Bioorganic & Medicinal Chemistry Letters 22 (2012) 5857–5862

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of liver selective non-steroidal glucocorticoid receptor antagonist as novel antidiabetic agents ☆

Kiran Shah [a,b], Dipam Patel [a], Pradip Jadav [a], Mubeen Sheikh [a], Kalapatapu V. V. M. Sairam [a], Amit Joharapurkar [a], Mukul R. Jain [a], Rajesh Bahekar [a,*]

[a] Zydus Research Centre, Sarkhej-Bavla N.H. 8A Moraiya, Ahmedabad 382210, India
[b] Department of Chemistry, Faculty of Science, M.S. University of Baroda, Vadodara 380002, India

ARTICLE INFO

Article history:
Received 7 May 2012
Revised 17 July 2012
Accepted 24 July 2012
Available online 31 July 2012

Keywords:
Glucocorticoid
Diabetes
Antagonist
Non-steroidal
Liver Selective

ABSTRACT

Series of benzyl-phenoxybenzyl amino-phenyl acid derivatives (**8a**–**q**) are reported as non-steroidal GR antagonist. Compound **8g** showed excellent h-GR binding and potent antagonistic activity (in vitro). The lead compound **8g** exhibited significant oral antidiabetic and antihyperlipidemic effects (in vivo), along with liver selectivity. These preliminary results confirm discovery of potent and liver selective passive GR antagonist for the treatment of T2DM.

In healthy person, glucose homeostasis is tightly controlled by two counter-regulating hormones; insulin (mediates glucose disposal) and glucagon (regulates hepatic glucose production (HGP)). Any imbalance between these two counter-regulating hormones results in hypo- or hyperglycemia (Type 2 diabetes mellitus; T2DM).[1]

Glucocorticoid receptor (GRα/β) is a member of the nuclear receptor (NR) super family that functions as ligand mediated transcription factors to control gene expression.[2] The GR consists of 3 domains, N-terminal trans-activation domain (Activation Function domain; AF-1), the central DNA Binding Domain (DBD) and the Ligand Binding Domain (LBD/Ligand-dependent Activation Function domain; AF-2).[3] The GR-LBD consists of twelve helices and four β-stands. Helices 4, 5, 8 and 9 create a cavity (binding pocket) where the ligand can bind, while helice-12 (H12) adopts close or open confirmations depending upon GR-interactions with agonist/antagonist respectively.[4] Activation of GR leads to either positive (trans-activation) or negative (trans-repression) regulation of gene expressions.[2e] GR agonists have been explored primarily as anti-inflammatory agents.[5] In contrast, the therapeutic potential of GR antagonist remains largely unexploited despite having a strong rational for its role in metabolic disorders.[6]

Glucocorticoids (GCs) raise blood glucose levels by antagonizing insulin action, thereby inhibits glucose disposal and promotes HGP (increase gluconeogenesis via PEPCK, TAT and G6Pase).[7] GR antagonism has been validated as a promising therapeutic target for regulating HGP, in animal models and humans, using a non-selective steroidal GR antagonist mifepristone (RU 486; **1**; Fig. 1).[8] However, the side-effects (hypercortisolemia and symptoms of adrenal insufficiency) of mifepristone are significant, including its abortifacient activity.[9] Thus, long-term systemic GR antagonism with a non-selective steroidal GR antagonist may not be viable approach for the treatment of T2DM.[10] In contrast, non-steroidal pharmacophores offers better opportunity to achieve improved therapeutic index and desirable selectivity over other NR.[11] Recently number of non-steroidal GR antagonists has emerged (compound **2** and **3**; Fig. 1), which showed reduced activity on the hypothalamic-pituitary-adrenal (HPA) axis while maintaining substantial antidiabetic activity.[12,13]

Ligands that compete with agonist GR binding and agonist receptor-complex DNA response element binding are called active antagonists, while ligands that only compete for agonist GR binding are known as passive antagonists.[14] Compound **2** (dibenzylamino-2-alkyl-phenyl-methanesulfonamide) represent passive GR antagonist, while compound **3** (benzoyl-phenoxy-phenyl acetic acid; KB285) has been documented as liver selective GR antagonist.[12,13]

In T2DM, therapeutic utility of systemic GR antagonist have been hampered by general blockage of GC action in all tissues.[15]

☆ ZRC communication no: 400 (*Part of PhD thesis work of Mr. Kiran Shah*).

