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Synthesis of chalcone derivatives as potential anti-diabetic agents

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ABSTRACT

Chalcones bearing electron donating or electron withdrawing substitutions were prepared and their glucose uptake activity was evaluated. Chalcone derivatives were synthesized in one step protocol with high purity and yield. Chalcones with chloro, bromo, iodo and hydroxy substitutions at position 2 on A-ring exhibited the highest activity with glucose medium concentration (210 to 236 mg/dl) compared to pioglitazone and rosiglitazone (230 and 263 mg/dl, respectively). Also chalcones with iodo substitution at position 3 on A-ring were comparably active (⩽ 238 mg/dl). The structure–activity relationship of the tested chalcones was studied and the findings were supported statistically

Metabolic syndrome and diabetes are growing health issues in the past decades. The associated complications of these disorders, such as cardiovascular diseases, peripheral vascular diseases, stroke, diabetic neuropathy, amputations, renal failure, and blindness result in increasing disability, reducing life expectancy, and enormous health costs.[1] Diabetes mellitus (DM) is a growing problem that threatens human health and life in both developed and third world countries. According to reports from the World Health Organization (WHO), around 250 million people are currently living with diabetes and this number is expected to be more than 366 million by 2030.[2,3] Consequently, the prevention and treatment of diabetes is considered a globally challenging problem.

Prevention and control of diabetes with diet, weight control, and physical activity are difficult tasks. Treatment of type 2 diabetes mellitus (T2DM) has centered on increasing blood insulin levels, either by direct insulin administration or using oral drugs that promote insulin secretion, decrease insulin resistance, or reduce the rate of carbohydrate absorption from the gastrointestinal tract. So far, the major drug categories currently used to treat T2DM have several side effects, especially for those patients with liver and renal functional disorders.[1,4] Therefore, developing a new drug category for diabetes is an ultimate goal for improving life quality of human beings.

Chalcone is a class of open-chain flavonoids that is not only biosynthesized by plants but also can be prepared synthetically.

The simplest chalcone can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of base.[5–7] Chalcones have shown a wide variety of pharmacological effects, including anti-inflammatory and anticancer activities.[5,8] Despite the comprehensive biological studies on chalcones, reports on their anti-diabetic activity are scarce.[5,8–15]

Significant advances have been made in the past few years in the isolation and preparation of several chalcone derivatives. Liu et al. synthesized a series of chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties which were active against Gram-positive and Gram-negative bacteria.[14] Shukla et al. synthesized chalcone fibrates with anti-dyslipidemic activity.[13] Jung et al. synthesized a group of 2-hydroxychalcones and chalconyl-thiazolidinediones and examined their anti-diabetic activity through evaluating their binding potential to peroxisome proliferator-activated receptor gamma (PPAR-γ).[11] PPAR-γ is a predominant molecular target for certain anti-diabetic drugs such as insulin-sensitizing thiazolidinediones (TZDs). In recent structure–activity relationship studies, it was reported that some chalcone derivatives stimulated expression and exhibited binding affinity to PPAR-γ.[9,13] Chalcones possess a unique skeleton that belong to flavonoid family with A, B rings linked by α,β-unsaturated carbonyl

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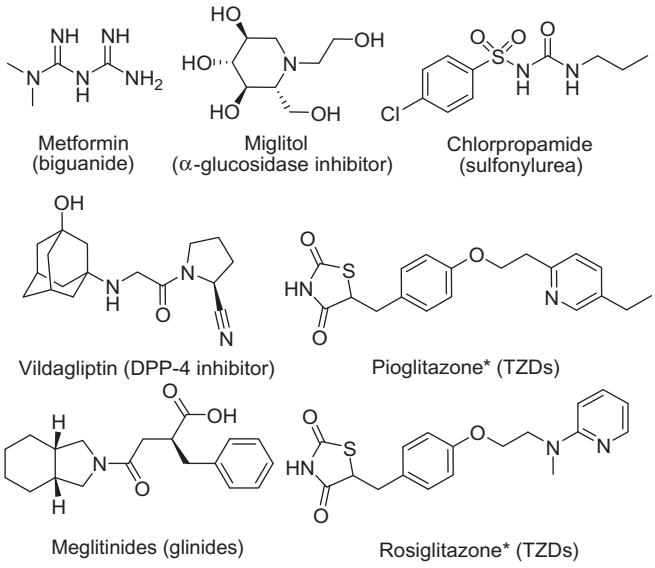


