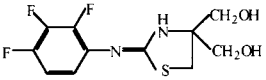
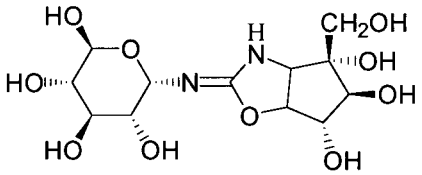
Table 1. Structure Formulas, Molecular Parameters, and Experimental and Calculated Trehalase Inhibitory Activities of Compounds 1−10

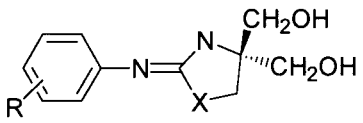
Compound	Structure Formula	Descriptor Variable				pIC_{50}	
		d_2 (Å)	V_1/V_2	D (Db)	Q_N	Exptl	Calcd[a]
1		4.550	3.212	3.633	0.186	4.21	4.05
2		4.554	3.624	2.995	0.163	3.08	2.97
3		4.547	2.970	1.193	0.154	3.58	3.62
4		4.551	3.281	0.603	0.181	3.24	2.77
5		4.553	3.142	1.844	0.158	3.05	3.53
6		4.553	3.552	1.302	0.155	2.21	2.48
7		5.092	3.353	1.041	0.140	4.45	4.50
8		5.098	3.703	0.509	0.138	3.62	3.60
9		4.773	3.379	2.031	0.155	4.07	3.81
10		4.768	3.626	2.203	0.153	3.15	3.34

[a] Calculated by eq 1.

Scheme 1. Structures of Trehazolin and Compounds 1−10



Trehazolin



X=O, R= **1**: 2-F; **3**: 4-F; **5**: 2,4-F_2; **7**: 3-Cl, 4-F; **9**: 2,3,4-F_3.
X=S, R= **2**: 2-F; **4**: 4-F; **6**: 2,4-F_2; **8**: 3-Cl, 4-F; **10**: 2,3,4-F_3.

MATERIALS AND METHODS

Instrumentation and Chemicals. All melting points (mp) were obtained with an electrothermal digital apparatus made in Shanghai and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WP-500SY (500 MHz) spectrometer with CD_3COCD_3 as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Infrared spectra were measured on a Nicolet FT-IR-20SX instrument using a potassium bromide (KBr) disk, scanning from 625 to 4000 cm^{-1}. Mass spectra were recorded under electron-impact (70 eV) conditions using a Hitachi M80 instrument. Combustion analyses for elemental composition were made with an Italian MOD 1106 analyzer. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F_{254}), and spots were visualized with ultraviolet (UV) light. All chemicals or reagents were purchased from standard commercial suppliers.

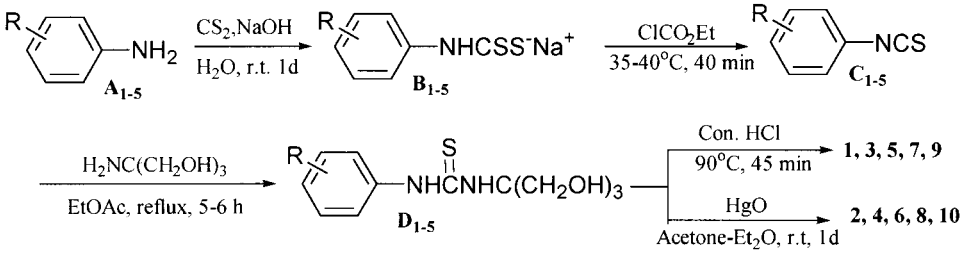
General Synthetic Procedure for Arylisothiocyanates (C_1−C_5). Scheme 2 illustrates the general synthesis route for the studied compounds and their intermediates. To a stirred mixture of carbon disulfide (45.6 g, 0.60 mol), sodium hydroxide (16.0 g, 0.40 mol), and water (80 mL) at 2−5 °C, was added dropwise the amine A_1−A_5 (0.40 mol) over a period of 30 min. After the mixture was stirred at 40−45 °C for 24 h, the lower layer of carbon disulfide was removed, and the upper aqueous layer containing intermediate B_1−B_5 was washed with benzene (3 × 100 mL). To this aqueous solution was added dropwise ethyl chloroformate (0.4 mol) at 35−40 °C, and the resulting mixture was stirred for about 40 min at the same temperature. Then the lower organic phase was separated, washed with water (3 × 50 mL), dried over anhydrous magnesium sulfate, and distilled in vacuo to give pure C_1−C_5.

