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Design, synthesis and insulin-sensitizing activity of indomethacin and diclofenac derivatives

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ABSTRACT

A series of aromatic acetic acid compounds were designed and synthesized on the basis of Non-steroidal anti-inflammatory drugs indomethacin and diclofenac. Compounds **5a, 7a, 5h, 7h** and **17** could strongly promote insulin-regulated differentiation of 3T3-L1 cells in vitro. They acted as full or partial PPARγ agonist, or improved insulin resistance through non-PPARγ pathway.

Type 2 diabetes mellitus (T2DM) is a debilitating metabolic disorder affecting over 100 million people worldwide,[1] which accounts for about 90% of all diabetes. With the total number projected to rise from 171 million in 2000 to 366 million in 2030,[2] diabetes has become a serious health issue threatening the world population, especially in the developed western countries.

Insulin resistance (IR) is an important marker for developing T2DM.[3] Improving insulin sensitivity can effectively keep blood glucose at an appropriate level, thus postpone or avoid the occurrence of undesired complications. Several thiazolidine-2, 4-diones insulin sensitizer such as troglitazone, pioglitazone, rosiglitazone have been launched into the market since 1997. All these agents share a common thiazolidine-2,4-dione(TZD) group, and are believed to mediate their effects via the activation of gamma isoform of the peroxisome proliferator-activated receptor (PPARγ).[4] However, the adverse toxicity profiles of selective PPARγ agonists have raised critical safety issues. Studies of the mechanisms responsible for these toxicities have not shown clearly whether these side effects identified are target- or compound-related.[5] We believe that identification of a novel non-TZD insulin-sensitizer with a better pharmacological profile could provide a suitable therapeutic option for T2DM.

Some recent studies have revealed that indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs) possess, to some extent, activities of insulin-elicited differentiation of preadipocyte cell line.[6] Indomethacin, although causing certain adverse reaction to the gastrointestinal system, is widely used as anti-inflammatory agents today. To the best of our knowledge, indomethacin's adverse reaction is due, mostly to its action mechanism, not its chemical structure. This intrigues us to design a set of new insulin-sensitizers on the basis of indomethacin for the sake of avoiding the possible structure-related adverse reactions. Therefore *N*-benzoyl-indoleacetic acid group in indomethacin structure was selected as the core structure in our original design. Some of the known heterocycle structures in thiazolidinedione insulin sensitizers were attached to this core structure via a linkage of the alkyloxy group, by replacing the 5-methoxy group. In addition to indomethacin, one derivative of another NSAIDs diclofenac was prepared as well.

Scheme 1 shows the synthesis of compounds **5(a–j)**. Demethylation of indomethacin with iodotrimethylsilane (TMSI) yielded compound **2**, and the key intermediate **3** was obtained via controlled benzylation of **2**. Condensation of **3** with desired heterocyclic alcohols via Mitsunobu reaction afforded compounds **4(a–j)**. Catalytic hydrogenolysis of **4(a–j)** with palladium on carbon gave the target compounds **5(a–j)**. Compounds **7(a–j)** were prepared by methyl esterification of **2**, followed by coupling with various heterocyclic alcohols. (Scheme 2).

The synthesis of diclofenac derivative **17** is shown in Scheme 3. Starting from *m*-nitrophenol, compound **10** was synthesized by four steps of methylation, reduction, diazotization and iodination. Condensation of **10** with 2,6-dichlorobenzenamine followed by chloroacetylation, Friedel–Crafts reaction and hydrolization afforded compound **14**. Esterification of **14** with methanol-*p*-methyl-

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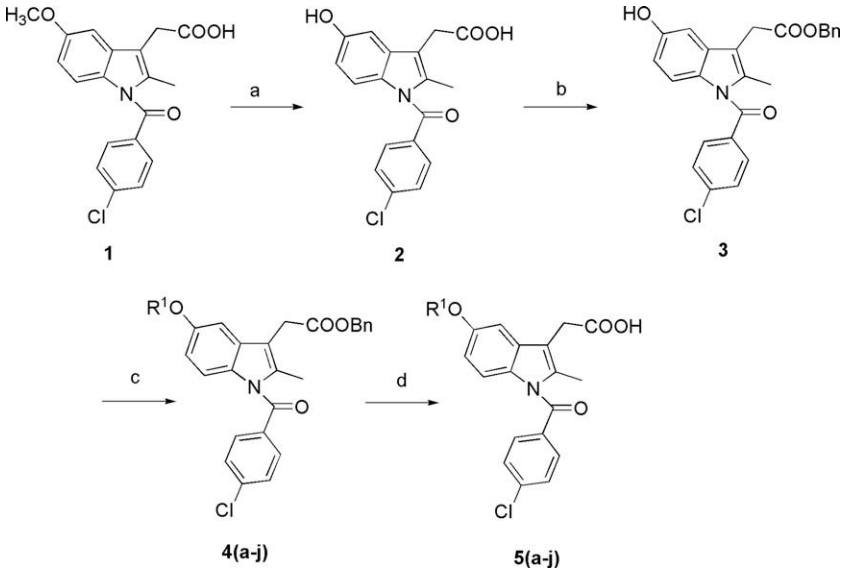
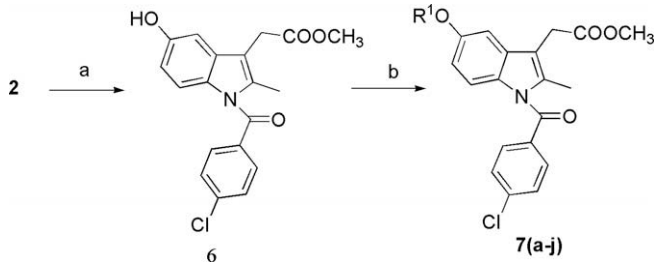