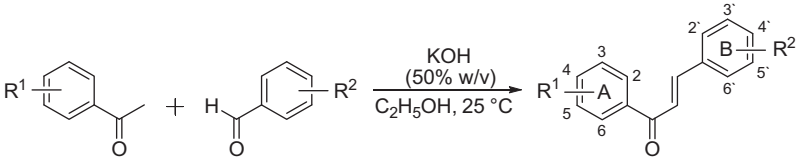
Figure 1. The structures of six major anti-diabetic drugs. *Used as positive controls.

system which is totally different from other anti-diabetic drugs in the world market (Fig. 1). In our previous report, we evaluated the anti-atherosclerotic effect of 2-hydroxy-4′-methoxychalcone (AN07, **1i**, Table 1) and investigated its mechanism of action.[9] The study revealed that the activity is mediated via stimulation of PPAR-γ mRNA and protein expression in human aortic smooth muscle cells. Therefore, we presumed that chalcone derivatives could potentially be used as anti-diabetic agents and subjected for further investigation.

In this study, cellular glucose uptake activity induced by different chalcone derivatives was evaluated. Most of the tested chalcone derivatives were prepared according to established protocol, and some of them were obtained commercially (see Supplementary data). [7] Acetophenones bearing hydroxy group and or halogens were reacted with substituted benzaldehydes in the presence of 50% (w/v) KOH/H_2O using ethanol as a solvent. Purification was done using column chromatography and some chalcones such as those bearing a 4-methoxy group on B-ring can even be recrystallized directly from ethyl acetate-methanol mixture, with acceptable yields ranging from 60%–80%.[16]

Adipose tissues are major sites for postprandial glucose uptake.[17] Therefore, in vitro anti-diabetic screening model based on measur-

Table 1
Chalcone derivatives and the value of glucose consumption in culture media



	A-Ring				B-Ring			Glucose (mg/dl)[a]		A-Ring				B-Ring			Glucose (mg/dl)[a]
	2	3	4	5	3′	4′	5′			2	3	4	5	3′	4′	5′	
1a	H	H	H	H	H	H	H	253 ± 9.9	**3e**	H	H	F	H	OCH_3	H	H	285 ± 1.6
1b	H	H	H	H	H	OCH_3	H	261 ± 3.2	**4a**	Cl	H	H	H	H	OCH_3	H	234 ± 24.4
1c	H	H	H	H	OCH_3	H	H	261 ± 7.2	**4b**	H	Cl	H	H	H	H	H	291 ± 9.2
1d	H	H	H	H	OBn	H	H	285 ± 8.2	**4c**	H	Cl	H	H	H	OCH_3	H	287 ± 13.4
1e	H	H	H	H	H	OBn	H	274 ± 13.0	**4d**	H	Cl	H	H	OCH_3	H	H	255 ± 27.1
1f	H	H	H	H	H	OH	H	263 ± 5.6	**4e**	H	H	Cl	H	H	H	H	295 ± 8.8
1g	H	H	H	H	H	$–OCH_2O–$		252 ± 18.1	**4f**	H	H	Cl	H	H	OCH_3	H	293 ± 7.8
1h	OH	H	H	H	H	H	H	269 ± 11.0	**4g**	H	H	Cl	H	OCH_3	H	H	272 ± 22.4
1i	OH	H	H	H	H	OCH_3	H	283 ± 21.4	**5a**	Br	H	H	H	H	H	H	249 ± 20.8
1j	OH	H	H	H	OCH_3	H	H	264 ± 7.8	**5b**	Br	H	H	H	H	OCH_3	H	230 ± 8.7
1k	OH	H	H	H	OBn	H	H	256 ± 13.1	**5c**	Br	H	H	H	OCH_3	H	H	249 ± 5.2
1l	OH	H	H	H	H	OBn	H	260 ± 3.8	**5d**	H	Br	H	H	H	H	H	299 ± 7.3
1m	OH	H	H	H	H	OH	H	267 ± 3.4	**5e**	H	Br	H	H	H	OCH_3	H	248 ± 8.0
1n	OH	H	H	H	H	$–OCH_2O–$		279 ± 7.6	**5f**	H	Br	H	H	OCH_3	H	H	262 ± 2.2
1o	OH	H	OH	H	H	H	H	273 ± 2.9	**5g**	H	H	Br	H	H	H	H	295 ± 5.8
1p	OH	H	OH	H	H	OCH_3	H	248 ± 0.4	**5h**	H	H	Br	H	H	OCH_3	H	261 ± 12.7
1q	OH	H	OH	H	OCH_3	H	H	274 ± 12.1	**5i**	H	H	Br	H	OCH_3	H	H	257 ± 10.0
1r	OH	H	OH	H	OBn	H	H	283 ± 2.6	**6a**	I	H	H	H	H	H	H	223 ± 13.8
1s	OH	H	OH	H	H	OBn	H	249 ± 12.2	**6b**	I	H	H	H	H	OCH_3	H	210 ± 3.7
2a	OH	H	H	F	OCH_3	H	H	297 ± 9.3	**6c**	I	H	H	H	OCH_3	H	H	210 ± 2.0
2b	OH	H	H	Cl	H	H	H	236 ± 17.5	**6d**	I	H	H	H	H	OBn	H	249 ± 3.6
2c	OH	H	H	Cl	H	OCH_3	H	282 ± 16.4	**6e**	H	I	H	H	OBn	H	H	238 ± 1.1
2d	OH	H	H	Br	OCH_3	H	H	266 ± 23.3	**6f**	H	I	H	H	H	H	H	294 ± 7.3
2e	OH	H	H	Br	H	H	H	253 ± 7.8	**6g**	H	I	H	H	H	OCH_3	H	233 ± 3.5
2f	H	H	H	Br	H	OCH_3	H	254 ± 17.6	**6h**	H	I	H	H	OCH_3	H	H	246 ± 11.3
2g	OH	H	H	Br	OCH_3	H	H	260 ± 3.3	**6i**	H	H	I	H	H	H	H	292 ± 5.3
2h	OH	H	H	Br	H	OBn	H	256 ± 1.3	**6j**	H	H	I	H	H	OCH_3	H	299 ± 3.8
2i	OH	H	H	Br	OBn	H	H	252 ± 0.1	**6k**	H	H	I	H	OCH_3	H	H	274 ± 22.5
3a	F	H	H	H	H	OCH_3	H	259 ± .3	Control								310 ± 4.0
3b	F	H	H	H	OCH_3	H	H	285 ± 4.4	Insulin (3.2×10^{-7} M)								294 ± 6.3
3c	H	F	H	H	H	OCH_3	H	298 ± 15.5	Rosiglitazone[b]								263 ± 23.9
3d	H	F	H	H	OCH_3	H	H	282 ± 10.1	Pioglitazone[b]								230 ± 13.5

