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Synthesis and evaluation of (*S*)-2-ethoxy-3-phenylpropanoic acid derivatives as insulin-sensitizing agents

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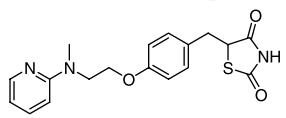
Abstract
A series of (*S*)-2-ethoxy-3-phenylpropanoic acid derivatives were synthesized and their insulin-sensitizing activities were evaluated in 3T3-L1 cells. Compounds **1b** and **1d** exhibited more potent insulin-sensitizing activity than rosiglitazone.

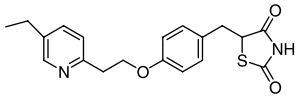
Keywords: *(S)-2-ethoxy-3-phenylpropanoic acid derivatives, type 2 diabetes, insulin-sensitizing agents*

Introduction

Type 2 diabetes is a complex metabolic disorder that affects between 6% and 20% of the populations in West industrialized societies. Type 2 diabetes is characterized by insulin resistance, hyperglycemia, and defects in insulin secretion and is usually associated with dyslipidemina, hypertension and obesity.[1–4] The peroxisome proliferator-activated receptors (PPARα, PPARγ, PPARδ) belong to the nuclear receptor superfamily and function as ligand-activated transcription factors[5]. These receptors play a key role controlling the expresssion of genes involved in lipid and carbohydrate metabolism in various tissues. Interest in the PPARs has increased with the discovery of insulin sensitizers (e.g., TZD, pioglitazone and rosiglitazone), which are potent and selective PPARγ agonists used in the treatment of type 2 diabetes. However, the success of these drugs has been hampered by cases of liver toxicity and side-effects such as fluid retention and weigh gain[6]. This has prompted the search for non-TZD ligands with different characteristics compared to the glitazones.



rosiglizatone



piglizatone

Materials and methods

We were interested in developing a series of non-thiazolidinedione insulin-sensitizing activites, which might surmount the hepatic toxicity problems associated with the known non-thiazolidinediones. A few (*S*)-2-ethoxy-3-phenylpropanoic acid derivatives could increase insulin-sensitizing activities and have been reported to be useful in the treatment of hyperglycemia and hyperlipidemia. Of them Ragaglitazar[7] is in phase III and Tesaglitazar[8] is in phase II clinical trials. Ragaglitazar (DRF-2725) has a binding affinity of 0.98 μM at hPPARα and 0.092 μM at hPPARγ and transactivates PPARα and PPARγ with EC_{50} values of 3.2 and 0.57 μM, respectively. Ragaglitazar also display good *in vivo* antidiabetic activity in db/ob mice and is reported to have 77% oral bioavailability in Wistar rats. Ragaglitazar was co-licensed by Novo Nordisk and completed phase II clinical trials. However, the clinical development of Ragaglitazar has been terminated because of an incidence of bladder tumors in rodents. Our ongoing efforts to find a drug substance in the non-TZD class. As we know, molecular structure in many drugs contains a bis-phenyl structural moiety, therefore, using the dual PPARα/γ Ragaglitazar as structural template and combining with the structural

