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Original article

Novel phthalazinone and benzoxazinone containing thiazolidinediones as antidiabetic and hypolipidemic agents

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Abstract – We report here the synthesis of a series of 5-[4-[2-[substituted phthalazinones-2(or 4)yl]ethoxy]phenylmethyl]thiazolidine-2,4-diones and 5-[4-[2-[2,3-benzoxazine-4-one-2-yl]ethoxy]phenylmethyl]thiazolidine-2,4-diones and their plasma glucose and plasma triglyceride lowering activity in db/db mice. In vitro PPARγ transactivation assay was performed in HEK 293T cells. In vitro and in vivo pharmacological studies showed that the phthalazinone analogue has better activity. **PHT46** (compound **5a**), the best compound in this series, showed better in vitro PPARγ transactivation potential than troglitazone and pioglitazone. In insulin resistant db/db mice, **PHT46** showed better plasma glucose and triglyceride lowering activity than the standard drugs. Pharmacokinetic study in Wistar rats showed good systemic exposure of **PHT46**. Subchronic toxicity study in Wistar rats did not show any treatment-related adverse effect. © 2001 Éditions scientifiques et médicales Elsevier SAS

phthalazinone / benzoxazinone / thiazolidinedione / PPARγ / plasma glucose / triglyceride lowering / diabetes mellitus / insulin resistance

1. Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is characterised by hyperglycemia, hyperinsulinemia and impaired insulin action [1]. Insulin resistance is considered to be the underlying mechanism in the pathogenesis of type 2 diabetes, which also leads to dyslipidemia, hypertension and obesity, termed together as metabolic syndrome [2, 3]. The combination of these disorders leads to the development of late diabetic complications like coronary artery disease, nephropathy, etc. [4, 5].

The advent of thiazolidinediones, which ameliorate insulin resistance and normalise elevated blood glucose levels has revolutionised the treatment of NIDDM [6]. Their mechanism of action is quite different from that of established antidiabetics such as sulfonylureas [7] and biguanides [8]. These compounds are synthetic, high affinity ligands of Peroxisome Proliferator Activated Receptor gamma (PPARγ), a member of the nuclear receptor superfamily which controls the expression of genes involved in lipid and carbohydrate metabolism in target tissues [9, 10]. It is believed that this class of compounds ameliorates insulin resistance through PPARγ activation. Many thiazolidinediones are in various stages of clinical development. Reports of hepatotoxicity and idiosyncratic deaths associated with troglitazone treatment have validated the need for safer insulin sensitisers to manage type 2 diabetes [11, 12].

We have previously reported [13] DRF-2189, which was designed based on the constrained conformation concept, conceptually obtained by cyclising the

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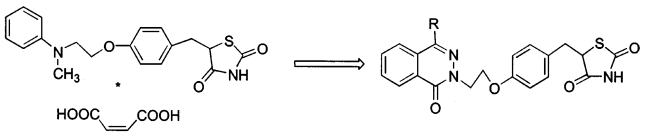


Figure 1. Conversion of rosiglitazone to phthalazinones.

methyl group on nitrogen of rosiglitazone with and without incorporation of a carbon or heteroatom. We envisioned that cyclisation with a hetero atom would lead to a lactam while preserving the link with the nitrogen atom of the lactam ring (*figure 1*), thus one can generate phthalazinone/benzoxazinone series of compounds, with potential insulin sensitising activity.

It is known that a heterocycle containing a carbonyl group is more efficacious than a simple heterocycle [14], so we anticipated that this type of modification will yield potent thiazolidinediones. In the present study, we are reporting some structure–activity relation studies (SAR) of thiazolidinediones having phthalazinones and benzoxazinones.

2. Chemistry

A general strategy for the synthesis of thiazolidine-2,4-diones is shown in *figure 2*. The starting materials phthalazinones were prepared by reported methods [15, 16]. The phthalazinones **2** were treated with 4-(2-bromoethoxy)benzaldehyde (**1**) [17] in the presence of K_2CO_3 in dimethyl formamide at 70–80 °C for 2–6 h to furnish the aldehyde **3**, which was reacted with thiazolidine-2,4-dione in the presence of piperidine benzoate to afford benzylidene thiazolidinedione analogues **4** in a good range of yields. The olefinic bond in **4** was hydrogenated in 1,4-dioxane using 10% Pd/C as catalyst to give 5-benzyl-2,4-thiazolidinediones (**5**), which were converted into their salts **6** by using metal (Na, K) methoxide in methanol (*figure 2*). Similarly, 2,3-benzoxazininone compounds **7**–**11** [18] (*figure 3*) were prepared following the protocol described for phthalazinones. Accordingly, 4-hydroxymethyl-2-methyl-1(2*H*)-phthalazinone (**12**) [19] (*figure 4*) was coupled with 4-fluorobenzaldehyde (NaH–DMF) at room temperature to give the aldehyde **13**, which was condensed with 2,4-thiazolidinedione followed by hydrogenation to afford the product **15**.

3. Pharmacology

Diabetic db/db mice, a commonly used animal model of NIDDM [20], were used to assess the plasma glucose and triglycerides lowering activity of these compounds. Troglitazone, pioglitazone and rosiglitazone were used as references in our studies.

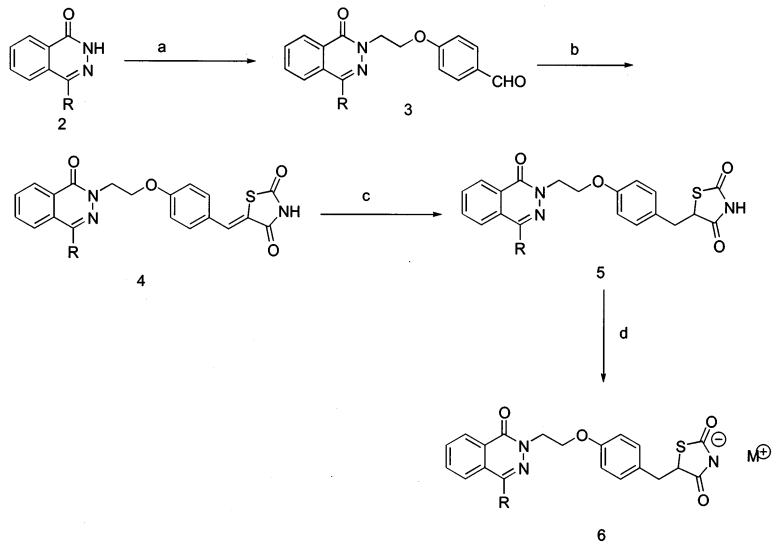


Figure 2. Synthesis of phthalazinone–thiazolidinediones **3**–**6**. Reagents: (a) DMF, K_2CO_3, 4-(2-bromoethoxy)benzaldehyde (**1**), 70 °C; (b) thiazolidine-2,4-dione (TZD), toluene, piperidine-benzoate, reflux; (c) 10% Pd/C–dioxane, r.t.; (d) MeOH, MOMe (M = metals like Na or K), r.t.

