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Novel Thieno Oxazine Analogues as Antihyperglycemic and Lipid Modulating Agents†

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Abstract—A series of phenyl acetic acid and α-hydroxy propionic acid derivatives were synthesized. In vivo studies of the compounds indicated compound **2c** as the most potent in one of the series, which has both glucose and lipid lowering properties. The syntheses and biological studies have been discussed.

Introduction

Non-insulin dependent diabetes mellitus (NIDDM), a major cause of mortality and morbidity in the population of the industrialized world, is a complex, chronic metabolic disorder characterized by insulin resistance in the liver and peripheral tissues.[1] Insulin resistance and associated disorders are being implicated increasingly in other pathophysiologic conditions such as obesity, hyperlipidemia, atherosclerosis and hypertension.[2] Thiazolidinediones (TZDs) are recently marketed insulin sensitizer antidiabetic agents that improve the blood glucose level in type 2 diabetes by the activation of peroxisome proliferator-activated receptor gamma (PPARγ),[3] a member of the nuclear hormone receptors family. But they are often poorly effective in improving the plasma lipid profile in type 2 diabetes patients.[4] Although TZDs have been introduced recently, there are several reports of undesirable side effects.[5] Fibrates class of drugs discovered a decade ago, are effective in reducing the serum triglyceride and increasing the high-density lipoprotein (HDL) cholesterol in humans.[6] Recent reports have indicated that it acts through activation of the PPARα isoform,[7] which is present predominantly in the liver. There are several recent observations where PPARα has also been implicated in their insulin sensitizing action.[8] Due to the tissue specific distribution of PPAR α and γ isoforms and their complimentary effect in lowering plasma lipid and glucose levels, it has been postulated that a dual activator of PPARs can reduce both plasma glucose (PG) and triglyceride (TG) to a considerable amount.

Design and Synthesis

A few phenyl acetic acids[9] and β-aryl α-hydroxy propionic acids[10] have been reported to be useful in the treatment of hyperglycemia and hyperlipidemia. Of them Ragaglitazar is in phase III and Tesaglitazar is in phase II clinical trials (see Fig. 1). As a part of our ongoing efforts to find a drug substance in the non-TZD class, which not only would improve the insulin sensitivity but, at the same time, effectively decrease the hyperlipidemia, we initiated a search for novel compounds that lower triglycerides and improve insulin sensitivity. Heterocycles containing a carbonyl group are reported to be more efficacious than a simple heterocycle[11] and so thieno[3,2-b][1,4]oxazinone[12] was selected as the heterocycle to obtain new phenyl acetic acids and β-aryl α-hydroxy propionic acid derivatives which indeed showed interesting blood glucose and triglyceride lowering activities in experimental mice models.

The present study describes the identification of a lead molecule by PPARα and PPARγ transactivation assays in conjugation with in vivo studies in a db/db mice model, which was then tested in a Swiss Albino Mice (SAM) model.

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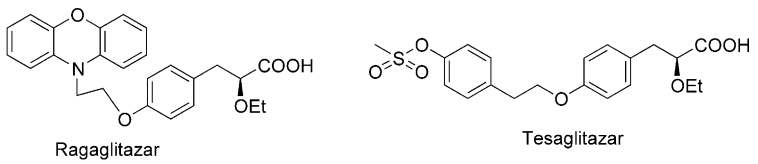


Figure 1.

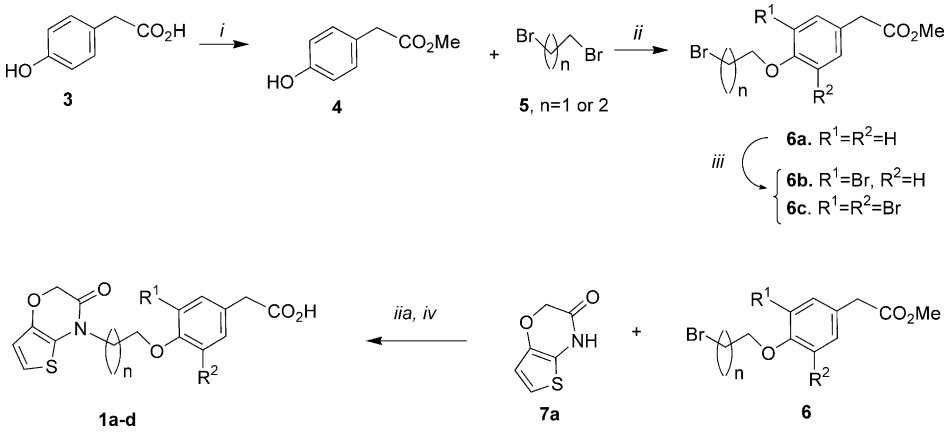
The compounds used in this study were synthesized by standard procedures as outlined in the scheme, and were characterized by ^{1}H NMR, IR and mass spectral analysis.[13] 4-Hydroxyphenyl acetic acid was esterified using methanolic HCl which on reaction with dibromo alkane **5** either in DMF with K_2CO_3[14] or in toluene using K_2CO_3 and tetrabutyl ammonium bromide gave **6a** which upon NBS treatment in CCl_4 afforded mono- and di-bromo derivatives **6b** and **6c**. Compound **6** was then alkylated with **7a**[12] and subsequently hydrolyzed to final acids **1a–d** (Scheme 1).