Data for C_1. 41.62 g, yield 68%; obtained as colorless liquid; bp 103−104 °C /8 Torr. R_f = 0.83 (petroleum ether). IR (KBr, cm^{-1}) υ_{max} 2033, 1601, 1598. Anal. Calcd. (%) for C_7H_4FNS: C, 54.89; H, 2.63; N, 9.14. Found: C, 54.71; H, 2.64; N, 9.20. *m/z* 153 (M^+).

Data for C_2. 44.06 g, yield 72%; obtained as colorless liquid; bp 95−97 °C /8 Torr. R_f = 0.85 (petroleum ether). IR (KBr, cm^{-1}) υ_{max} 2040, 1590, 1504. *m/z* 153 (M^+).

Data for C_3. 60.88 g, yield 89%; obtained as colorless liquid; bp 85−86 °C /8 Torr. R_f = 0.86 (petroleum ether). IR (KBr, cm^{-1}) υ_{max} 2049, 1602, 1501. Anal. Calcd. (%) for C_7H_4FNS: C, 49.12; H, 1.77; N, 8.18. Found: C, 49.22; H, 1.76; N, 8.19. *m/z* 171 (M^+).

Scheme 2. General Synthetic Route for Fluorine-Containing 4,4-Dihydroxylmethyl-2-anilinooxazolines and Thiazolines (1−10)



A_1, B_1, C_1, D_1: R=2-F; **A_2, B_2, C_2, D_2**: R= 4-F; **A_3, B_3, C_3, D_3**: R=2,4-F_2;

A_4, B_4, C_4, D_4: R= 3-Cl, 4-F; **A_5, B_5, C_5, D_5**: R=2,3,4-F_3;

Data for ***C₄***. 61.50 g, yield 82%; obtained as colorless liquid; bp 110−112 °C /8 Torr. R_f = 0.80 (petroleum ether). IR (KBr, cm^{-1}) υ_{max} 2000, 1580, 1480. *m/z* 187 (M^+), 189 ($M+2^+$).

Data for ***C₅***. 65.02 g, yield 86%; obtained as colorless liquid; bp 80−82 °C /8 Torr. R_f = 0.85 (petroleum ether). IR (KBr, cm^{-1}) υ_{max} 2018, 1500, 1490. *m/z* 189 (M^+).

General Synthetic Procedure for Fluorine-Substituted *N*-Phenyl-*N′*-tri(hydroxylmethyl) methylthioureas (D_1−D_5). A mixture of the corresponding arylisothiocyanate **(C_1−C_5)** (0.02 mol), tri (hydroxylmethyl)methylamine (2.662 g, 0.022 mol), and ethyl acetate (30 mL) was refluxed for 12 h. After the mixture cooled, it was concentrated under reduced pressure to give the crude products, which were dissolved in water (20 mL), stirred for 30 min, extracted with ethyl acetate (3 × 20 mL), and dried over anhydrous magnesium sulfate. The resulting colorless liquid was concentrated under reduced pressure to give the desired intermediates **(D_1−D_5)** for the following reaction.

Data for ***D₁***. 3.67 g, yield 67%; obtained as light yellow oily product. R_f = 0.40 (ethyl acetate).

Data for ***D₂***. 3.12 g, yield 57%; obtained as yellow sticky solid. R_f = 0.45 (ethyl acetate).

Data for ***D₃***. 3.39 g, yield 58%; obtained as light yellow oily product. R_f = 0.40 (ethyl acetate).