Scheme 1. Reagents: (a) iodotrimethylsilane, $CHCl_3$, 61%; (b) benzyl bromide, K_2CO_3, DMF, 47%; (c) R^1–OH, Ph_3P, diethyl azodicarboxylate, Et_2O; (d) H_2, Pd–C(10%), THF.



Scheme 2. Reagents: (a) $SOCl_2$, CH_3OH, 44%; (b) R^1–OH, Ph_3P, diethyl azodicarboxylate, Et_2O.

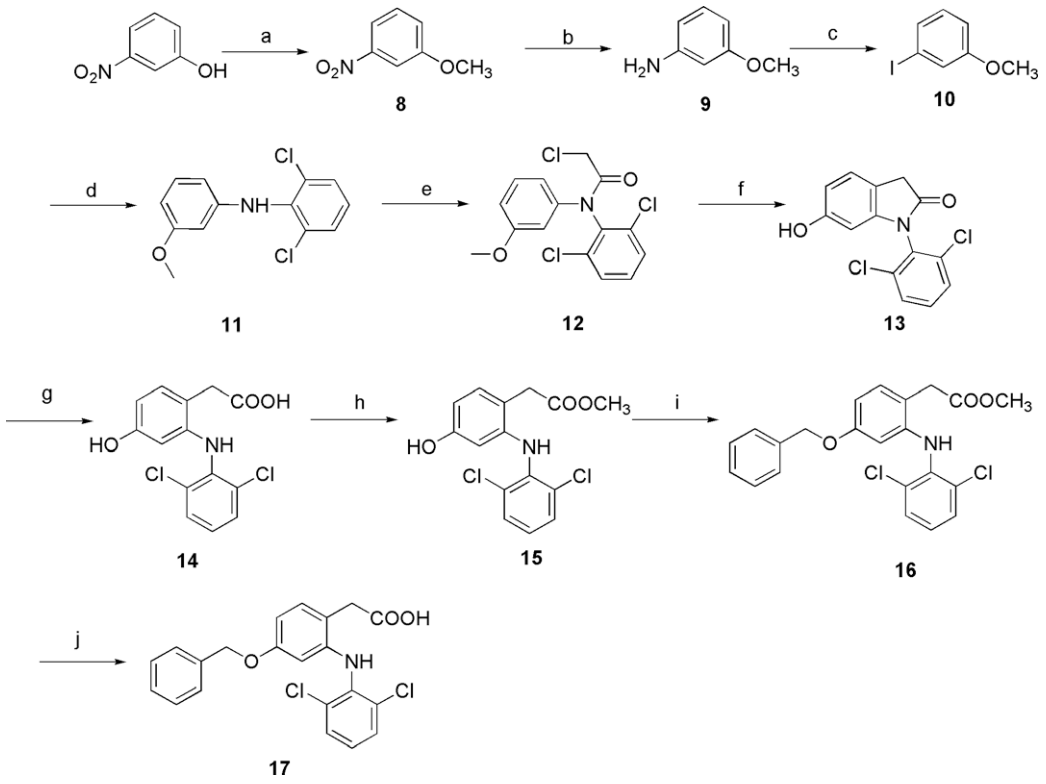
benzenesulfonic acid gave intermediate **15**. Benzylation of **15** with benzyl bromide followed by hydrolysis produced the target compound **17**.