[a]Data are means ± SD of $N = 3$ determinations.
[b]Chalcone derivatives, rosiglitazone and pioglitazone were using the same concentrations 30 μg/ml.

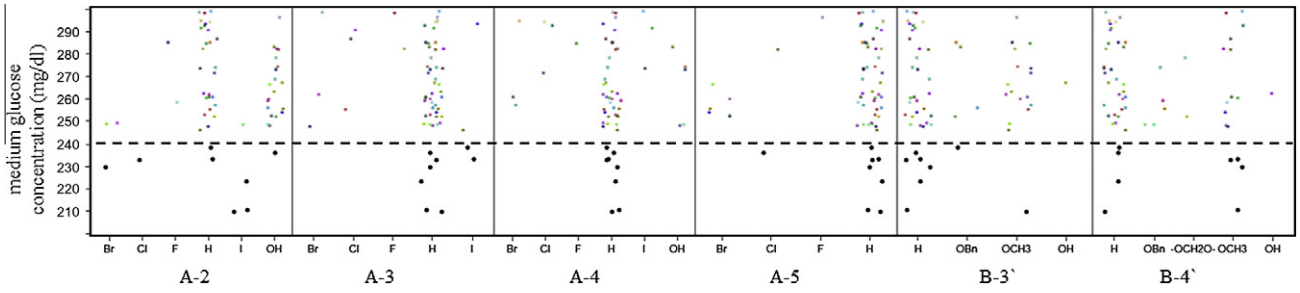


Figure 2. Scatterplot of structure–activity relationship. *X*-axis: substitutions on A and B ring (Ring-Position); *Y*-axis: culture medium glucose concentration.

Table 2
Multi-way ANOVA for chalcones SAR

Source	Nparm		DF	Sum of squares	*F* ratio	Prob > *F*
A-Ring	2′	5	5	6870.2235	4.8792	0.0016*
	3′	4	4	2612.6151	2.3193	0.0756
	4′	5	5	1859.3364	1.3205	0.2775
	5′	3	3	1811.7209	2.1444	0.1117
B-Ring	3′	2	2	1038.6301	1.8441	0.1728
	4′	4	4	799.7002	0.7099	0.5905

Nparm: nonparametric statistics; DF: degree of freedom.
* p value <0.05.