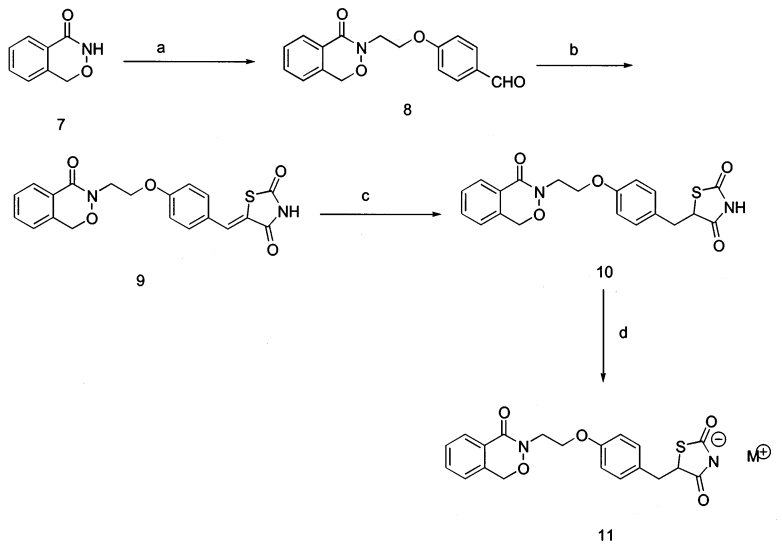


Figure 3. Synthesis of 5-[4-[2-[4-oxo-3,4-dihydro-1*H*-2,3-benzoxazine-3-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione (**10**). Reagents: (a) DMF, K_2CO_3, 4-(2-bromoethoxy)benzaldehyde (**1**), 70 °C; (b) TZD, toluene, piperidine-benzoate, reflux; (c) 10% Pd/C–dioxane, r.t.; (d) MeOH, MOMe(M = metals like Na or K), r.t.

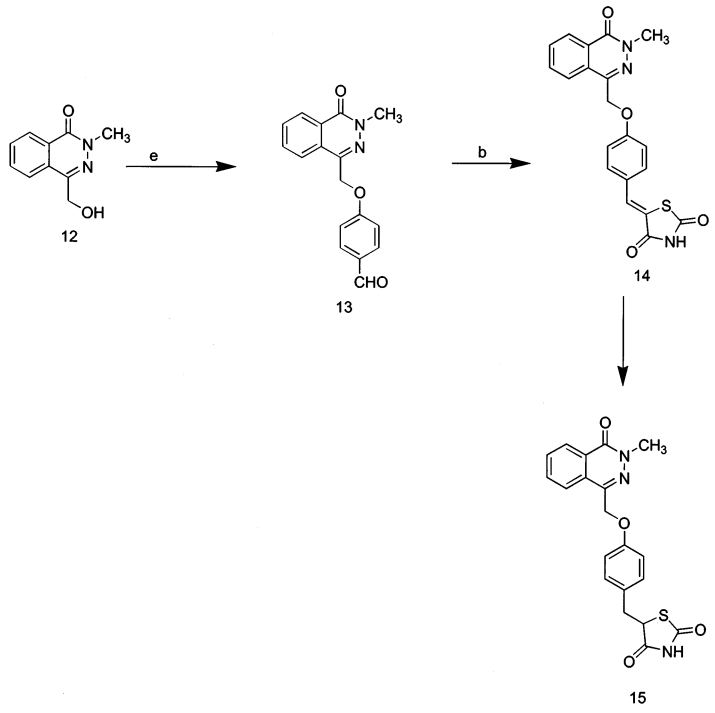
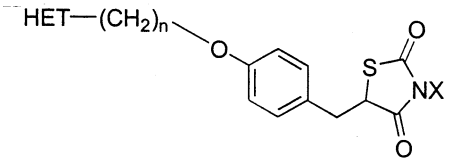
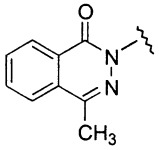
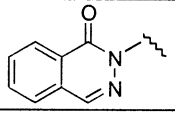
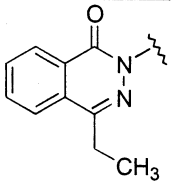
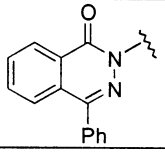
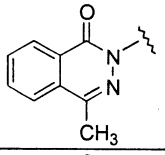
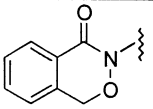
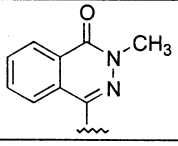


Figure 4. Synthesis of 5-[4-[[2-methyl-1-oxo-1,2-dihydrophthalazin-4-yl]methoxy]phenylmethyl]thiazolidine-2,4-dione (**15**). Reagents: (b) TZD, toluene, piperidine-benzoate, reflux; (c) 10% Pd/C–dioxane, r.t.; (e) NaH, DMF, 4-fluorobenzaldehyde, r.t.

Table I. SAR studies of phthalazinone and benzoxazinone–thiazolidinediones **5**–**15**.