Data for ***D₄***. 2.21 g, yield 59%; obtained as yellow sticky solid. R_f = 0.40 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3250, 3050, 2880, 1600, 1530, 1500, 1450, 1050, 870, 820, 770. ^{1}H NMR (CD_3COCD_3) δ 7.93 (m, 1H, ArH), 7.46 (m, 1H, ArH), 7.27 (t, J = 9.0 Hz, 1H, ArH), 3.85 (s, 6H, CH_2OH).

Data for ***D₅***. 1.78 g, yield 47%; obtained as light yellow oily product. R_f = 0.40 (ethyl acetate).

General Synthetic Procedure for Fluorine-Containing 4,4-Dihydroxylmethyl-2-anilino thiazolines (2, 4, 6, 8, 10). The fluorine-substituted *N*-phenyl-*N*-tri(hydroxylmethyl)-methylthiourea **(D_1−D_5)** (0.01 mol) was dissolved in concentrated HCl (10 mL). The reaction mixture obtained was stirred for 45 min at 90 °C. After cooling to room temperature, the mixture was neutralized by using NaOH (10 N) in an ice bath. The resulting precipitate was filtered and recrystallized from absolute ethanol to give the desired products (**2**, **4**, **6**, **8**, and **10**).

Compound ***2***. 1.39 g, yield 57%; obtained as white powdery crystals; mp 171−172 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3150, 2920, 2880, 1630, 1605, 1495, 1230. ^{1}H NMR (CD_3COCD_3) δ 7.36 (m, 1H, ArH), 7.05−7.09 (m, 2H, ArH), 7.00 (m, 1H, ArH), 3.73 and 3.65 (ABq, J = 10.9 Hz, 4H, CH_2-OH), 3.28 (s, 2H, SCH_2). Anal. Calcd. (%) for $C_{11}H_{13}FN_2O_2S$: C, 51.56; H, 5.08; N, 10.94. Found: C, 51.42; H, 4.95; N, 10.93.

Compound ***4***. 1.36 g, yield 56%; obtained as white powdery crystals; mp 202−203 °C. R_f = 0.40 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3300, 3100, 2880, 1620, 1500, 1200, 1050, 840. ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 2H, ArH), 7.00 (t, J = 8.9 Hz, 2H, ArH), 3.69 and 3.66 (ABq, J = 11.0 Hz, 4H, CH_2OH), 3.29 (s, 2H, SCH_2). Anal. Calcd. (%) for $C_{11}H_{13}FN_2O_2S$: C, 51.56; H, 5.08; N, 10.94. Found: C, 51.34; H, 4.97; N, 11.09.

Compound ***6***. 1.12 g, yield 43%; obtained as white powdery crystals; mp 207−208 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3280, 3100, 2880, 1640, 1500, 1240, 1050, 850, 820, 720. ^{1}H NMR (CD_3COCD_3) δ 7.36 (m, 1H, ArH), 6.99 (t, J = 9.7 Hz, 1H, ArH), 6.88 (t, J = 8.5 Hz, 1H, ArH), 3.73 and 3.67 (ABq, J = 11.0 Hz, 4H, CH_2OH), 3.28 (s, 2H, SCH_2). Anal. Calcd. (%) for $C_{11}H_{12}F_2N_2O_2S$: C, 48.18; H, 4.38; N, 10.22. Found: C, 48.35; H, 4.33; N, 10.07.

Compound ***8***. 1.28 g, yield 46%; obtained as white powdery crystals; mp 195−196 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3280, 3100, 2880, 1640, 1495, 1195, 1050, 870, 820, 770. ^{1}H NMR (CD_3COCD_3) δ 7.26−7.40 (m, 2H, ArH), 7.17 (t, J = 9.0 Hz, 1H, ArH), 3.71 and 3.67 (ABq, J = 10.9 Hz, 4H, CH_2OH), 3.32 (s, 2H, SCH_2). Anal. Calcd. (%) for $C_{11}H_{12}$-$ClFN_2O_2S$: C, 45.44; H, 4.13; N, 9.64. Found: C, 45.21; H, 4.28; N, 9.49.