* Corresponding author. Tel.: +91 2717 665555; fax: +91 2717 665355.
E-mail address: rajeshbahekar@zyduscadila.com (R. Bahekar).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.07.078

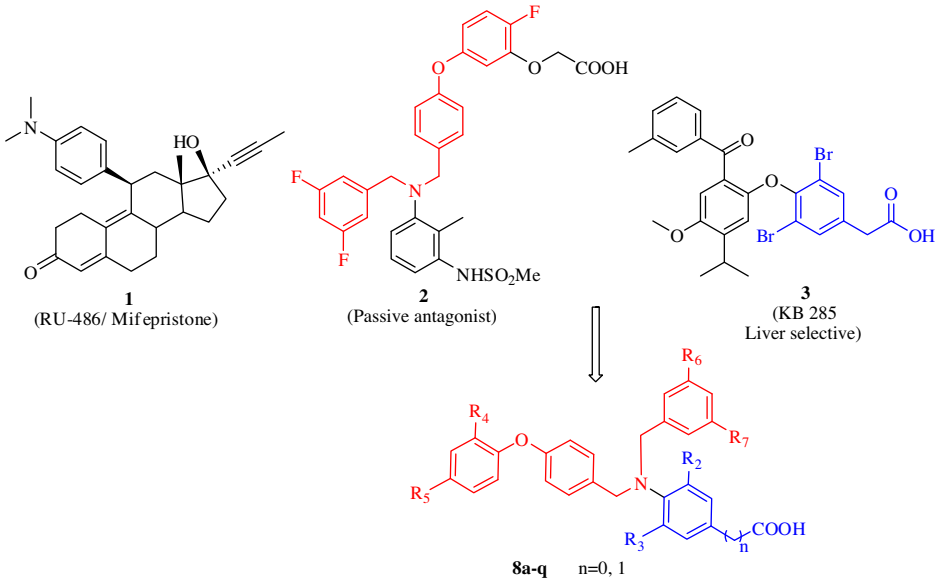
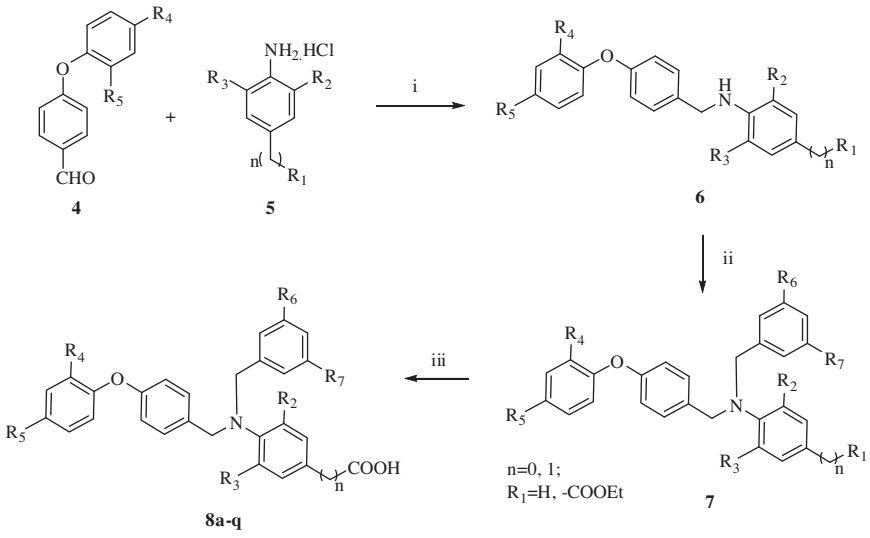