Comp. no:	HET	DB	n	X	M.P. °C	Dose (mg/kg)	% Redn. in PG	% Redn. in TG
5a		no	2	H	170	10	72	68
5b		no	2	H	165	10	54	NE
5c		no	2	H	174	10	7	NE
5d		no	2	H	198	10	12	NE
6b		yes	2	Na	280	10	21	NE
11		no	2	Na	255	10	37	60
15		no	1	H	234	10	15	NE

Male db/db mice were treated with the compounds for 6 days and plasma glucose and triglyceride levels were measured. Percentage reduction was calculated according to the formula: $1-[(TT/OT)/(TC/OC)]\times 100$; TT: test day treated, OT: zero day treated, TC: test day control, OC: zero day control; HET: heterocycle; DB: double bond; NE: no effect; PG: plasma glucose; TG: plasma triglyceride.

Male Wistar rats were used for pharmacokinetic studies to check the systemic exposure of the interesting compound. Wistar rats of both sexes were used for subchronic oral toxicity studies. Since thiazolidinediones are known to activate nuclear receptor PPARγ, we have used a cell-based reporter assay to check the activity of these compounds on PPARγ.

Table II. Effect of **PHT46**, troglitazone and pioglitazone in PPAR transactivation assay.

Concentration (μM)	**PHT46** (fold activation)	Troglitazone (fold activation)	Pioglitazone (fold activation)
0.250	1.7	1.8	1.6
0.500	3.9	2.3	2.4
1.0	5.4	3.7	3.8
5.0	11.0	5.5	6.0

HEK 293T cells transfected with GAL4-PPARγ1 and Pgl2-gal4 × 5-Luc plasmids (Section 6.2). Transfected cells were treated with **PHT46**, troglitazone and pioglitazone at concentrations mentioned. Each data point represents mean of two experiments.

4. Results and discussion

The synthesis of phthalazinone derived thiazolidinedione analogues **5** and **15** and benzoxazinone derivatives **10** and **11** was achieved by the synthetic protocols described earlier. Compounds **5** and **10** were prepared via a common route, but for compound **15** slight modification was done at an intermediate step to prepare **13** which was obtained from 4-fluorobenzaldehyde. Compound **10** could not be tested because of its gummy oil nature but its sodium salt **11** was tested for biological activity. These compounds were evaluated in db/db mice at 10 mg kg^{-1} dose (p.o.) for 6 days and plasma glucose and triglycerides levels were measured. The results after 6 days of treatment (*table I*) suggest that phthalazinone thiazolidinediones **5c**, **5a**, **15**, **5d**, **6b** showed poor plasma glucose (PG) reductions. However, phthalazinone **5b** (R = H) showed interesting glucose reduction (54%), but no triglycerides reduction at 10 mg kg^{-1} dose whereas **5a** ($R = CH_3$) showed both impressive plasma glucose (61%) and triglyceride (40%) lowering activity. The benzoxazinone derivative of thiazolidinedione **10** was prepared as its sodium salt **11** and it showed a moderate plasma glucose reduction (37%), but impressive triglycerides reduction (60%). In vitro PPARγ transactivation assay of **5a** showed more potent activity than **11** (11-fold vs. 6-fold at 1 μM). In view of our interest in potent PPARγ agonist, thiazolidinediones having both sugar and triglyceride lowering activity, we further explored the phthalazinone series. In view of the propensity of the racemisation of the C-5 chiral centre of thiazolidine-2,4-dione at physiological pH [21], no attempts were made to synthesise these compounds in optically pure forms.

It appears that the methyl derivative of phthalazinone with a linker from one of the nitrogen atom adjacent to carbonyl **5a** (named as **PHT46**) showed a better profile compared to other derivatives. Substitution by phenyl (**5d**) or ethyl (**5c**) groups leads to the loss of both glucose and triglycerides lowering activity. Interestingly, the substitution by hydrogen (**5b**) retains the glucose lowering activity but not the triglycerides lowering potential. On the other hand, interchanging the methyl group and linker positions as seen in compound **15** leads to the loss of biological activity. We believe that this could be due to the size [13, 22] of the substitution group, as methyl is smaller and more planar compared to ethyl, phenyl and a 4-position substituted compound. The unsaturated analogue **6b** showed less euglycemic activity, as compared to the saturated analogue **5**. In view of its potent in vitro and in vivo activity, **PHT46** (**5a**) was selected for further evaluation.

A comparative dose–response study was performed to evaluate the PPARγ transactivation potential of **PHT46** with troglitazone and pioglitazone as standard compounds. Interestingly, **PHT46** showed much better PPARγ activation than both the standard compounds in HEK 293T cells (*table II*). In an in vivo dose–response study in db/db mice, **PHT46** showed dose-dependent reduction in plasma glucose and triglycerides levels after 10 days of treatment (*table*

Table III. Effect of **PHT46** on plasma glucose and triglyceride levels in db/db mice.

Group	Plasma Glucose (mg/dl)	Plasma triglyceride (mg/dl)
Control	419.06 ± 19.46	135.85 ± 18.48
1 mg kg^{-1}	347.13 ± 98.13	75.42 ± 5.45
3 mg kg^{-1}	221.20 ± 58.60*	66.65 ± 6.40*
10 mg kg^{-1}	118.70 ± 5.15*	56.01 ± 1.61*

Male db/db mice were treated with **PHT46** at the doses mentioned for 10 days. Values are mean ± S.E. ($n = 5$). *$P <$ 0.05 vs. control (ANOVA).

Table IV. Dose–response studies of selected thiazolidinediones in db/db mice.

Compound	Dose ($mg\,kg^{-1}\,day^{-1}$)	Percent reduction in plasma glucose	Percent reduction in plasma triglyceride
PHT46	1	24.2 ± 3.6	33.5 ± 6.5
	3	45.1 ± 1.5	57.3 ± 3.1
	10	72.6 ± 2.6	58.5 ± 2.9
Troglitazone	100	37.2 ± 8.5	38.4 ± 9.2
	200	45.5 ± 3.5	57.2 ± 2.6
	400	59.8 ± 3.6	62.7 ± 2.8
Pioglitazone	10	19.4 ± 3.5	14.7 ± 1.9
	30	38.5 ± 2.4	36.1 ± 1.7
	100	46.2 ± 2.9	46.9 ± 1.6

Male db/db mice were treated with the compounds orally at the doses mentioned for 10 days. Values are mean ± S.E. ($n = 5$). Percentage reduction was calculated as mentioned in *table I*.

