DESIGN AND SYNTHESES OF 2-OXIRANECARBOXYLATE DERIVATIVES AND THEIR HYPOGLYCEMIC ACTIVITIES

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[e]Department of Pharmacy, Sham Yook University, Seoul 139-742, Korea

Abstract : A series of 2-oxiranecarboxylate derivatives was prepared as carnitine palmitoyl transferase I (CPT- -I) inhibitors for the development of new antidiabetic agents. The syntheses and biological activities were reported. The most promising derivative, **15b** showed 2.5 times more hypoglycemic activity and 2 times lower acute toxicity compared to Etomoxir (**3**).

Introduction

2-Oxiranecarboxylate derivatives such as Palmoxirate[1] (**1**), Clomoxir[2] (**2**) and Etomoxir[3,4] (**3**) were reported as potent hypoglycemic agents in fasted animals and humen[5] (Figure 1). These compounds inactivate carnitine palmitoyl transferase I (CPT I), which is a rate limiting enzyme for transport of long chain acyl CoA into the mitochondria matrix for fatty β-oxidation[6]. The mode of inactivation involves the irreversible binding with CPT I through a stable covalent modification[7]. The inactivation of CPT I inhibits fatty acid oxidation, which gradually increases the utilization of glucose and finally the following decrement of gluconeogenesis leads hypoglycemic activity[8,9,10].
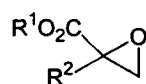
Palmoxirate (1) R^1 = Me R^2 = $CH_3(CH_2)_{12}CH_2$-

Clomoxir (2) R^1 = Et R^2 = Cl—⟨ ⟩—$CH_2(CH_2)_3CH_2$-

Etomoxir (3) R^1 = Et R^2 = Cl—⟨ ⟩—$OCH_2(CH_2)_4CH_2$·

Figure 1

Etomoxir has been most widely studied as a CPT I inhibitor in the series of 2-oxiranecarboxylates[4,11]. It was reported that **3** is 7 and 15 times more effective compared with tolbutamide and buformin, respectively which are currently clinically using as hypoglycemic agents[4]. Although Etomoxir had quite potent hypoglycemic effect, the drug development research was discontinued by its long term toxicity such as myocardial hypertrophy[6]. As part of our program directed toward the development of new antidiabetic agents which have more potent activity and lower toxicities, we designed and synthesized a new series of 2- oxiranecarboxylate derivatives as CPT I inhibitors by modification of **3**. The structure-activity relationship studies were carried out by comparision of the hypoglycemic activities of prepared derivatives.

Design and Synthesis

Based on previous studies, the oxirane ring in **3** appeared to be essential for drug action[9]. So we planned to modify the side chain of Etomoxir as a strategy for our SAR study. As shown in Scheme 1 and 2, we designed a new series of 2-oxiranecarboxylate derivatives (**15a-j**) by replaceing the phenyl group in **3** with various heterocyclic groups such as thiophene and furane. Also the length of the side chain was changed by increasing of the carbon number between heterocycle ring and oxygen.
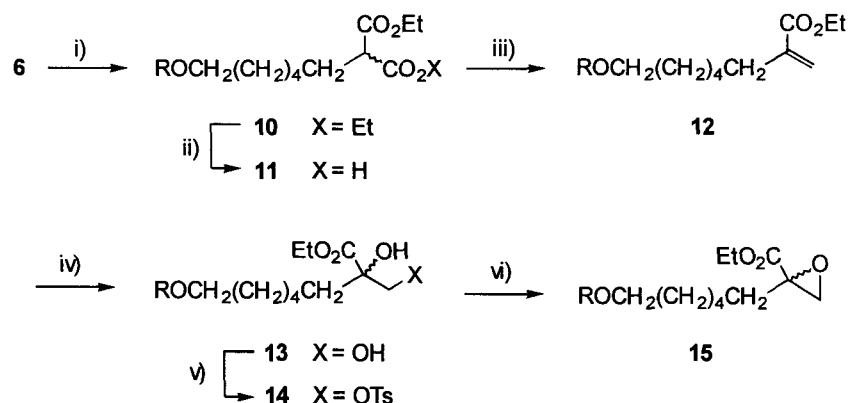
Scheme 1
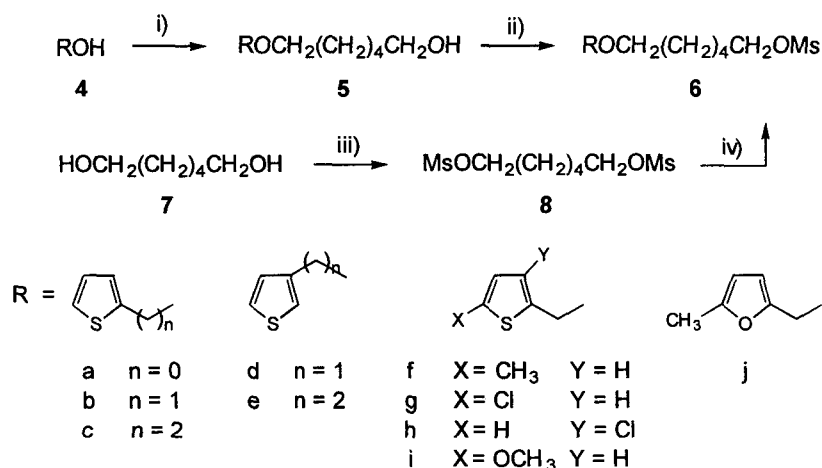
Reagents: i) MsCl/TEA/THF, rt, 1 h; then 7/NaH.THF, rt, 16 h (44%), ii) MsCl/TEA/THF, rt, 1 h (96-100%),

iii) MsCl(2.2 eq.)/TEA/THF, rt, 1 h, (100%), iv) 4/NaH/THF, rt, 16 h (50-78%)