The insulin-sensitizing activity of all above synthesized compounds was screened by measuring the triglyceride accumulation resulting from insulin-regulated differentiation of 3T3-L1 cells.[7] Rosiglitazone was chosen as a positive control. The activities of the screened compounds are given as percentages to the data obtained from rosiglitazone, with the insulin-sensitizing activity of which at 1 μM concentration designated as 100%. (Table 1). The EC_{30} values (effective concentration for 30% enhancement of insulin-induced triglyceride accumulation in 3T3-L1 cells) of some compounds were also given. Compounds with good insulin-sensitizing activities, including **5a**, **7a**, **5f**, **7f**, **5h**, **7h** and **17,** were chosen and evaluated for activity at human PPARγ by using an established cellbased transactivation assay in U2OS cells.[8] The results (listed in Table 1) were compared with the reference compound rosiglitazone. The activating efficacy of the tested compounds was judged by the maximal activation and given as the percentage to the data obtained from the reference compound. The activating potency of the tested compounds was expressed as EC_{50} (the concentration at which 50% of the maximum activation was observed).

As described in Table 1, **5a**,[11] **7a**, **5h** and **7h**[12] exhibited more potent insulin-sensitizing activity than rosiglitazone, indicated by their higher EC_{30} values, and **7d**, **7f**, **7j** and **17**[13] also showed comparable activities. The most potential triglyceride accumulation enhancement at 1 μM was given by compound **7h**, 1.38-fold higher than that of rosiglitazine. It should be noted that in general the esters displayed better activities than the corresponding carboxylic acids. With the elongation of left lipophilic chain, the insulin-sensitizing effect was enhanced. Comparisons between **5f** and **5h**, **7f** and **7h**, **5h** and **5i**, **7h** and **7i**, show that *para* electron-donating substitutions on the phenyl ring also potentially increase the efficiency, which agrees well with literature observations on other types of insulin-sensitizers reported previously.[9] This may be due to additional sterical effects introduced by the substitution groups, which could provide a more suitable conformation for their binding, Comparing **5f** and **5j**, **7f** and **7j**, we found that a *meta* electron-withdrawing and electron-donating conjugated substitution on the phenyl ring resulted in an increase of the insulin-sensitizing activity.

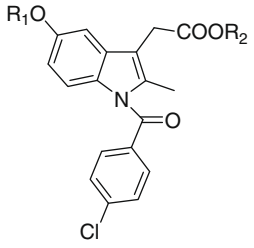
Compound **7h** possesses the strongest PPARγ agonist efficacy (E_{max}) in the transactivation assays, higher than that of the positive control rosiglitazone, and compounds **5f**, **7f**, **5h** show comparable transactivation activities as well. These findings are consistent with the results we reported in a previous paper where phenyl or biphenyl oxazolylethanol were used as replacement.[9] Surprisingly, compounds **5a** and **7a**, despite their highly insulin-sensitizing activities in 3T3-L1 cells, were inactive in the transactivation assays. This finding indicates that, **5a** and **7a** may improve their insulin resistances via a mechanism different from that of PPARγ agonist. Exceptional behaviors were also observed for diclofenac derivative **17**, with a comparable potent insulin-sensitizing activity in 3T3-L1 cells, while exhibiting only weak PPARγ agonist activity in the transactivation assays. Given its maximal activity in transactivation assays, compound **17** can be assumed to be a PPARγ partial agonist. In the research of novel insulin-sensitizer, PPARγ partial agonist was believed to possess many advantages in safety aspect compared to PPARγ full agonist.[10] For these reasons compounds **7a** and **17** can be taken as leading compounds for future insulin-sensitizer research work.

In summary, we have demonstrated that NSAIDs indomethacin and diclofenac derivatives possess potent insulin-sensitizing in 3T3-L1 cells, via functioning as full or partial PPARγ agonists. Surprisingly, some compounds show even improved insulin resistance, probably via a non-PPARγ pathway. It is worth anticipating that more new type of compounds with potent insulin-sensitizing activity can be discovered on the basis of other NSAIDs. Compounds **5a**, **7a**, **7h** and **17**, possessing good insulin-sensitizing activity, can be taken for further pharmacological evaluations, or



Scheme 3. Reagents: (a) CH_3I, K_2CO_3, acetone, 98%; (b) Fe–HCl, EtOH; 95.5%; (c) (1) H_2SO_4, THF; (2) H_2SO_4, $NaNO_2$ aqueous; (3) KI aqueous, 68.%; (d) 2,6-dichlorobenzenamine, Cu, CuI, K_2CO_3, NMP, 58%; (e) 2-chloroacetyl chloride; (f) $AlCl_3$, 71%; (g) 10% NaOH aqueous, 75%; (h) methanol, *p*-methylbenzenesulfonic acid, 72%; (i) benzyl bromide, K_2CO_3, 75%; (j) EtOH–NaOH, HCl aqueous, 36%.