Figure 1. Selected GR antagonist and design strategy of compounds **8a–q**



Scheme 1. Synthesis of compounds **7** and **8a–q**. Reagents and conditions: (i) AcOH, DCE, 85 °C,16 h; $NaBH(OAc)_3$, 26 °C, 24 h, 80%; (ii) Bzl-Br, i-Pr_2NEt, DMF, 90 °C, 12 h, 78%; (iii) LiOH, THF–H_2O, 26 °C, 90%.

In contrast a liver-specific GR antagonist would be expected to decrease HGP and improve glucose disposal with minimal risk of peripherally driven side effects.[16] The above considerations have led us to design liver-selective non-steroidal GR antagonist as a novel therapeutic approach for the effective treatment of T2DM.

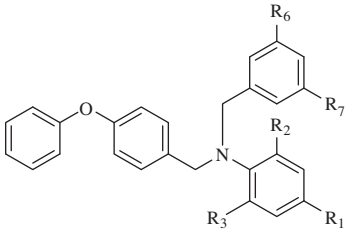
Liver-selective non-steroidal GR antagonists (**8a–q**) were designed as hybrid of compounds **2** and **3** (Fig. 1), wherein tolylmethane sulfonamide of compound **2** was specifically replaced with phenyl acetic acid (acid functionality as a liver selective component of compound **3**).[12,13] Also oxyacetic acid component along with tolylmethane sulfonamide of compound **2** was not introduced in new series to overcome oral bioavailability (hydrophilicity due to di-acids) and sulfonamide instability issues.[17]

All the compounds were tested for their binding affinity to the h-GR ([^{3}H]-dexamethasone radioligand binding assay) and the functional antagonistic activities were determined using GRAF (inhibition of dexamethasone-induced ALP expression) and TAT (inhibition of dexamethasone induced TAT expression in freshly isolated rat hepatocytes) assays.[18] Furthermore, based on the in vitro results, highly potent and selective compound (**8g**) was subjected in vivo, to assess its antidiabetic effect and pharmacokinetic (PK) profile.

Synthesis of the title compounds **8a–q** was carried out as depicted in Scheme 1, following the modified literature procedure.[19] Treatment of substituted phenoxy benzaldehydes (**4**) with aniline (**5**) in the presence of acetic acid lead to the formation of Schiff base (in-situ).[20] Reductive amination of Schiff base with sodium triacetoxyborohydride ($NaBH(OAc)_3$) provides substituted phenoxybenzyl aniline (**6**). Alkylation of the **6** with substituted benzylbromide, in presence of base (ethyl diisopropylamine (iPr_2NEt)) yields substituted *N*-benzyl phenoxybenzyl aniline (**7**). Hydrolysis of **7** with lithium hydroxide (LiOH) provides substituted benzyl-phenoxybenzyl amino-phenyl acids (**8a–q**).

All the titled compounds and intermediates were characterized by their physical, analytical and spectral data (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined within ±0.04%

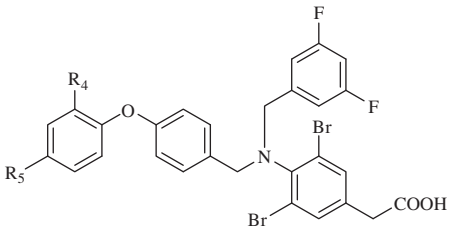
Table 1
h-GR binding IC_{50}, GRAF IC_{50} and rat TAT IC_{50} results for **7** & **8a–e**