In another set of experiments, compound **8a**[15] was converted to mono- and di-bromo derivatives (**9a**, **9b**); and mono-chloro derivative **9c** following a similar procedure as for **6b** and **6c** above. The compounds **9a–c** were then allowed to react with **7b**, obtained by the 1,2-dibromoethane reaction on **7a**, to produce corresponding esters which on hydrolysis afforded the corresponding acids **2a**,**b**,**d** (Scheme 2).

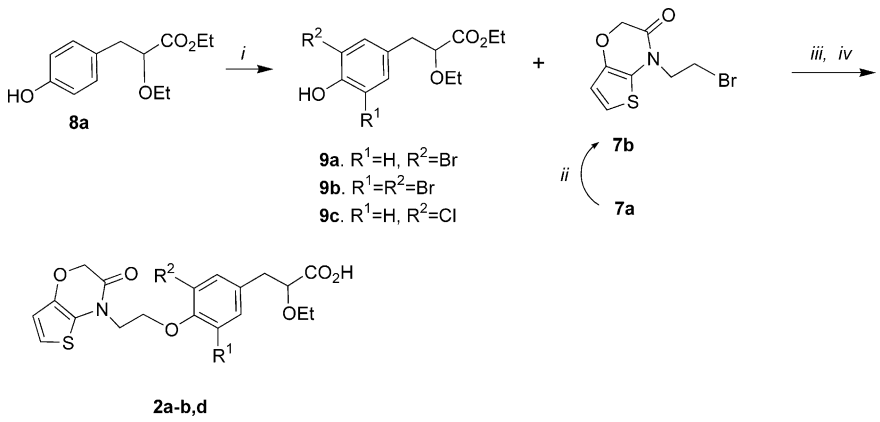
Compounds **2c**,**e–f** were synthesized as follows. Hydroxy groups of compound **10** (see Scheme 3) were protected by benzylation using benzyl bromide, which on Wittig-coupling gave the corresponding enes **12**. Hydrogenation of **12** followed by alkylation using 1,2-dibromoethane afforded bromoethoxy half esters **13**. Coupling of compounds **13** with **7a** using potassium carbonate yielded the corresponding esters **14** which on hydrolysis using sodium carbonate in MeOH–water provided the end products **2c**,**e–f**.