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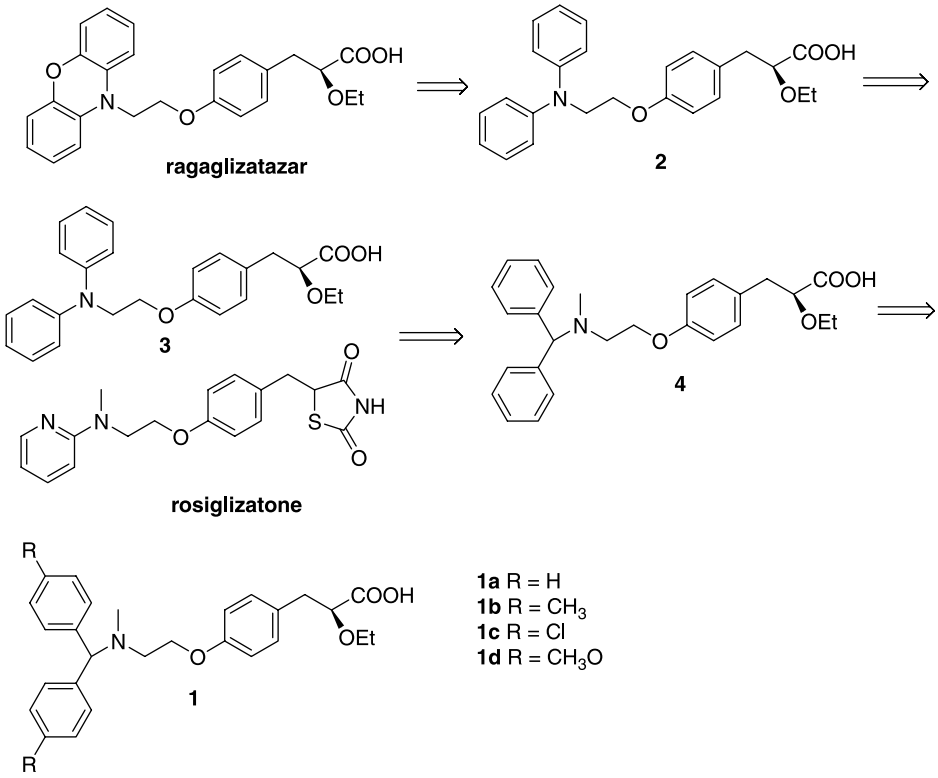
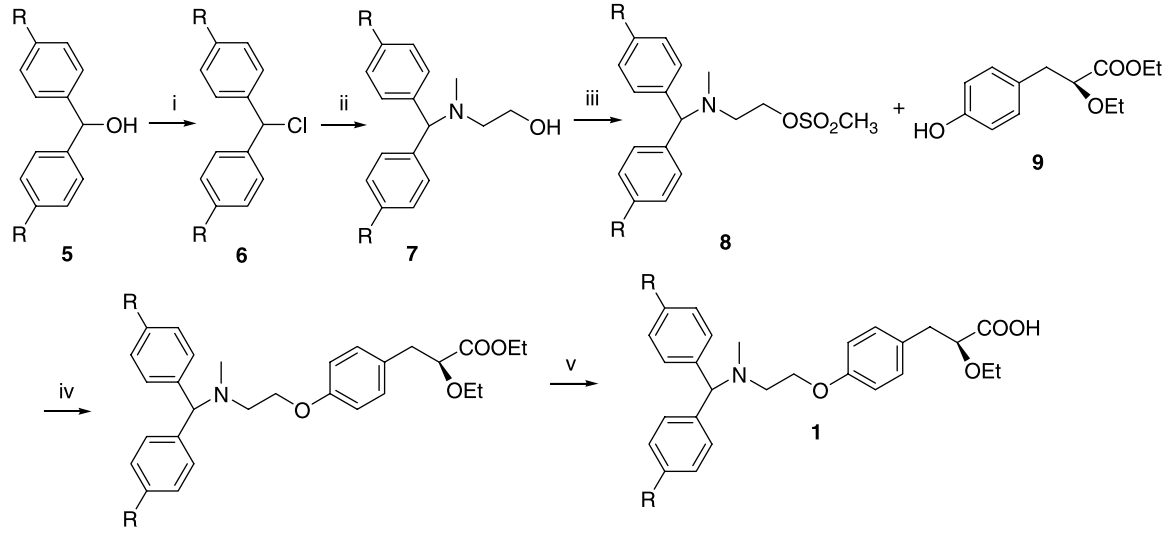


Scheme 1. Design Strategy for new compounds.

characteristic of rosiglitazone (Scheme 1), we demonstrate that insulin-sensitizing agents can be designed by using (*S*)-2-ethoxy-3-phenylpropanoic acid as the acidic moiety and a bis-phenyl structure as the moiety lipophilic of the molecule. A few *bis*-phenyl compounds **1a–1d** were designed and synthesized, and their insulin-sensitizing activites were evaluated with 3T3-L1 cells *in vitro*.

Compounds **(1a–1d)** were synthesized as outline in Scheme 2. Intermediate **(8)** was prepared starting from diaryl methanols by chlorination, condensation with 2-methylaminoethanol, and mesylation[7]. Compound **8** condensed with ethyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxy-propionate **(9)**[7] gave the ethyl esters of desired products. Hydrolysis of the esters afforded the target compounds.

All starting compounds were used as received from commercial sources without further purification. Melting points were determined on XRC-1 micro-melting point aparatus and are uncorrected. Column chromatography was carried out on silica (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.).