Compound ***10***. 1.17 g, yield 42%; obtained as white powdery crystals; mp 202−204 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3280, 3100, 2950, 2880, 1630, 1505, 1220, 1045, 815. ^{1}H NMR (D_2O) δ 7.36 (m, 1H, ArH), 7.05 (m, 1H, ArH), 3.75 and 3.67 (ABq, J = 10.9 Hz, 4H, CH_2OH), 3.30 (s, 2H, SCH_2). Anal. Calcd. (%) for $C_{11}H_{11}F_3N_2O_2S$: C, 45.21; H, 3.77; N, 9.59. Found: C, 45.19; H, 3.66; N, 9.67.

General Synthetic Procedure for Fluorine-Containing 4,4-Dihydroxylmethyl-2-anilino oxazolines (1, 3, 5, 7, 9). The fluorine-substituted *N*-phenyl-*N*-tri(hydroxylmethyl)-methylthiourea **(D_1−D_5)** (0.01 mol) was dissolved in CH_3-$COCH_3$/Et_2O (1:6, v/v) (30 mL). To the reaction mixture was added in batch yellow HgO (15 g, 0.07 mol). After 24 h, the resulting mixture was filtered through silicon gel, and the filtrate was concentrated under reduced pressure to give the crude products, which were purified on TLC plates to give the desired products (**1**, **3**, **5**, **7**, and **9**).

Compound ***1***. 1.19 g, yield 49%; obtained as white powdery solid; mp 156−157 °C. R_f = 0.40 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3150, 2920, 2880, 1670, 1605, 1495, 1250, 1050, 745. ^{1}H NMR (CD_3COCD_3) δ 7.60 (m, 1H, ArH), 7.02−7.08 (m, 2H, ArH), 6.93−6.97 (m, 1H, ArH), 4.29 (s, 2H, OCH_2), 3.64 (s, 4H, CH_2OH). Anal. Calcd. (%) for $C_{11}H_{13}FN_2O_3$: C, 55.00; H, 5.42; N, 11.67. Found: C, 55.06; H, 5.33; N, 11.63.

Compound ***3***. 1.31 g, yield 54%; obtained as white powdery solid; mp 135−136 °C. R_f = 0.40 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3220, 3100, 2930, 2880, 1650, 1600, 1510, 1220, 1050, 840. ^{1}H NMR (CD_3COCD_3) δ 7.57 (dd, J = 8.3 and 4.7 Hz, 2H, ArH), 7.00 (m, 2H, ArH), 4.23 (s, 2H, OCH_2), 3.64 and 3.62 (ABq, J = 11.0 Hz, 4H, CH_2OH). Anal. Calcd. (%) for $C_{11}H_{13}FN_2O_3$: C, 55.00; H, 5.42; N, 11.67. Found: C, 55.16; H, 5.37; N, 11.58.

Compound ***5***. 1.36 g, yield 52%; obtained as white powdery solid; mp 147−148 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3300, 3070, 2890, 1710, 1600, 1510, 1255, 1050, 840, 820, 800. ^{1}H NMR (CD_3COCD_3) δ 7.44 (m, 1H, ArH), 6.92 (t, J = 9.9 Hz, 1H, ArH), 6.88 (t, J = 8.6 Hz, 1H, ArH), 4.30 (s, 2H, OCH_2), 3.66 and 3.60 (ABq, J = 11.1 Hz, 4H, CH_2OH). Anal. Calcd. (%) for $C_{11}H_{12}F_2N_2O_3$: C, 51.16; H, 4.65; N, 10.85. Found: C, 51.23; H, 4.44; N, 10.79.

Compound ***7***. 1.08 g, yield 39%; obtained as white powdery solid; mp 150−151 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3250, 3100, 2930, 2840, 1650, 1610, 1505, 1260, 1020, 860, 820, 790. ^{1}H NMR (CD_3COCD_3) δ 7.80 (m, 1H, ArH), 7.37 (m,

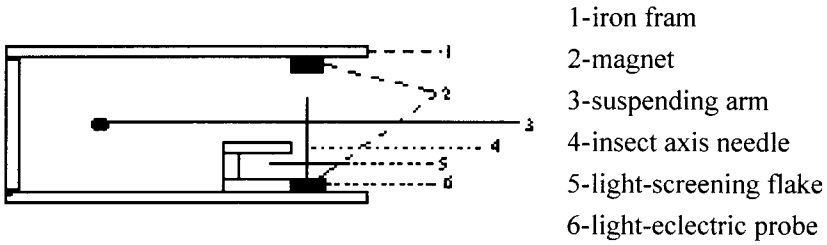


Figure 1. Flight mill for determining the flight ability of wild fruitfly.