Results and Discussion

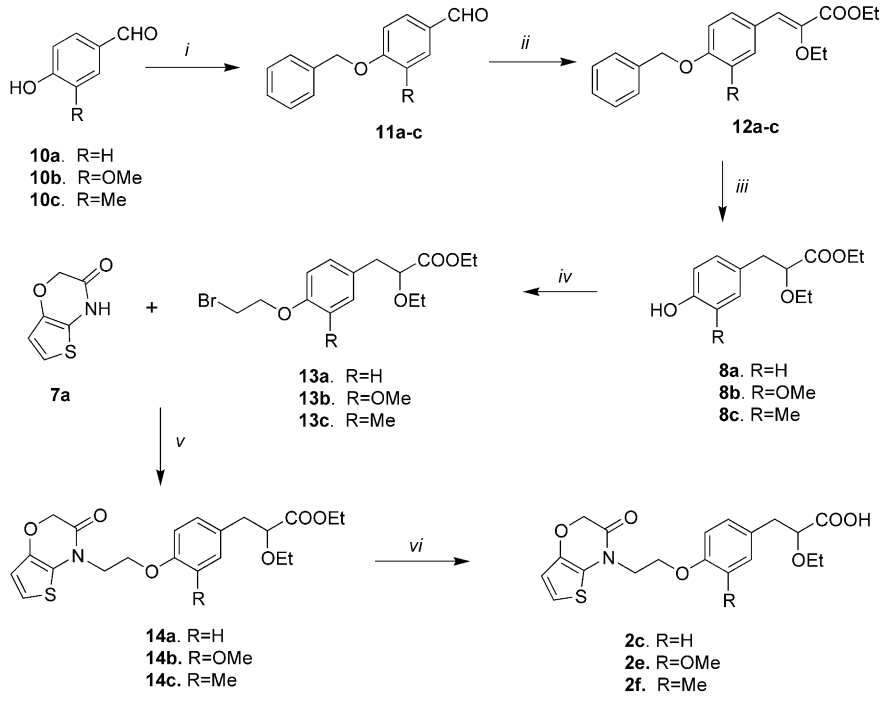
The commonly used diabetic db/db mice model of NIDDM[16] was used for the assessment of the PG and TG. Pioglitazone (which showed 3.8-fold activity at 1 μM concentration) was used as the reference standard for PPARγ and fenofibrate (which showed 1.5-fold activity at 50 μM concentration) was used as standard for PPARα transactivation assay[17] in our studies. The synthesized phenyl acetic acid analogues **1a–d** were evaluated for PPAR activity in transactivation assay, and in db/db mice at 3 mg/kg dose (po) for 6 days to measure PG and TG lowering activity.[10a] Compounds **1b** and **1c** showed impressive PPARγ activity whereas compounds **1a** and **1d** showed poor PPARγ activity. Only compound **1b** showed good PPARα activity. In agreement with these in vitro results, in vivo results, after 6 days of treatment (Table 1), show that **1a** and **1d** have poor PG and TG lowering activity. Compound **1c** has a better plasma glucose reducing effect but still there is no improvement for triglyceride lowering. However, **1b** showed both impressive PG (39%) and TG (43%) reductions at 3 mg/kg dose whereas the standard compound pioglitazone showed 39% PG and 36% TG reduction, respectively, at 30 mg/kg dose after 6 days of treatment. It is seen from the structure of this series of compounds that the optimum length of the linker is ethylene ($n = 1$) and the bromo substituted phenyl acetic acid derivatives are showing poor triglycerides lowering effect although the *o*-dibromo phenyl acetic derivative **1c** has a similar PG reducing property as that of **1b**. On the other hand, the β-aryl α-hydroxy propionic acid derivatives **2a–c** showed very good PPARα activity, whereas **2c** showed dual PPARα and γ activity. In agreement with these in vitro results, in vivo results, after 6 days of treatment (Table 2), show that triglyceride lowering activity under in vivo treatment but only **2c** has shown a substantial reduction in plasma glucose level, the standard used being the same pioglitazone at the same conditions. Interestingly, in this series, the effect of bromo substitution is just the reverse of that of



Scheme 1. Reagents: (i) Methanolic HCl, rt, 2 h; (ii) (a) DMF, K_2CO_3, rt, 24 h; OR; (b) toulene, K_2CO_3, Bu_4NBr, 100 °C, 40 h; (iii) NBS, CCl_4, 55–60 °C, 3 h; (iv) Na_2CO_3, MeOH-H_2O, rt, 18 h.

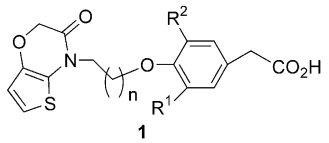


Scheme 2. Reagents: (i) NBS or NCS, CCl_4, 55–60 °C, 3 h; (ii) 1,2-dibromoethane, DMF, K_2CO_3, rt, 24 h; (iii) DMF, K_2CO_3, rt, 24 h; (iv) Na_2CO_3, MeOH–H_2O, rt, 18 h.



Scheme 3. Reagents: (i) DMF, BnBr, K_2CO_3, rt, 16–30 h; (ii) THF, NaH, $(OEt)_2P(O)CH_2(OEt)CO_2Et$, 0 °C-rt, 16–40 h; (iii) dioxane, 10% Pd–C, H_2, 60 psi, 30–40 h; (iv) Acetone, 1,2-dibromoethane, K_2CO_3, reflux, 5 days; (v) DMF, K_2CO_3, rt, 20–48 h; (vi) MeOH–H_2O, Na_2CO_3, rt, 48 h.