Compds.	R_1	R_2	R_3	R_6	R_7	h-GR IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat TAT IC_{50}, μM[a]
7	H	H	H	H	H	>10000	ND	ND
8a	-COOH	H	H	H	H	>9000	ND	ND
8b	$-CH_2COOH$	H	H	H	H	1910	ND	ND
8c	$-CH_2COOH$	Br	Br	H	H	1200	ND	ND
8d	$-CH_2COOH$	Br	Br	F	H	820	ND	ND
8e	$-CH_2COOH$	Br	Br	F	F	722	1050	3.9
2[b]	Abbott (Std)					4.6	432	1.88
3[c]	KB285 (Std)					20	410	2.49

[a] Values are means of three experiments (ND = not determined).
[b] Lit. Values: h-GR, GRAF & TAT IC_{50}: 4.8 nM, 440 nM & 1.9 μM, respectively (Compd No: 12; Ref[12]JMC 2005).
[c] Lit. Values: h-GR, GRAF & TAT IC_{50}: : 19 nM, 400 nM & 2.5 μM respectively (Ref[13a]; WO199963976).

Table 2
h-GR binding IC_{50}, GRAF IC_{50} and Rat TAT IC_{50} results for **8f–q**



Compd	R_4	R_5	h-GR IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat TAT IC_{50}, μM[a]
8f	F	H	145	46	2.1
8g[d]	F	F	4.5	211	0.9
8h	Cl	H	235	512	3.0
8i	Cl	Cl	96	391	2.6
8j	Br	H	801	1171	NA
8k	Br	Br	879	1423	NA
8l	Me	H	1278	ND	ND
8m	Me	Me	1642	ND	ND
8n	OMe	H	1256	ND	ND
8o	OMe	OMe	1601	ND	ND
8p	CF_3	H	298	598	3.3
8q	CF_3	CF_3	111	421	2.9
2[b]	Abbott (Std)		4.6	432	1.88
3[c]	KB285 (Std)		20	410	2.49

[a] Values are means of three experiments (ND = not determined; NA = not active, >10 μM).
[b] Lit. Values: h-GR, GRAF & TAT IC_{50}: 4.8 nM, 440 nM & 1.9 μM, respectively (Compd No: 12; Ref[12]; JMC 2005).
[c] Lit. Values: h-GR, GRAF & TAT IC_{50}: : 19 nM, 400 nM & 2.5 μM respectively (Ref[13a]; WO199963976).
[d] InVP16-GR assay, **8g** showed no effect upto 100 μM concentrations.

of theoretical values. Overall, **8a–q** were prepared in good yield, under the mild reaction conditions. The percentage yield in the final step was found to be in the range of 60–80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **8a–q** (see Supplementary data for analytical and spectral data).

The in vitro binding affinity to the h-GR and the functional antagonistic activities were determined, to establish the Structure–Activity Relationship (SAR).[18] As shown in Table 1, initially six compounds (**7** and **8a–e**) were prepared to assess role of acid functionality (R_1), effect of halogen (Br/F) on phenyl (R_2/R_3)/ benzyl (R_6/R_7) ring system. Compound **8a** (R_1 = –COOH) showed weak binding, **8b** (R_1 = $–CH_2–COOH$) showed moderate binding, while **7** (R_1 = –H) showed weakest h-GR binding. So acidic functionally at R_1 is essential for good GR binding and compared to carboxylic acid (**8a**), acetic acid derivative (**8b**) showed improved GR binding affinity.

Among **8c-e** tested, **8c** (R_1 = $–CH_2–COOH$; R_2 & R_3 = Br) showed good binding, **8d** (R_1 = $–CH_2–COOH$; R_2 & R_3 = Br; R_6 = F) showed better binding, while **8e** (R_1 = $–CH_2–COOH$; R_2 & R_3 = Br; R_6 & R_7 = F) showed best h-GR binding (IC_{50} = 722 nM), indicated that dibromo substitutions on phenyl ring and difluoro substitutions on benzyl ring are most favorable. Compounds **8c–e** were specifically designed with R_2/R_3 and R_6/R_7 as Br and F substituents respectively, to establish and validate our primary lead (**8e**) with previously reported and optimized substituents of compounds **2** and **3** at corresponding positions.