III). This study indicates that the treatment with **PHT46** brings down the plasma glucose to the normal level. **PHT46** was compared with troglitazone and pioglitazone in a 10-day dose–response study in db/db mice. **PHT46** at 3 $mg\,kg^{-1}$ dose showed similar efficacy to pioglitazone at 100 $mg\,kg^{-1}$ and troglitazone at 200 $mg\,kg^{-1}$ doses (*table IV*). **PHT46** was also compared with rosiglitazone at maximal effective dose in a 10-day treatment schedule in db/db mice. In this study, **PHT46** at 10 $mg\,kg^{-1}$ dose showed 72% reduction of plasma glucose and 58% reduction of triglyceride, whereas rosiglitazone at 30 $mg\,kg^{-1}$ showed 67% reduction in plasma glucose and 40% reduction in triglyceride. These studies indicate that **PHT46** is more potent and efficacious than the standard compounds.

Subchronic (14 days) oral administration of **PHT46** to Wistar rats did not show any significant change in body weight (control male rats: 192±7; female rats: 144±5; **PHT46** treated male rats: 191±8; female rats: 150±5 g) and food consumption throughout the experiment. The haematology examination after 14 days showed non-significant reduction in haemoglobin (6%) and packed cell volume (5%). There was a non-significant increase (6%) of heart and liver weight. The changes observed in haematology and organ weights were within the acceptable limit. The biochemical and histological findings are not suggestive of any treatment related pathology.

To check the systemic exposure of **PHT46**, a single dose oral pharmacokinetic study was performed in male Wistar rats, which indicated that the compound has good oral exposure and pharmacokinetic parameters (*table V*, *figure 5*).

5. Conclusion

Insulin resistance is considered as the primary defect in NIDDM patients. Insulin resistance not only leads to hyperglycemia, but also to hyperlipidemia. Thiazolidinediones are known to ameliorate insulin resistance through their action on PPARγ. In this communication, we have shown that our phathalazinone derivatives of thiazolidinediones have interesting insulin sensitising property. **PHT46**, the best compound in this series is a potent PPARγ activator and shows both glucose and triglycerides lowering activity. The compound shows better efficacy than the reference thiazolidinediones (i.e. troglitazone, pioglitazone and rosiglitazone) in both in vitro and in vivo animal studies. Subchronic toxicity study in Wistar rats did not show any treatment related adverse effect. It is important to note that an efficient control of triglyceride levels in type 2 diabetic patients is considered to be beneficial in diabetes related complications, especially atherosclerosis and cardiovascular disease, as such a drug that ameliorates insulin resistance and also lowers triglyceride is preferable for treatment of

Table V. Pharmacokinetic parameters of **PHT46** in Wistar rats.

$AUC_{(0-\infty)}$ ($\mu g\,h\,mL^{-1}$)	13.17
C_{max} ($\mu g\,mL^{-1}$)	4.15
T_{max} (h)	3.00
K_{el} (h^{-1})	0.42
$t_{1/2}$ (h)	1.67

Male Wistar rats were administered with a single dose of **PHT46** at 10 $mg\,kg^{-1}$, p.o. Each value is mean from $n = 3$ animals.

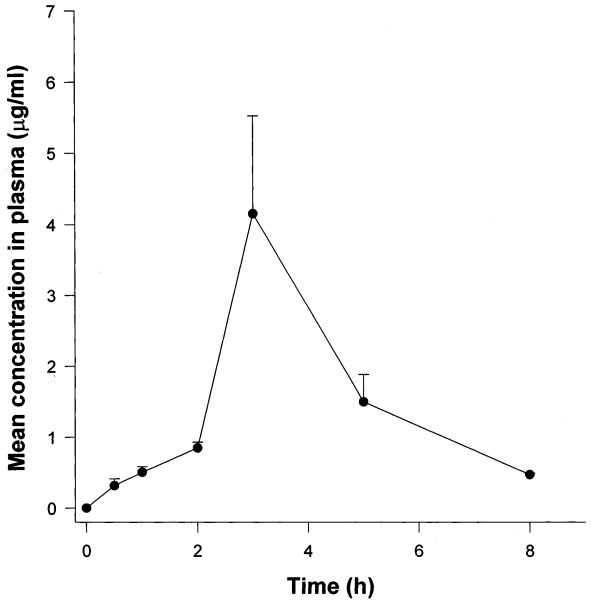


Figure 5. Plasma concentration profile of **PHT46**. Male Wistar rats were treated with 10 mg kg^{-1} of **PHT46** and plasma samples were collected at different time points. Values are expressed as mean ± S.D. ($n = 3$).

type 2 diabetes. Therefore, **PHT46** by virtue of its potent antidiabetic and triglyceride lowering property is a potential drug for the treatment of metabolic syndrome. Further developmental work is in progress.

6. Experimental protocols

6.1. Synthesis

Thin layer chromatography was performed on precoated silica gel plates (F254, Merck). Chromatography was performed on silica gel (SRL 100–200 mesh). Melting points were recorded in a Veego Melting Point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer and are reported as parts per million (ppm) downfield to TMS. The infrared spectra were recorded in a Perkin–Elmer FT-IR 1600 spectrometer. 4-(2-Bromoethoxy)benzaldehyde (**1**) and phthalazinones [15, 16] were prepared according to reported literature methods [17], while 2(1*H*)-phthalazinone was obtained from Merck.

6.1.1. General method of preparation of 4-(2-heterocyclyl ethoxy)benzaldehyde

To a stirred suspension of K_2CO_3 (82.5 mmol) in anhydrous dimethylformamide (50 mL) at 25–30 °C was added a solution of the respective phthalazinone (41.25 mmol) in dry DMF (100 mL). The reaction mixture was stirred for 30 min at 25 °C and 4-(2-bromoethoxy)benzaldehyde (**1**) (41.25 mmol) was added. The reaction mixture was immersed in a pre-heated oil bath at 70 °C and stirred at 65–70 °C for 24 h. The reaction mixture was cooled to room temperature (r.t.) and water (50 mL) was added. The reaction mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were washed with water and brine. Drying the organic extract over anhydrous Na_2SO_4 followed by concentration afforded the crude solids, which were recrystallised from methanol to afford the pure aldehydes in good yields.