Scheme 2. Synthesis of Compound (1) $SOCl_2$, benzene, reflux; ii) $CH_3NHCH_2CH_2OH$, 100°C; iii) CH_3SO_2Cl, CH_2Cl_2, Et_3N, rt., 88 ~ 92%; iv) toluene, K_2CO_3, reflux; v) MeOH, NaOH, rt.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer with TMS as internal standard. IR spectra were obtained on a Perkin Elmer spectrum one FT-IR spectrometer (KBr disc). ESI-MS and HRESI-MS were obtained on a Finnigan LCQ^{DECA} and a Bruler Bio-TOF IIIQ respectively.

Synthesis

To a solution of diaryl methanol **5a** (1.84 g, 10 mmol) in benzene (15 mL) was added dropwise thionyl chloride (2.38 g, 20 mmol) and refluxed for 12 h. The solution was concentrated under reduced pressure to give **6a**. 2-Methylaminoethanol (4 mL) was then added and the mixture was heated to 100°C for 8 h. After the completion (monitored by TLC), water (50 mL) was added to the reaction mixture, and extracted with ethyl acetate (20 mL × 4). The extract was washed with water, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatrography (petroleum-ethyl acetate, 10/1) to afford **7a** 1.06 g (44%) as a colorless liquid. IR (KBr) ν_{max}: 3401, 3060, 3026, 2965, 2879, 2847, 1598, 1492, 1452, 1268, 1080, 1017, 759, 746, 706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 2.18 (s, 3H), 2.56 (t, 2H, $J = 7.2$ Hz), 3.64 (t, 2H, $J = 7.2$ Hz), 4.50 (s, 1H), 7.21 (t, 2H, $J = 7.2$ Hz), 7.32 (t, 4H, $J = 7.6$ Hz), 7.39 (d, 4H, $J = 7.2$ Hz,); ESI-MS *m/z* (%): 243 ([M + H + 1]$^+$, 8), 242 ([M + H]$^+$, 67).

General procedure for compound 1a–1d. A mixture of ethyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)propionate **(9)** (0.357 g, 1.5 mmol), mesylate of **7** (**8**, 1.5 mmol), potassium carbonate (0.828 g, 6 mmol) and toluene (15 mL) was refluxed for 12 h. After completetion, monitored by TLC, the reaction mixture was poured into ice water and the toluene layer was seperated while the aqueous layer was extracted with toluene. The combined organic layer was washed with water and concentrated under reduced pressure to afford an oil, which was dissolved in methanol (20 mL), and 2 mol/L sodium hydroxide (1.5 mL) aqueous solution was added slowly into the solution at room temperature. The reaction mixture was stirred for 8 h. The progress of the reaction was monitored by TLC. After completion of the hydrolysis, the reaction mixture was diluted with water (40 mL) and washed with ether to remove the impurities. The aqueous layer was adjusted to pH 2.5-3.0 with dilute hydrochloric acid and extracted with ethyl acetate (20 mL × 3). The combined organic solution were washed with water and concentrated under reduced pressure to give **1** as an colorless solid.

1a: Yield (82%), Mp 57–58°C, $[\alpha]_D^{25} = -9.0$ (MeOH, $c = 0.1$); IR (KBr) ν_{max}: 3430, 3028, 2973, 2923, 2870, 1730, 1612, 1583, 1512, 1454, 1241, 1178, 1021, 924, 827, 706 cm^{-1}; ESI-MS *m/z* (%): 434 ([M + H]$^+$, 100), 433 ([M$^-$], 28), 432 ([M–H]$^-$, 100); ^{1}H NMR ($CDCl_3$) δ: 1.20 (t, 3H, $J = 6.8$ Hz, $-OCH_2CH_3$), 2.30 (s, 3H, $-NCH_3$), 2.81–2.83 (m, 2H, $-NCH_2CH_2O$-), 2.95 (dd, 1H, $J = 14.4$, 7.6 Hz, $-CH_2CH$-), 3.09 (dd, 1H, J = 14.4, 4.2 Hz, $-CH_2CH$-), 3.46 ~ 3.51 (m, 1H, $-OCH_2CH_3$), 3.56 ~ 3.61 (m, 1H, $-OCH_2CH_3$), 4.06 ~ 4.09 (m, 3H, $-NCH_2CH_2O$-, $-CH_2CH$-), 4.51 (s, 1H, $-CHNCH_3$-), 6.79 (d, 2H, $J = 8.4$ Hz), 7.13 (d, 2H, $J = 8.4$ Hz), 7.19 (t, 2H, $J = 7.2$ Hz), 7.29 (t, 4H, $J = 7.6$ Hz), 7.45 (d, 4H, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$) δ: 15.1, 37.5, 41.5, 53.8, 66.3, 67.0, 75.9, 79.8, 114.3, 127.0, 128.1, 128.4, 130.5, 142.8, 157.9, 172.8; HRESI-MS *m/z*: 456.2147 ([M + Na]$^+$, calcd. for $C_{28}H_{29}NNO_4Na$: 456.2145).