1H, ArH), 7.17 (t, J = 9.0 Hz, 1H, ArH), 4.27 (s, 2H, OCH_2), 3.61 (s, 4H, CH_2OH). Anal. Calcd. (%) for $C_{11}H_{12}ClFN_2O_3$: C, 48.09; H, 4.37; N, 10.20. Found: C, 47.93; H, 4.52; N, 10.33.

Compound **9**. 1.28 g, yield 46%; obtained as white powdery solid; mp 50−52 °C. R_f = 0.35 (ethyl acetate). IR (KBr, cm^{-1}) υ_{max} 3300, 3080, 2950, 2900, 1700, 1510, 1270, 1050, 820. ^1H NMR (CD_3COCD_3) δ 7.36 (m, 1H, ArH), 6.98 (m, 1H, ArH), 4.52 (s, 2H, OCH_2), 3.69 and 3.65 (ABq, J = 11.4 Hz, 4H, CH_2-OH). Anal. Calcd. (%) for $C_{11}H_{11}F_3N_2O_3$: C, 47.83; H, 3.99; N, 10.14. Found: C, 47.66; H, 3.71; N, 10.38.

Bioassays. *Trehalase Inhibitory Activity In Vitro.* The experiment was made according to the standard method (*15*) described as follows: To a solution of 1.08 μg of porcine trehalase (Sigma) in 20 μL of 50 nM phosphate buffer (pH 5.5) was added 20 μL of DMSO containing the compound, then the mixture was incubated at 37 °C for 15 min. To the mixture was added 80 μL of treahalose solution (3 nM in the same buffer). After the reaction mixture was incubated at 37 °C for 1.5 h, 0.5 mL of 1.8% $Ba(OH)_2$ and 0.5 mL of 2% $Zn(OH)_2$ were added, and then the mixture was centrifuged for 5 min. To the resulting supernatant solution containing glucose was added 1 mL of the 124036 color-producing reagent containing 10 units of glucose oxidase from *Aspergillus spp.*, 0.8 units of peroxidase from *horseradish,* and 1 mg of ABTS. After the mixture was left standing at room temperature for 30 min, the absorbance at 660 nm was measured, and the amount of glucose in the solution formed was determined on the basis of the standard glucose-absorbance curve. The inhibition of trehalase was calculated by the following equation: inhibition of trehalase (%) = $[1 - A/B] \times 100$, where A and B indicate the amount of glucose in the solution with and without compound, respectively. The concentration is defined as mole of compound per liter of solution. Trehalase inhibitory activity data of the synthesized compounds were taken as the dependent variable and were expressed as pIC_{50} (i.e., $-\log IC_{50}$, M^{-1}). For each compound, at least 5 different concentrations needed to be tested to make the result reliable. These data of pIC_{50} are presented in Table 1.

Larvicidal Activity. Compounds **1**−**10** were screened for their larvicidal activity toward the wild fruitfly *Drosophila melanogaster*. The test method was similar to that described previously (*16*). One hundred *Drosophila* eggs were placed in a 25-cm^3 bottle containing an 8-cm^3 solution of the egg culture media (a standard yeast, corn meal, and agar mixture) and the compound (in a dose of 200 ppm) to be screened (dissolved in DMSO). After 10−14 days the adults would appear and be counted. The larvicidal activity data in Table 2 are presented as total percentage kill, which is the difference between the number of eggs placed on the media and the number of living adults.