6.1.2. 4-[2-[4-Methyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]benzaldehyde (*3a*)

This compound was prepared by the general procedure using 4-methyl-1(2*H*)phthalazinone (6.6 g, 41.25 mmol), aldehyde **1** (9.4 g, 41.25 mmol) and K_2CO_3 (11.3 g, 82.5 mmol) in 77% yield (9.7 g), m.p.: 90 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.50$ (s, 3H), 4.50 (t, $J = 5.66$ Hz, 2H), 4.64 (t, $J = 5.66$ Hz, 2H), 7.03 (d, $J = 8.50$ Hz, 2H), 7.20–7.90 (m, 5H), 8.46 (d, $J = 8.10$ Hz, 1H), 9.85 (s, 1H).

6.1.3. 4-[2-[1-Oxo-1,2-dihydrophthalazin-2-yl]ethoxy]benzaldehyde (*3b*)

This compound was prepared by the general procedure using 1(2*H*)-phthalazinone (0.8 g, 5.48 mmol), aldehyde **1** (1.25 g, 5.48 mmol) and K_2CO_3 (1.5 g, 10.96 mmol) in 44% yield (0.7 g). ^{1}H-NMR ($CDCl_3$): $\delta = 4.53$ (t, $J = 5.70$ Hz, 2H), 4.69 (t, $J = 5.70$ Hz, 2H), 7.04 (d, $J = 8.72$ Hz, 2H), 7.69–7.87 (m, 5H), 8.19 (s, 1H), 8.46 (d, $J = 8.10$ Hz, 1H), 9.87 (s, 1H).

6.1.4. 4-[2-[4-Ethyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]benzaldehyde (*3c*)

This compound was prepared by the general procedure using 4-ethyl-1(2*H*)-phthalazinone (0.45 g, 2.6 mmol), aldehyde **1** (0.6 g, 2.6 mmol) and K_2CO_3 (0.72 g, 5.2 mmol) in 54% yield (0.45 g), m.p.: 110 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 1.36$ (t, $J = 7.05$ Hz, 3H), 2.98 (q, $J = 7.05$ Hz, 2H), 4.50 (t, $J = 5.70$ Hz, 2H), 4.68 (t, $J = 5.70$ Hz, 2H), 7.02 (d, $J = 8.72$ Hz, 2H), 7.70–7.92 (m, 5H), 8.51 (d, $J = 8.10$ Hz, 1H), 9.21 (s, 1H).

*6.1.5. 4-[2-[4-Phenyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]benzaldehyde (**3d**)*

This compound was prepared by the general procedure using 4-phenyl-1(2*H*)-phthalazinone (1.30 g, 5.85 mmol), aldehyde **1** (1.34 g, 5.85 mmol) and K_2CO_3 (1.60 g, 11.7 mmol) in 85% yield (2.0 g), m.p.: 136 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 4.56$ (t, $J = 5.70$ Hz, 2H), 4.75 (t, $J = 5.70$ Hz, 2H), 7.03 (d, $J = 8.72$ Hz, 2H), 7.55 (bs, 5H), 7.65–7.85 (m, 5H), 8.55 (d, $J = 8.10$ Hz, 1H), 9.86 (s, 1H).

*6.1.6. 4-[2-[4-Oxo-3,4-dihydro-(1H)-2,3-benzoxazin-3-yl]ethoxy]benzaldehyde (**8**)*

This compound was prepared by the general procedure using 4-oxo-3,4-dihydro-(1*H*)-2,3-benzoxazin (0.28 g, 1.87 mmol), aldehyde **1** (0.43 g, 1.87 mmol) and K_2CO_3 (0.52 g, 3.74 mmol), m.p.: 102 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 4.23$ (t, $J = 5.0$ Hz, 2H), 4.35 (t, $J = 5.0$ Hz, 2H), 5.11 (s, 2H), 7.04 (d, $J = 8.70$ Hz, 2H), 7.10 (d, $J = 7.00$ Hz, 1H), 7.40–7.60 (m, 2H), 7.80 (d, $J = 8.70$ Hz, 2H), 8.10 (d, $J = 7.20$ Hz, 1H), 9.89 (s, 1H).

*6.1.7. 4-[[2-Methyl-1-oxo-1,2-dihydrophthalazin-4-yl]methoxy]benzaldehyde (**13**)*

To a solution of 4-hydroxymethyl-2-methyl-1(2*H*)phthalazinone (0.35 g, 1.84 mmol) in dry DMF (30 mL) was added NaH (0.088 g, 3.68 mmol) in portions over 30 min at 25–30 °C. This was followed by dropwise addition of 4-fluorobenzaldehyde (0.228 g, 1.84 mmol) at 0–15 °C and the resulting reaction mixture was stirred at r.t. for 6 h. Ice (200 g) was added to the reaction mixture which was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated to afford the title compound **13** (0.3 g, 50%), m.p.: 168–170 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 3.88$ (s, 3H), 5.39 (s, 2H), 7.18 (d, $J = 8.72$ Hz, 2H), 7.72–8.00 (m, 5H), 8.48 (d, $J = 8.10$ Hz, 1H), 9.92 (s, 1H).

*6.1.8. General method to prepare 5-[4-[2-(heterocyclyl)-ethoxy]phenylmethylene]thiazolidine-2,4-dione (**4**)*

A mixture of 4-[2-(heterocyclyl)ethoxy]benzaldehyde **3** (10 mmol), thiazolidine-2,4-dione (10 mmol), benzoic acid (1.3 mmol) and piperidine (1.5 mmol) in 25 mL of toluene was refluxed for 1 h with continuous removal of water using a Dean Stark water separator. The reaction mixture was cooled to r.t. and the resultant crystalline compound filtered, washed with water and dried to afford the pure compound **4** (70–99%).