1b: Yield (78%), Mp 66–67°C, $[\alpha]_D^{25} = -8.2$ (MeOH, $c = 0.1$); IR ν_{max} (KBr) cm^{-1}: 3431, 3028, 2974, 2924, 2872, 1730, 1612, 1584, 1512, 1454, 1404, 1299, 1242, 1177, 1032, 835, 744, 720; ESI-MS *m/z* (%): 485 ([M + Na]$^+$, 26), 463 ([M + H]$^+$, 100), 461 ([M-H]$^-$, 100); ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, $J = 6.8$ Hz, $-OCH_2CH_3$), 2.29 (s, 6H, -PhCH_3), 2.32 (s, 3H, $-NCH_3$), 2.84–2.86 (m, 2H, $-NCH_2CH_2O$-), 2.95 (dd, 1H, $J = 14.4$, 7.6 Hz, $-CH_2CH$-), 3.07 (dd, 1H, $J = 14.4$, 4.2 Hz, $-CH_2CH$-), 3.42 ~ 3.47 (m, 1H, $-OCH_2CH_3$), 3.57 ~ 3.62 (m, 1H, $-OCH_2CH_3$), 4.03 ~ 4.06 (m, 3H, $-NCH_2CH_2O$-, -CH_2CH-), 4.52 (s, 1H, -$CHNCH_3$-), 6.77 (d, 2H, $J = 8.4$ Hz), 7.09 (d,, 2H, $J = 8.4$ Hz), 7.13 (d, 2H, $J = 8.4$ Hz), 7.27 (d, 2H, $J = 7.6$ Hz), 7.33 (d, 2H, $J = 7.2$ Hz), 7.44 (d, 2H, $J = 7.2$ Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 15.1, 21.0, 37.8, 41.3, 53.7, 66.0, 66.8, 75.6, 80.0, 114.3, 127.0, 128.0, 128.5, 128.7, 129.2, 130.5, 157.7, 174.1; HRESI-MS *m/z*: 501.2046 ([M + K]$^+$, calcd. for $C_{29}H_{36}KNO_4$: 501.2041).

1c: Yield (85%), Mp 70–71°C, $[\alpha]_D^{25} = -10.5$ (MeOH, $c = 0.1$); IR (KBr) ν_{max}: 3436, 3030, 2974, 2923, 2870, 1728, 1611, 1588, 1511, 1488, 1408, 1242, 1177, 1013, 803 cm^{-1}; ESI-MS *m/z* (%): 502 ([M + H]$^+$, 100), 502 ([M-H + 2]$^-$, 67), 500 ([M-H]$^-$, 100); ^{1}H NMR ($CDCl_3$): δ 1.19 (t, 3H, $J = 6.8$ Hz, $-OCH_2CH_3$), 2.32 (s, 3H, $-NCH_3$), 2.85–2.86 (m, 2H, $-NCH_2CH_2O$-), 2.95 (dd, 1H, $J = 14.3$, 7.4 Hz, $-CH_2CH$-), 3.07 (dd, 1H, $J = 14.3$, 4.1 Hz, $-CH_2CH$-), 3.43 ~ 3.49 (m, 1H, $-OCH_2CH_3$), 3.59 ~ 3.63 (m, 1H, $-OCH_2CH_3$), 4.06 ~ 4.08 (m, 3H, -CH_2CH-, NCH_2CH_2O-), 4.54 (s, 1H, $-CHNCH_3$-), 6.79 (d, 2H, $J = 8.4$ Hz), 7.15 (d, 2H, $J = 8.4$ Hz), 7.39 (d, 4H, $J = 7.2$ Hz), 7.45 (d, 4H, $J = 7.2$ Hz); HRESI-MS *m/z*: 524.1342 [M + Na]$^+$, calcd. for $C_{27}H_{29}Cl_2NNaO_4$: 524.1366.