Table 3
Pharmacokinetic (PK) study parameters[a] and liver selectivity of **8g**

Tmax (h)	Cmax (μg/ml)	$T_{1/2}$ (h)	AUC (0-∞) (h μg/ml)	%F[b]	0.5h liver level (μg/ml)	Liver to Plasma ratio
0.51 ± 0.01	0.88 ± 0.09	5.96 ± 0.61	1.39 ± 0.10	10.68	21.82 ± 1.18	24.8

[a] In male SD rats ($n = 6$), compound **8g** were administered orally (po) at 20 mpk dose and plasma concentration was analyzed by LC–MS, values indicate Mean ± SD.
[b] Oral bioavailability (%F) was calculated wrt to iv AUC (13.02 ± 3.21 h μg/ml) administered at 20 mpk dose, iv.

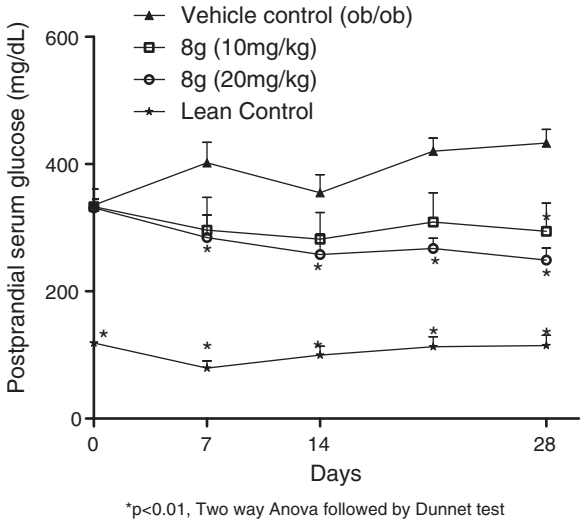


Figure 2. Antidiabetic activity of **8g** in ob/ob mice, repeated dosing

In GRAF and TAT functional assays, only primary lead (**8e**) were screened and it showed moderate GR antagonistic activities (IC_{50} = 1.05 μM (GRAF) and 3.8 μM (TAT)).[18] As a positive control, compounds **2** and **3** were subjected for in vitro GR binding and functional antagonistic activity assays and the IC_{50} values of **2** and **3** were found to be comparable with reported values (Table 1).

To convert primary lead (**8e**) to optimize lead, additional twelve compounds (**8f–q**) were prepared, mainly by incorporating suitable substitutions at R_4 and R_5 position of phenoxy ring of **8e** and subjected in vitro for GR binding and functional antagonistic assays (Table 2). Compound **8f** (R_4 = F & R_5 = H) showed significant improvement in GR binding, while **8g** (R_4 & R_5 = F) showed single digit nanomolar binding affinity (IC_{50} = 4.5 nM), which was found to be comparable with reference standard compound **2** (IC_{50} = 4.6 nM).[12] In GRAF and TAT assays, GR antagonistic activity of **8f** was found to be comparable with compounds **2** and **3**, while **8g** showed ~two-fold stronger GR antagonistic activity (IC_{50} = 211 nM (GRAF) and 0.8 μM (TAT)), compared to **2** (IC_{50} = 432 nM (GRAF) and 1.88 μM (TAT)).