*6.1.9. 5-[4-[2-[4-Methyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (**4a**)*

This compound (3.3 g, 91%) was prepared according to the general procedure using **3a** (2.75 g, 8.93 mmol) and thiazolidine-2,4-dione (1.05 g, 8.93 mmol), m.p.: 202 °C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.60$ (s, 3H), 4.48 (t, $J = 5.30$ Hz, 2H) 4.55 (t, $J = 5.30$ Hz, 3H), 6.95 (d, $J = 8.30$ Hz, 2H), 7.40 (d, $J = 8.30$ Hz, 2H), 7.72 (s, 1H), 7.75–7.90 (m, 3H), 8.49 (d, $J = 8.10$ Hz, 1H).

*6.1.10. 5-[4-[2-[1-Oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethylene]thiazolidin-2,4-dione (**4b**)*

This compound (533 mg, 80%) was prepared according to the general procedure using **3b** (500 mg, 1.7 mmol) and thiazolidine-2,4-dione (200 mg, 1.7 mmol), m.p.: 242 °C. ^{1}H-NMR (DMSO+$CDCl_3$): $\delta = 4.50$ (t, $J = 5.25$ Hz, 2H), 4.58 (t, $J = 5.25$ Hz, 2H), 7.05 (d, $J = 8.72$ Hz, 2H), 7.46 (d, $J = 8.72$ Hz, 2H), 7.88–7.98 (m, 3H), 8.06 (s, 1H), 8.33 (d, $J = 7.20$ Hz, 1H), 8.35 (s, 1H). 12.3 (bs, 1H, D_2O exchangeable).

*6.1.11. 5-[4-[2-[4-Ethyl-1,2-dihydrophthalazine-2-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (**4c**)*

This compound (432 mg, 82%) was prepared according to the general procedure using **3c** (400 mg, 1.24 mmol) and thiazolidine-2,4-dione (145 mg, 1.24 mmol), m.p.: 230 °C. ^{1}H-NMR (DMSO+$CDCl_3$): $\delta = 1.31$ (t, $J = 7.38$ Hz, 3H), 2.98 (q, $J = 7.38$ Hz, 2H), 4.38–4.65 (m, 4H), 7.05 (d, $J = 8.72$ Hz, 2H), 7.45 (d, $J = 8.72$ Hz, 2H), 7.80–7.98 (m, 3H), 7.80–7.98 (m, 3H), 8.09 (s, 1H), 8.35 (d, $J = 7.15$ Hz, 1H), 12.34 (bs, 1H, D_2O exchangeable).

*6.1.12. 5-[4-[2-[4-Phenyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (**4d**)*

This compound (1.10 g, 87%) was prepared according to the general procedure using **3d** (1.0 g, 2.7 mmol) and thiazolidine-2,4-dione (316 mg, 2.7 mmol), m.p.: 224 °C. ^{1}H-NMR (DMSO+$CDCl_3$): $\delta = 4.55$ (t, $J =$ 5.25 Hz, 2H), 4.65 (t, $J = 5.25$ Hz, 2H), 7.04 (d, $J = 8.72$ Hz, 2H), 7.44 (d, $J = 8.72$ Hz, 2H), 7.40–7.60 (m, 5H), 7.60–7.89 (m, 3H), 7.96 (s, 1H), 8.45 (t, $J = 7.30$ Hz, 1H), 12.20 (bs, 1H, D_2O exchangeable).

*6.1.13. 5-[4-[2-[4-Oxo-3,4-dihydro-1H-2,3-benzoxazin-3yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (**9**)*

This compound (310 mg, 67%) was prepared according to the general procedure using **8** (350 mg, 1.18 mmol) and thiazolidine-2,4-dione (138 mg, 1.18 mmol), m.p.: 182 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 4.18$ (t, $J = 5.40$

Hz, 2H), 4.35 (t, $J = 5.40$ Hz, 2H), 5.14 (s, 2H), 7.04 (d, $J = 8.70$ Hz, 2H), 7.20 (d, $J = 7.40$ Hz, 1H), 7.44 (d, $J = 8.70$ Hz, 2H), 7.50–7.60 (m, 2H), 7.71 (s, 1H), 8.02 (d, $J = 7.40$ Hz, 1H), 12.15 (bs, 1H, D_2O exchangeable).

6.1.14. 5-[4-[[2-Methyl-1-oxo-1,2-dihydrophthalazin-4-yl]methoxy]phenylmethylene]thiazolidine-2,4-dione ***(14)***

This compound (yield: 250 mg, 98%) was prepared according to the general procedure using **13** (190 mg, 0.65 mmol) and thiazolidine-2,4-dione (75 mg, 0.65 mmol), m.p.: 262 °C. ^{1}H-NMR ($CDCl_3$+DMSO-d_6): $\delta = 3.79$ (s, 3H), 5.47 (s, 2H), 7.28 (d, $J = 8.72$ Hz, 2H), 7.62 (d, $J = 8.72$ Hz, 2H), 7.70 (s, 1H), 7.90–8.15 (m, 3H), 8.35 (d, $J = 7.25$ Hz, 1H).

6.1.15. General procedure for preparation of 5-[4-[2-[heterocyclyl]ethoxy]phenylmethyl]thiazolidine-2,4-diones ***(5)***

A solution of thiazolidine-2,4-dione **4** (1.0 g) in 1,4-dioxane (35 mL) was hydrogenated in the presence of 10% Pd/C (2.0 g) at 60 PSI for 36–60 h at r.t. The mixture was filtered through a bed of celite and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel using a mixture of ethyl acetate and petroleum ether as an eluent to yield pure compound **5**.

6.1.16. 5-[4-[2-[4-Methyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione ***(5a)***

This compound was prepared according to the general procedure using **4a** (9.0 g, 22 mmol) and 10% Pd/C (18 g) in 80% yield (7.2 g), m.p.: 170 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 2.60$ (s, 3H), 3.07 (dd, $J = 14.12$, 9.50 Hz, 1H), 3.42 (dd, $J = 14.12$, 3.70 Hz, 1H), 4.40 (t, $J = 6.10$ Hz, 2H), 4.46 (dd, $J = 9.50$, 3.70 Hz, 1H), 4.61 (t, $J = 6.10$ Hz, 2H), 6.88 (d, $J = 8.50$ Hz, 2H), 7.11 (d, $J = 8.50$ Hz, 2H), 7.68–7.91 (m, 3H), 8.51 (d, $J = 8.10$ Hz, 1H).