Flight-Inhibition Activities. Compounds **1**−**10** were also tested for their flight-inhibition activity toward the adult of wild fruitfly *Drosophila melanogaster*. The fruitflies were fed with corn culture media containing compound for 48 h, then their flight ability was tested on a computer-monitored flight mill system (*17*) as follows: The flight tests were measured at 25 °C under natural light. The wild fruitfly was anesthetized with ether for 2 min and then adhered with glue, on its abdomen, to the termini of the suspension arm of the flight mill (Figure 1). After approx. 2−3 min, the wild fruitfly would begin to fly. The flight time, flight distance, and flight speed were recorded on a computer. The percentage inhibition of flight was calculated by using the following equation: inhibition of flight (%) = $[1 - C/D] \times 100$, where C indicates the flight distance of the wild fruitfly fed with the test compound, and D indicates the flight distance of the normal wild fruitfly. All these test results are listed in Table 2.

Table 2. Larvicidal and Flight-Inhibition Activities of Compounds 1−10

compound	larvicidal activity[a] (200 ppm, %)	flight inhibition[a,b] (200 ppm, %)
1	45.2	30.7
2	66.5	45.4
3	56.3	35.7
4	60.4	37.2
5	17.2	2.7
6	35.1	1.7
7	65.0	37.2
8	76.0	49.7
9	50.4	16.6
10	65.3	26.9

[a] Determined in 95% confidence. [b] Against adult of wild fly.

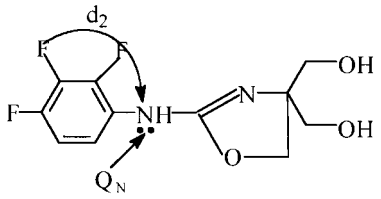


Figure 2. Demonstration of the defined length d_2 (e.g., compound **9**).

Descriptor Variables. The molecular shape parameter V_1/V_2, where V_1 and V_2 stand for the nonpolar surface area and polar surface area (Å^2) of water solvation shell, respectively, and the defined length d_2 (Figure 2) were calculated by using a molecular modeling program, PCMODEL (4th ed., June 1990) of Serena Software (Burlingame, CA). Before the above parameters of a compound were calculated, its spatial molecular conformation was optimized with PCMODEL to acquire its most relaxed conformation. For electronic parameters, the dipole of the compound and the net charge on N atom (Q_N) (Figure 2) were calculated by using the PM3-SCF-MO method with Hyperchem Software (1993 ed.) of Hypercube, Inc. (Gainesville, FL). Also, the molecular total energy (E_t), the energy of the lowest unoccupied molecular orbital (E_{LUMO}), and the energy of the highest occupied molecular orbital (E_{HOMO}) were calculated. However, the latter three parameters were not listed because they gave poor correlation with pIC_{50}. Before electronic parameters of a compound were calculated, its spatial molecular conformation was also optimized with Hyperchem to acquire its most relaxed conformation. All descriptor data are listed in Table 1.

Multiple Linear Regression (MLR) Analysis. Regression analysis was done with the computer software package Origin (version 5.0) of Microcal Software, Inc. (Northampton, MA). The regression was done in a stepwise manner, with the parameter minimizing the sum of squared deviations being introduced step-by-step and the analysis being stopped when the introduction of the new parameter was no longer statistically significant as evidenced by F test.

RESULTS AND DISCUSSION

Synthesis. Arylisothiocyanates were synthesized from arylamines according to the newly reported method (*18*): they have a typical strong N=C=S absorption peak at $\upsilon_{max} = 2040\ cm^{-1}$. Both thiazoline and oxazoline were synthesized from the same intermediate thiourea, which could be readily synthesized by the reaction of the corresponding arylisothiocyanate with the relevant primary amine. When thiourea was treated with concentrated HCl, the product proved to be thiazoline. When treated with yellow HgO, oxazoline turned out to be the end product. In the infrared spectra of the intermediate thiourea, the typical absorption peak at 2040 cm^{-1} disappears, and instead, some new typical peaks appear at $\upsilon_{max} = 1130$ and 1530 cm^{-1}, representing the absorption by C=S and N−H. The final product thiazoline or oxazoline has another two characteristic absorption peaks at 1620 cm^{-1} and 1200−1250 cm^{-1}, representing the functional group C=N and =C−O(S)−C−, respectively. In comparison with the infrared spectra of thiourea, the absorption at 1130 cm^{-1} disappears, which explains that the functional group C=S is concerned in the formation of thiazoline and oxazoline. All thiazolines and oxazolines were recrystallized and obtained in a very pure form for the biological assays.