Table 1. PG, TG, PPARα and PPARγ of the compounds of formula **1**

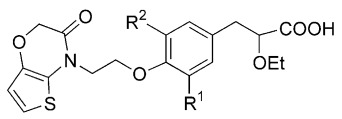


	R^1	R^2	*n*	PG[a]	TG[a]	PPARα[b]	PPARγ[c]
1a	H	H	2	14	NE[d]	0.5	5.0
1b	H	H	1	39	43	5.0	14.0
1c	Br	Br	1	43	15	2.0	15.0
1d	Br	H	1	21	14	1.3	8.0

[a]Percentage reduction in db/db mice at 3 mg/kg dose.
[b]PPARα activity measured at 50 μM.
[c]PPARγ activity at 1 μM concentration.
[d]NE, no effect.

the phenyl acetic acid series. Both mono- and *o*-dibromo compounds **2a**,**b**,**d** and **e** showed poor PG lowering activity, even when the chain length was maintained at 2 carbon units i.e.; ethylene bridge. Compound **2f** has a better plasma glucose reducing effect, which is a true reflection of its PPARγ value in transactivation assay but there is very little improvement for triglyceride lowering. In in vitro PPAR transactivation assays (both PPARα and PPARγ) **2c** showed more potent dual PPARα and PPARγ activity than the standards used. Compound **2c** showed 8-fold PPARα activation and 16 fold PPARγ activity, respectively. In this series also unsubstituted compound **2c** showed impressive PG (47%) and TG (56%) reduction, which is best in the two series and much better with respect to standard pioglitazone. Compound **2c**, which is the best one between both the series, was taken up for further study. We had

Table 2. PG, TG, PPARα and PPARγ of the compounds of formula **2**



2

	R^1	R^2	PG[a]	TG[a]	PPARα[b]	PPARγ[c]
2a	Br	H	NE[d]	62	8.0	1.2
2b	Br	Br	NE[d]	48	5.0	1.6
2c	H	H	47	56	8.0	16.0
2d	Cl	H	21	27	2.5	7.0
2e	OMe	H	NE[d]	NE[d]	0.5	1.2
2f	Me	H	52	19	1.5	18.0

[a]Percentage reduction in db/db mice at 3 mg/kg dose.
[b]PPARα activity measured at 50 μM.
[c]PPARγ activity at 1 μM concentration.
[d]NE, no effect.

resolved compounds **2a–c** to their respective *S*- and *R*-enantiomers. Since we did not see any enrichment of activities in *S*-enantiomers we also observed the decrease in activities for *R*-enantimers, we only report here the values of racemic compounds.

Triglyceride lowering potential of **2c** was further studied in Swiss Albino mice, a moderate hypertriglyceridemic model.[18] Results indicate that **2c** brought about 38% reduction of plasma triglyceride when administered at 3 mg/kg/day dose for 6 days. The standard fenofibrate showed 36% triglyceride reduction in the same model when administered at 30 mg/kg/day dose for 6 days. Pioglitazone did not show any significant activity in this model.

Conclusion

To summarize, a series of phenyl acetic acid and α-hydroxy propionic acid derivatives were synthesized, of which compound **2c** is the most potent one. Compound **2c** is a dual activator of PPARα and PPARγ and showed better activity than the standard drugs pioglitazone and fenofibrate.

Acknowledgements

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13. All the compounds were fully characterized. Structural data for **2c** is presented here: mp 84 °C; ^{1}H NMR ($CDCl_3$) δ 7.13 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.3 Hz, 2H), 6.71 (d, J = 5.5 Hz, 1H), 6.62 (d, J = 5.5 Hz, 1H), 4.62 (s, 2H), 4.28–4.08 (m, 4H), 4.02 (dd, J = 7.2, 4.4 Hz, 1H), 3.69–3.32 (m, 2H), 3.11–2.84 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H); Mass *m*/*e*: M^+ = 391.
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18. An inbreed colony (at our own animal house) of Swiss Albino Mice (SAM) of 21–29 g body weight, moderately hypertriglycerimic, has been used for screening the

compounds. Animals were treated orally with 3 mg/kg/day of **2c** for 6 days. The control animals were treated with the vehicle (0.25% carboxymethyl-cellulose, 10 mL/kg) only. Animals were bled through retro orbital sinus on day-1 and day-6 of the experiment. Plasma samples were prepared and triglyceride levels were measured by using a commercial kit (Linco Research Lab., USA). For calculations of percentage reduction of triglycerides, standard method[19] was applied.

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