6.1.17. 5-[4-[2-[1-Oxo-1,2-dihydrophthalazin-2-yl]-ethoxy]phenylmethyl]thiazolidine-2,4-dione ***(5b)***

This compound was prepared by the general procedure using **4b** (385 mg, 0.98 mmol) and 10% Pd/C (770 mg) in 52% yield (200 mg), m.p.: 165 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 3.08$ (dd, $J = 14.12$, 9.32 Hz, 1H), 3.40 (dd, $J = 14.12$, 3.97 Hz, 1H), 4.42 (t, $J = 5.81$ Hz, 2H), 4.46 (dd, $J = 9.32$, 3.97 Hz, 1H), 4.65 (t, $J = 5.81$ Hz, 2H), 6.88 (d, $J = 8.72$ Hz, 2H), 7.11 (d, $J = 8.72$ Hz, 2H), 7.60–7.90 (m, 3H), 8.07 (bs, 1H, D_2O exchangeable), 8.20 (s, 1H), 8.45 (d, $J = 8.62$ Hz, 1H).

6.1.18. 5-[4-[2-[4-Ethyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione ***(5c)***

This compound was prepared according to the general procedure using **4c** (430 mg, 1.02 mmol) and 10% Pd/C (860 mg) in 46% yield (200 mg), m.p.: 174 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 1.33$ (t, $J = 7.50$ Hz, 3H), 2.96 (q, $J = 7.50$ Hz, 2H), 3.04 (d, $J = 14.11$, 9.59 Hz, 1H), 3.40 (dd, $J = 14.11$, 3.92 Hz, 1H), 4.32 (t, $J = 5.90$ Hz, 2H), 4.42 (dd, $J = 9.59$, 3.92 Hz, 1H), 4.59 (t, $J = 5.90$ Hz, 2H), 6.85 (d, $J = 8.72$ Hz, 2H), 7.08 (d, $J = 8.72$ Hz, 2H), 7.60–7.80 (m, 3H), 8.02 (bs, 1H, D_2O exchangeable), 8.46 (d, $J = 8.41$ Hz, 1H).

6.1.19. 5-[4-[2-[4-Phenyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione ***(5d)***

This compound was prepared according to the general procedure using **4d** (1.0 g, 2.1 mmol) and 10% Pd/C (2.0 g) in 60% yield (600 mg), m.p.: 198 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 3.07$ (1H, dd, $J = 14.12$, 9.6 Hz), 3.44 (dd, $J = 14.12$, 3.74 Hz, 1H), 4.42 (dd, $J = 9.60$, 3.74 Hz, 1H), 4.46 (t, $J = 5.90$ Hz, 2H), 4.72 (t, $J = 5.90$ Hz, 2H), 6.89 (d, $J = 8.62$ Hz, 2H), 7.11 (d, $J = 8.62$ Hz, 2H), 7.45–7.70 (m, 5H), 7.70–7.90 (m, 3H), 8.00 (bs, 1H, D_2O exchangeable), 8.55 (d, $J = 8.30$ Hz, 1H).

6.1.20. 5-[4-[[2-Methyl-1-oxo-1,2-dihydrophthalazin-4-yl]methoxy]phenylmethyl]thiazolidine-2,4-dione ***(15)***

This compound was prepared according to the general procedure using **14** (1.0 g, 2.54 mmol) and 10% Pd/C (2.0 g) in 50% yield (500 mg), m.p.: 234 °C. ^{1}H-NMR ($CDCl_3$): $\delta = 3.08$ (dd, $J = 14.11$, 9.44 Hz, 1H), 3.46 (dd, $J = 14.11$, 3.74 Hz, 1H), 3.86 (s, 3H), 4.46 (dd, $J = 9.44$, 3.74 Hz, 1H), 5.28 (s, 2H), 6.99 (d, $J = 8.12$ Hz, 2H), 7.19 (d, $J = 8.12$ Hz, 2H), 7.88–7.92 (m, 2H), 7.99 (d, $J = 7.30$ Hz, 1H), 8.42 (d, $J = 7.30$ Hz, 1H), 11.70 (bs, 1H, D_2O exchangeable).

6.1.21. 5-[4-[2-[4-Oxo-3,4-dihydro-1H-2,3-benzoxazin-3-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione ***(10)***

This compound (310 mg, 67%) was prepared as a gummy oil according to the general procedure using **9** (250 mg, 0.63 mmol) and 10% Pd/C (500 mg) in 40% yield (100 mg), m.p.: 140 °C. ^{1}H-NMR ($CDCl_3$): $\delta =$ 3.12 (dd, $J = 14.11$, 4.89 Hz, 1H), 3.41 (dd, $J = 14.11$, 3.73 Hz, 1H), 4.18 (t, $J = 4.50$ Hz, 2H), 4.24 (t, $J = 4.50$ Hz, 2H), 4.50 (dd, $J = 9.22$, 3.83 Hz, 1H), 5.09 (s, 2H), 6.88 (d, $J = 8.60$ Hz, 2H), 7.21 (d, $J = 8.60$ Hz, 2H), 7.40–7.60 (m, 3H), 8.00 (bs, 1H, D_2O exchangeable), 8.08 (d, $J = 7.20$ Hz, 1H).

*6.1.22. Sodium salt of 5-[4-[2-[4-methyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethylene]-thiazolidine-2,4-dione (**6b**)*

To 5-[4-[2-[4-methyl-1-oxo-1,2-dihydrophthalazin-2-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (**4**) (200 mg, 0.49 mmol) in dry methanol (5 mL) was added NaOMe (freshly prepared from 45 mg of Na metal in 1 mL of dry methanol) and the mixture stirred at r.t. for 1 h. The reaction mixture was diluted with 40 mL of dry diethylether and the white precipitate thus formed was filtered and washed with methanol–ether (1:3) and dried under vacuum, to give a white compound **6b** (180 mg, 86%), m.p.: 280 °C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.60$ (s, 3H), 4.42 (t, $J = 5.20$ Hz, 2H), 4.50 (t, $J = 5.20$ Hz, 2H), 6.94 (d, $J = 8.72$ Hz, 2H), 7.38 (d, $J = 8.72$ Hz, 2H), 7.70–7.90 (m, 3H), 7.88 (s, 1H), 8.34 (d, $J = 7.13$ Hz, 1H).