1d: Yield 77%, Mp 78–79°C, $[\alpha]_D^{25} = -6.8$ (MeOH, $c = 0.1$); IR (KBr) ν_{max}: 3423, 2959, 2929, 2837, 1717, 1609, 1583, 1511, 1463, 1442, 1300,

Table I. Screening data of insulin-sensitizing activity for(*S*)-2-Ethoxy-3-phenylpropanoic acid derivatives.

Compound	R	Insulin-sensitizing activity	
		Triglyceride accumulation at 1 μM[a]	EC_{30}[b] μM)
1a	H	96	3.82×10^{-2}
1b	H	113	9.43×10^{-3}
1c	Cl	87	8.92×10^{-1}
1d	MeO	125	7.45×10^{-3}
rosiglitazone	–	100	2.06×10^{-2}

[a] % activity of rosiglitazone at 1 μM. Values are means of three experiments. [b] Effective concentration (μM) for 30% enhancement of insulin-induced triglyceride accumulation in 3T3-L1 cells.

1245, 1178, 1112, 1031, 820 cm^{-1}; ESI-MS *m/z* (%): 494 ($[M + H]^+$, 31), 493 ($[M^-]$, 30), 492 ($[M-H]^-$, 100); ^1H NMR ($CDCl_3$): δ 1.18 (t, 3H, $J = 7.0$ Hz, $-OCH_2CH_3$), 2.29 (s, 3H, $-NCH_3$), 2.82-2.83 (m, 2H, $-NCH_2CH_2O$-), 2.95 (dd, 1H, $J = 14.4$, 7.3 Hz, $-CH_2CH$-), 3.07 (dd, 1H, $J = 14.4$, 3.7 Hz, $-CH_2CH$-), 3.46 ~ 3.48 (m, 1H, $-OCH_2CH_3$), 3.57 ~ 3.62 (m, 1H, $-OCH_2CH_3$), 3.76 (s, 6H, $-OCH_3$), 4.04 ~ 4.06 (m, 3H, $-CH_2CH$-, NCH_2-CH_2O-), 4.47 (s, 1H, $-CHNCH_3$-), 6.77 (d, 2H, $J = 8.4$ Hz), 6.81 (d, 4H, $J = 8.4$ Hz), 7.13 (d, 2H, $J = 8.4$ Hz), 7.33 (d, 4H, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$): δ 15.1, 27.2, 37.7, 41.2, 53.6, 55.2, 66.0, 66.7, 74.4, 80.0, 113.9, 114.1, 114.3, 128.7, 129.1, 130.5, 134.7, 157.6, 158.5, 173.9; HRESI-MS *m/z*: 516.2336 $[M + Na]^+$, calcd. for $C_{29}H_{35}NNaO_6$: 516.2357.

Insulin-sensitizing Assay

All compounds were screened for insulin-sensitizing activity by measuring the triglyceride accumulation resulting from insulin-regulated differentiation of 3T3-L1 cells[9]. Confluent 3T3-L1 cells were incubated in 5% fetal calf serum with isobutylmethylxanthine (0.5 mM) and dexamethasone (1 μM) for 48 h. Cultures were then incubated in Dulbecco's modified Eagle's medium/2% fetal calf serum for 4 days with insulin (10 ng/mL) and the test compounds. Cellular triglycerides were extracted with 2-propanol and assayed by the enzymatic method using a commercially available kit (MPR2 Triglycerides GPO-PAP, Boehringer Mannheim). The marketed insulin-sensitizing drug rosiglitazone was selected as positive control. All compounds'activities in Table 1 are given as a percentage of rosiglitazone response for insulin-sensitizing activity at 1 μM concentration, and the EC_{30} values (effective concentration for 30% enhancement of insulin-induced triglyceride accumulation in 3T3-L1 cells) of some compounds are also given.

Discussions

As indicated in Table 1, compounds **1b** and **1d** exhibited more potent insulin-sensitizing activity than rosiglitazone according to their EC_{30} values. The primary structure-activity relationship study of these compounds indicated that the substituents at the *p*-position of the bis-phenyl moiety plays a key role in the potency of their activity, and the eletron-donating substituents **(1b, 1d)** lead to enhancement of the activity, whereas, eletron-withdrawing substituents reduce the activity **(1c)**.

Conclusions

In summary, we have developed a novel class of (*S*)-2-ethoxy-3-phenylpropanoic acid derivatives which possess potent insulin-sensitizing activity. These results have encouraged the continued synthesis and evaluation of compounds in the chemical series with the purpose of increased *in vitro* potency, while maintaining acceptable parameters of pharmaceutical suitability.

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