Table 1
Data of insulin-sensitizing activity and PPARγ activity in vitro for compounds



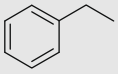
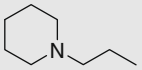
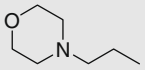
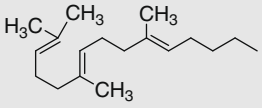
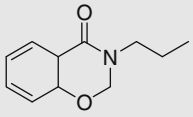
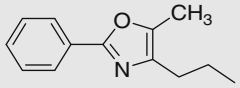
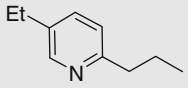
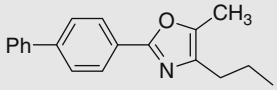
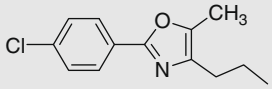
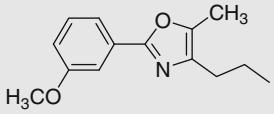
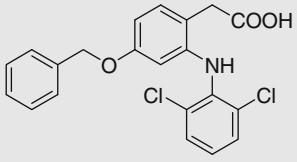
Compd	R_1	R_2	Insulin-sensitizing activity		PPAR-γ	
			Triglyceride accumulation at 1μM[a]	EC30(M)[b]	EC50(M)	% max
5a		H	126	9.51×10^{-9}	ia[c]	ia
7a		CH_3	134	6.56×10^{-9}	ia	ia
5b		H	30	–		
7b		CH_3	33	–		
5c		H	36	–		
7c		CH_3	42	–		
5d		H	74	6.92×10^{-7}		
7d		CH_3	81	2.88×10^{-7}		

Table 1 (*continued*)

Compd	R_1	R_2	Insulin-sensitizing activity		PPAR-γ	
			Triglyceride accumulation at 1μM[a]	EC30(M)[b]	EC50(M)	% max
5e		H	41	–		
7e		CH_3	47	–		
5f		H	36	6.17×10^{-6}	1.23	79
7f		CH_3	80	2.75×10-7	0.62	74
5g		H	42	5.89×10^{-6}		
7g		CH_3	50	1.70×10^{-6}		
5h		H	129	3.31×10^{-8}	0.04	134
7h		CH_3	138	1.23×10^{-8}	0.02	141
5i		H	38	–		
7i		CH_3	57	2.00×10^{-6}		
5j		H	76	3.75×10^{-7}		
7j		CH_3	85	3.16×10^{-7}		
17			98	2.51×10^{-7}	6.03	35
	Rosiglitazone		100	1.05×10^{-7}	0.01	100

[a] % Activity of rosiglitazone at 1 μM, Values are means of three experiments.
[b] Effective concentration for 30% enhancement of insulin-induced triglyceride accumulation in 3T3-L1 cells.
[c] ia: inactive.

as interesting leading compounds for further studies due to their presumably different acting mechanism.

Acknowledgments

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10. Anne, R.; Rémy, H.; Dean, W. H.; Jean, C. F.; Bart, S. *Biochim. Biophys. Acta* **2007**, *1771*, 1065.
11. *Data of* **5a**: white crystals, mp 170–173 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.8 Hz, 2H), 7.38 (m, 5H), 7.05 (d, J = 2.4 Hz, 1H), 6.86 (d, J = 9.2 Hz, 1H), 6.75 (dd, J = 11.2 Hz, 1H), 5.07 (s, 2H), 3.68 (s, 2H), 2.39 (s, 3H). Anal. Calcd. for $C_{25}H_{20}ClNO_4$: C, 69.20; H, 4.65; N, 3.23. Found: C, 69.35; H, 4.51; N, 3.50.
12. *Data of* **7h**: white crystals, mp 113–114 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 7.2 Hz, 4H), 7.46 (m, 4H), 7.38 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 8.8 Hz, 1H), 6.67 (dd, J = 8.8 Hz, 1H), 4.30 (t, J = 6.8 Hz, 2H), 3.70 (s, 3H), 3.65 (s, 2H), 3.03 (t, J = 6.4 Hz, 2H), 2.41 (s, 3H), 2.37 (s, 3H); Anal. Calcd. for $C_{37}H_{31}ClN_2O_5$: C, 71.78; H, 5.05; N, 4.52. Found: C, 71.89; H, 5.22; N, 4.29.
13. *Data of* **17**: brown solid, mp 149–150 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.59 (s, 2H), 4.91 (s, 2H), 5.78 (t, 1H), 6.49–6.52 (dd, J = 2.4 Hz, 4.4 Hz, 1H), 7.09 (d, J = 7.2 Hz, 1H), 7.18–7.24 (m, 1H), 7.27–7.31 (m, 4H), 7.51 (d, J = 8.0 Hz, 7H). Anal. Calcd. for $C_{21}H_{17}Cl_2NO_3$: C, 62.70; H, 4.26; N, 3.48. Found: C, 60.52; H, 4.30; N, 4.29.