Biological Activity. The experiments in vitro (Table 1) showed that compounds **1**−**10** had moderate trehalase inhibitory activity around pIC_{50} 2.21−4.45; and compound **7** had the highest bioactivity. For oxazolines and thiazolines, the compounds with 2,4-F_2 have lower inhibition activity to trehalase in vitro, but those with 3-Cl, 4-F groups showed higher activity. And for all cases, the inhibition activities of oxazolines are higher than those of thiazolines. Although their bioactivities were slightly lower than those of natural trehazolin analogues, they were structurally simple and easily prepared; at least providing an alternative approach to artificial trehalase inhibitor.

As the above compounds with some hydrophobicity have moderate inhibition activities to trehalase in vitro, we hoped that they would have the ability to affect the metabolism process of blood sugar of insects in vivo to show interesting bioactivity. Interestingly, they did not show any insecticidal activity to the adult of wild fruitfly under the experimental conditions, implying, at least, that these compounds were not toxic to them (at 200-ppm level, for 10−14 days). However, compounds **1**−**10** did show interesting flight-inhibition activity toward the adult of wild fruitfly *Drosophila melanogaster* after they were fed with corn culture media containing compounds for 2 days; and compound **8** was the most potent. In addition, it was found that compounds **1**−**10** have obvious larvicidal activity, at the 200-ppm level, toward the wild fruitfly *Drosophila melanogaster*; and compound **8** was most potent also in this experiment. This might be because the larva could not acquire the essential amount of energy required for living during their feeding with corn culture media containing compounds for about 10−14 days. This might be the first time that trehalase inhibitor showed bioactivity in vivo through inhibition of trehalase. For compounds **1**−**10**, their differences in biological activities among trehalase inhibition in vitro, larvicidal, and insect flight-inhibition in vivo might be attributed to differences in their hydrophobicities, besides structural changes.

Results of the multiple regression analysis between structural parameters and their inhibition of trehalase in vitro are given below along with the statistical values (n = number of compounds; r = correlation coefficient; s = standard deviation; F = significance index with respect to the equation). The figures in parentheses are the confidence intervals of the regression coefficient and intercept. Equation 1 is significant at the 99% level on the basis of results of the statistical F test.

It was found that the effects of the parameters on the activity were in the following order: $d_2 > V_1/V_2 > D > Q_N$. All the 10 synthesized compounds were included in the regression analysis to derive the following regression equation:

$$pIC_{50} = -5.1087 + 0.3755D + 3.1741d_2 - 2.0707V_1/V_2 \quad (1)$$

$$(\pm 2.7092)\ (\pm 0.1245)\ (\pm 0.6242)\ (\pm 0.5291)$$

$$n = 10,\ s = 0.335,\ r = 0.830,\ F = 9.79$$

Experimental values and values calculated by eq 1 are given in Table 1. Equation 1 explains that the activities of oxazoline and thiazoline depend on the lipophilicity (V_1/V_2), the electronic parameter (D), and the spatial parameter (d_2). The higher the values of D and d_2, the higher the activity. The lower the value of V_1/V_2, the worse the lipophilicity, so the higher the activity.

CONCLUSIONS

Compounds **1**−**10** showed moderate trehalase inhibition in vitro, and obvious larvicidal activity and insect-flight inhibition activity in vivo. Their differences in the above biological activities might be attributed to the differences in their hydrophobicities, besides structural changes.

The inhibitory activity of fluorine-containing 4,4-dihydroxylmethyl-2-anilinooxazolines and thiazolines against trehalase in vitro depends on the following factors: the defined length (d_2), the lipophilicity (V_1/V_2), the dipole (D), and the net charge on N atom (Q_N).

The substituents at *m*-position show a remarkable increase in the activity, compared with those of unoccupied compounds. In general, oxazolines possess much higher activity than corresponding thiazolines, as the former have higher value of dipole, more net charge on N atom, and lower lipophilicity (V_1/V_2).

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