ing glucose consumption after 24 h in culture medium of 3T3-L1 adipocytes was developed.[3,18–21] In our preliminary screening results, it was found that the substitution on A-ring is crucial for promoting cellular glucose consumption. Accordingly, 60 chalcone derivatives with and without substitutions on A-ring were examined in our developed model. Two anti-diabetic clinical drugs, pioglitazone and rosiglitazone were used as positive controls with culture medium glucose concentrations of 230 and 263 mg/dl, respectively. Chalcones which lowered glucose level (n = 3) below 240 mg/dl were regarded as active candidates (Table 1). Structure–activity relationship (SAR) analyses data are shown in the scatter plot (Fig. 2). The *X*-axis of the scatter plot represents different substitutions on A and B rings of the tested chalcones, and the *Y*-axis represents the effect of substitution on culture medium glucose concentration. Chalcones with hydroxy (**2b**), chloro (**4a**), bromo (**5b**) and iodo (**6a**, **6b** and **6c**) substitutions at position 2 on A-ring exhibited good activity with culture glucose medium concentrations ranging from 210 to 236 mg/dl. Additionally, chalcones with iodo substitution at position 3 on A-ring (**6e** and **6g**) were active with comparable results (238 and 233 mg/dl, respectively). It is noteworthy to state that methoxy or benzyloxy substitution on B ring also positively affected chalcones activity (**2b** and **2c**, **5a**, **5b**, and **5c** as well as **6g** and **6h**).

The analysis of multi-way ANOVA test was performed using JMP 9.0.0 (SAS Institute, Cary, NC). The results showed that substitution (fluoro, chloro, bromo, iodo, hydroxy and hydrogen) at position 2 of A-ring, significantly affected the glucose uptake activity in the cell model (p = 0.0016; Table 2). The statistical results by Student *t*-test supported our hypothesis that the iodo functionality of 2-iodochalcones has an important role in glucose uptake activity (p value = 0.0010, 0.0002 and 0.0006 for iodo vs fluoro, hydroxy and hydrogen, respectively). On the other hand, bromo and chloro substitution at position 2 on A-ring showed borderline significance (p value = 0.0392 and 0.0488 for bromo vs fluoro and hydroxy, respectively; p value = 0.0611 for chloro vs fluoro). These data suggest that the glucose uptake activity of chalcone is significantly affected by iodo substitution at position 2 on A-Ring.

In conclusion, the relationship between the structural features of chalcone derivatives and the cellular glucose consumption in 3T3-L1 adipocytes culture medium was studied. Active compounds (**2b**, **4a**, **5b**, **6a**, **6b** and **6c**) shared a common feature with substitution at position 2 of A-ring, suggesting the significance of substitution at this position for glucose uptake activity. Additionally, compounds (**6e** and **6g**) with iodo substitution at position 3 on A-ring also showed great potential in reducing glucose medium concentration. The SAR of the examined chalcones was studied and statistical results supported our findings. Currently a series of detailed mechanistic studies on these active chalcones are under investigation in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.108.

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16. To mixture of acetophenone and benzaldehyde in ethanol, equal amount of 50% (w/v) KOH/H_2O was added. After overnight stirring at room temperature, the reaction was quenched with distilled water and the solution was partitioned with ethyl acetate. After removal of organic phase under vacuum, the residue was purified by column chromatography or crystallization.
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21. Equal amounts (5×10^5 cells) of 3T3-L1 pre-adipocytes (BCRC#60159; Bioresource Collection and Research Center, Taiwan) were seeded and cultured in normal D-glucose (100 mg/dl) DMEM with 10% FBS, 100 U/ml of penicillin and 100 l g/ml of streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. When the cell density reached 100% confluence, 3T3-L1 preadipocytes were induced to be differentiated by treating the culture with 450 mg/dl D-glucose, 0.32 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine and 1 μM dexamethasone for 2 days. Then, the culture medium of the differentiated adipocytes was changed to DMEM containing 300 mg/dl D-glucose with or without the administration of tested compounds. After 24 h, the anti-diabetic activity was determined by measuring the medium glucose concentration using a Roche Cobas Integra 400 Chemistry Analyzer (Roche Diagnostics, Taipei, Taiwan). The coefficient of variation (CV) of the analyzer was 0.62–0.92% within-run and 1.1–1.2% between days. To confirm whether our in vitro model was sufficient to measure the glucose-lowering effect, insulin, pioglitazone and rosiglitazone maleate (RSZ) were used as positive controls. The insulin powder was dissolved in 0.01 M acetic acid (pH 3.0) to provide a 10^{-2} M stock solution and then diluted in distilled water. Compounds were dissolved in DMSO to make 50 μg/μl stock solutions and then diluted in DMSO; the final concentration of DMSO in the medium was 0.1%.