Scheme 2

Reagents: i) diethylmalonate/NaH/THF, reflux, 16 h (63-100%), ii) KOH (1.0 eq.)/EtOH, rt, 1 h (60-89%), iii) Eschenmoser's salt/NaH/THF, reflux, 16 h (70-84%), iv) NMO/OsO$_4$/Acetone/H$_2$O/t-BuOH, rt, 1 h (100%), v) TsCl/Pyr, rt, 3 h (79-100%), iv) K$_2$CO$_3$/EtOH, rt, 5 h (86-100%)

The syntheses of 2-oxirancarboxylate derivatives (**15a-j**) were accomplished in 6 steps starting from mesylate **6a-j** which could be prepared by two methods (Scheme 1). The mesylation of **4b** and **4f** followed by ether formation with 1,6-hexandiol (**7**) gave corresponding alchol **5b** and **5f** *in situ*. Then the second mesylation of **5b**, **5f** were performed to get **6b** and **6f**, respectively. The other alkylating agents (**6a**, **6c-e**, **6g-j**) could be obtained by more efficient method. 1,6-hexandiol was dimesylated to give **8** and by using **8**, the alcohols (**4a**, **4c-e**, **4g-j**) could be directly converted to **6a**, **6c-e**, **6g**, respectively. Diethyl malonate was alkylated with **6a-j**, followed by partial hydrolysis with one equivalent of KOH to give the half esters[12] (**11a-j**). Treatment of half esters with Eschenmoser's salt[13] in the presence of NaH produced ethyl 2-methylenecarboxylates (**12a-j**), which were dihydroxylated with 4-methylmorpholine *N*-oxide (NMO) and OsO$_4$[14] to afford the 2,3-dihydroxypropionates (**13a-j**). The following tosylation and intramolecular cyclization with excess K$_2$CO$_3$ furnished the desired ethyl oxiranecarboxylates (**15a-j**) (Scheme 2).

Biological Assay

The hypoglycemic activity test was performed as follows. Male sprague-Dawley rats (200-250 g) were housed in stainess-steel cages in a room maintained at 20-24°C with a 12 h light/dark cycle. The rats received food and water *ad libitum* except for the specified periods. Diabetes was induced using streptozotocin (STZ) according to the method of Reaven *et al.*[15,16]. After a 24 h-fast, rats were injected intravenously with 45 mg/kg STZ (Sigma Chem Co., St. Louis, MO) which was freshly prepared in a cold 0.1 M citrate buffer (pH 4.5). Antidiabetic effects were studied only using the rats showing serum glucose levels of over 350 mg/dl on day 7 after STZ

administration. Vehicle or synthetic compounds (50 mg/kg) dissolved in 5% ethanol-saline were administered orally. Blood samples were obtained 1 and 2 h after drug administration and serum glucose concentrations were determined using an enzymatic kit from Young-Dong Pharm. Corp (Seoul, Korea).

Results and Discussion

The hypoglycemic activities of the prepared derivatives compared with Etomoxir (3) were listed in Table I. Generally the replacement of benzene ring in 3 with thiophene showed comparable hypoglycemic activity with 3. Especially 15b showed the most potent hypoglycemic activity (75.9%) and the activity of 15e-h (30.7 - 42.2%) was similar to 3 (31.0%).

Table 1. The hypoglycemic activity of prepared 2-oxiranecarboxylate derivatives

No.	Hypoglycemic activity (%)	No.	Hypoglycemic activity (%)
3	31.0		
15a	21.4	15f	33.7
15b	75.9	15g	32.7
15c	5.6	15h	30.7
15d	9.3	15i	5.9
15e	42.2	15j	6.8

Inspection of Table 1 showed that the hypoglycemic activity were dramatically changed by the length of alkyl linker chain between thiophene ring and oxygen (15a-c). For example the hypoglycemic activity of 15a, 15b and 15c were 21.4%, 75.9% and 5.6% , respectively. Similar result was observed in 3-substituted thiophene derivatives (15d, 15e). 15e showed 42.2% of hypoglycemic activity, whereas 15d showed only 9.3% of hypoglycemic activity. It is suggested that there is optimal length of the linker chain to maximize the binding interaction with CPT I. 3- or 5-Substituted derivatives with chlorine (15g, 15h) and methyl (15f) in thiophene ring resulted in comparable activities (32.7 - 33.7%) with 3, whereas substitution with 5-methoxy (15i) led loss of activity (5.9%). Replacement of furan mioety with thiophene moiety drastically decreased hypoglycemic activity (15j 6.8% ; 15f 33.7%). Among the synthesized derivatives, 15b which showed the highest hypoglycemic activity was selected and the LD_{50} was evaluated. The LD_{50} of 15b was 487 mg/kg and that of etomoxir was 250 mg/kg. The 2.5-fold higher hyperglycemic activity and 2-fold lower acute toxicity of 15b compared with Etomoxir encouraged us to proceed with preclinical study for a new antidiabatic drug.

Conclusion

A series of 2-oxiranecarboxylate derivatives bearing thiophene or furan moiety were prepared and their hypoglycemic activities were reported. Among this series,

15b showed the most potent hypoglycemic activity (75.9%) compared with **3** (31%). Also The LD_{50} of **15b** (478 mg/kg) was 2 times lower than **3** (250 mg/kg).   These finding gave us promising possibility to develope a new potent and low toxic antidiabatic drug.   The preclinical study of **15b**   is currently being investigated.

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