*6.1.23. Sodium salt of 5-[4-[2-[4-oxo-3,4-dihydro-1H-2,3-benzoxazin-3-yl]ethoxy]phenylmethyl]-thiazolidine-2,4-dione (**11**)*

This compound (50 mg, 48%) was prepared according to the previous method using **10** (100 mg, 0.344 mmol) and NaOMe (0.688 mmol), m.p.: 255 °C. ^{1}H-NMR (DMSO-d_6): $\delta = 2.65$ (dd, $J = 10.47$, 3.23 Hz, 1H), 2.55 (dd, $J = 10.47$, 4.27 Hz, 1H), 4.00–4.19 (m, 3H), 4.21 (t, $J = 5.07$ Hz, 2H), 5.15 (s, 2H), 6.85 (d, $J = 8.38$ Hz, 2H), 7.10 (d, $J = 8.38$ Hz, 2H), 7.34 (d, $J = 7.47$ Hz, 1H), 7.45–7.70 (m, 2H), 7.91 (d, $J = 7.47$ Hz, 1H).

6.2. Pharmacological methods

6.2.1. Materials

The standard compounds, troglitazone and pioglitazone, were synthesised in house by the published procedure and were found to be 99% pure. Plasma glucose and triglyceride were measured spectrophotometrically using commercially available kits (Point Scientific, USA). Carboxymethylcellulose (CMC) was obtained from LOBA Chemicals Pvt. Ltd, Mumbai, India.

6.2.2. Animals and treatment

Male C57 BL/Ks J-db/db mice were from the breeding stock of the DRF animal house generated from an original stock of Jackson Laboratories, Maine, USA. All animals were maintained under 12 h light and 12 h dark cycles at 25±1 °C. All animals were given standard chow (National Institute of Nutrition, India) and water ad libitum. All animal experiments were carried out in accordance with internationally valid guidelines. All experimental protocols were approved by the DRF animal ethics committee.

In preliminary studies db/db mice (8–9 weeks) were treated with drugs (10 mg kg^{-1}) for 6 days. Animals in control groups received vehicle only (0.25% CMC, 10 mL kg^{-1}). During the dose–response study, db/db mice were treated with **PHT46** at 1, 3 and 10 mg kg^{-1} doses for 10 days. Blood samples were collected from animals (in fed state) under mild ether anaesthesia from retro-orbital sinus 1 h after drug administration. Plasma samples were separated for glucose, triglycerides, free fatty acids and insulin measurement.

For the subacute toxicity study, 6–8 week old Wistar rats (obtained from National Institute of Nutrition, Hyderabad) of either sex (110–160 g) were divided randomly into two groups, each consisting of four males and four females. **PHT46** was administered orally daily at 100 mg kg^{-1} dose, while the control group received the vehicle only. On the day of termination (14th day), blood was collected from retro-orbital sinus under light ether anaesthesia for haematological parameters followed by autopsy and macroscopic examination.

6.2.3. PPAR transactivation assay

The response element (UASGAL4×5) is present upstream of pFR-Luc reporter (Promega, WI, USA) that contains Simian virus early promoter for luciferase assay. GAL4 fusions were made by fusing the human PPARγ1 ligand binding domain (amino acids: 174–475) to the C-terminal end of the yeast GAL4 DNA binding domain (amino acids: 1–147) of the pM1 vector. The pAdVantage vector was used to enhance luciferase expression.

HEK 293T cells were transfected with relevant plasmids by superfect as per the instruction manual [23]. Forty-two hours after transfection, cells were treated for 18 h with the test compounds. DMSO (1:1000) was used as blank. Luciferase activity was determined as fold activation relative to untreated cells by using a Luclite kit (Packard Instrument Co, Meriden, CT, USA) in a Packard Top Count (Packard Instrument Co.).

6.2.4. Pharmacokinetic studies

All studies were carried out in male Wistar rats obtained from the National Institute of Nutrition (Hyderabad, India). The animals (200–225 gm) were fasted 12 h before starting the experiment and they had free access to water throughout the experiment period. Animals were fed 3 h after drug administration.

6.2.4.1. Single dose pharmacokinetics

Animals were administered the drug at 10 mg kg^{-1} per orally as 0.5% CMC suspension and about 0.30 mL of blood sample was collected into heparinised microfuge tubes at different time points from retro-orbital sinus. To 0.1 mL of plasma, internal standard (another thiazolidinedione) was added and drugs were extracted with a suitable solvent mixture. The solvent was evaporated and the residue was reconstituted with a mobile phase and injected into the HPLC system. The samples were analysed by reverse-phase HPLC to generate plasma concentration time profiles.

Pharmacokinetic parameters such as $AUC_{(0-\infty)}$, K_{el}, half-life, C_{max} and t_{max} were calculated using non-compartmental model analysis. $AUC_{(0-\infty)}$ is the area under the plasma concentration versus time curve extrapolated to infinity, K_{el} is the elimination rate constant, C_{max} is the observed maximum plasma concentration and t_{max} is the time at which maximum concentration (C_{max}) is reached.

6.2.4.2. HPLC assay

The HPLC system includes a Waters LC Module-1, Millennium software and a Suplecosil C_{18} (ODS) column (5 μm, 4.6 mm×250 mm). Analysis of **PHT46** was carried out using 0.05 M NaH_2PO_4 buffer (pH 4.0)/methanol (30:70) as the mobile phase at a flow rate of 1 mL min^{-1}. The eluate from the column was monitored by a UV detector (Waters LC Module) set at 280 nm. Under these conditions retention times for **PHT46** and IS were, respectively, 13.7 and 9.9 min. Absolute recovery was >95%, the limit of quantification was 0.1 μg mL^{-1} and the response was linear up to 50 μg mL^{-1}.

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