Compounds **8h** and **8i** (mono and dichloro substituents) showed significant improvement in GR binding affinity and antagonistic activity (GRAF and TAT assays), compared to **8e** and among **8h** and **8i**, compound **8i** was found to be more potent. **8j** and **8k** (mono and dibromo substituents) showed no improvement in GR binding affinity and antagonistic activity (GRAF assay), compared to **8e** and among **8j** and **8k**, compound **8k** was found to be less potent, indicates that bulky substitutions at R_4 and R_5 positions are not favorable. Compounds **8l–o** (mono and di-methyl/methoxy substituents) showed no improvement in GR binding affinity, compared to **8e** and among **8l–o**, compounds **8m** and **8o** were found to be least potent, indicates that methyl/methoxy substitutions at R_4 and R_5 positions are not favorable. Compounds **8p** and **8q** (mono and di-triflurometyl substituents) showed significant improvement in GR binding affinity and antagonistic activities (GRAF and TAT assays), compared to **8e** and among **8p** and **8q**, compound **8q** was found to be more potent.

In general, substitutions with halogens (mono/di) at R_4 and R_5 positions were found to be favorable, however, increase in the size (bulk) of halogen groups (F/Cl/Br) resulted in decreased GR binding affinity and antagonistic activities. Substitutions with electron donating groups (OMe/Me) at R_4 and R_5 positions showed weak binding and antagonistic activities, relative to that of un-substituted, while substitution with electron withdrawing groups (F/Cl/CF_3) showed significant improvement in binding and antagonistic activities, relative to that of un-substituted.

Based upon in vitro binding and functional antagonistic activities results, **8g** was considered as optimized lead among eighteen compounds (**7** and **8a–q**) prepared. **8g** was tested for its binding affinity to related nuclear hormone receptors (h-PR, h-MR, h-AR, h-ER(α/β) and TR(α/β)) so as to assess its GR selectivity over other NR.[18] Compound **8g** displayed excellent selectivity for GR over related nuclear hormone receptors (>200-fold selective over h-PR, h-MR, h-AR, h-ER(α/β) and TR(α/β)).

A functional (VP16-GR) assay is used to evaluate the active or passive antagonistic nature of **8g**.[21] VP16 is a transcriptional activation domain that, if present in the nucleus, activates GRE's. Human liver hepatoma (HuH7) cells were transiently transfected with VP16-GR fusion protein expression plasmid and a reporter (GRE-Lue).[12] In this assay passive antagonist show little or no response while active antagonists robustly stimulate luciferase expression. In VP16-GR assay, **8g** showed no effect upto 100 μM concentrations, indicates that it is a passive antagonist.

Compound **8g** was subjected for PK and various pharmacodynamic (PD) profiling studies (in vivo).[22,23] In a single dose (20 mpk, po/iv) PK study (male SD rats; $n = 6$), **8g** showed rapid Tmax (0.5 h), extended half-life ($T_{1/2}$: ~6 h), good Cmax, AUC and oral bioavailability (%F 10.68%), Table 3.[22] To evaluate liver selectivity of **8g**, a single dose (20 mpk, po) PK study was carried out in male SD rats ($n = 6$) and at Tmax (0.5 h), liver concentrations of **8g** were determined and compared with Tmax/Cmax plasma concentration (Table 3). The PK profile of **8g** suggests a high degree of liver selectivity and based upon liver to plasma ratio, **8g** was found to be ~25-fold liver selective.

Table 4
28 days repeat dose study in ob/ob mice with **8g**

Compd (Animals)	Dose (mpk)	Insulin (ng/mL)	HbA1c (%)	TG (mg/dL)	FFA (mEq/L)	Cholesterol (mg/dL)	Change in B.Wt. (Day28-0)	AST (U/L)	ALT (U/L)
Vehicle (ob/ob)	-	13.6±2.3	9.3±0.4	292.5±17.5	1.96±0.09	331.2±17.9	11.7±1.1	332.2±39.1	259.4±18.6
8g (ob/ob)	10	14.1±3.9	6.7±0.4	214.3±22.3	1.32±0.13	266.6±18.3	11.9±0.8	345.3±53.2	261.0±21.5
8g (ob/ob)	20	12.3±2.1	6.0±0.4	173.5±19.6	1.17±0.05	219.5±11.9	10.9±0.9	353.2±45.2	247.5±27.6
Vehicle(lean)	-	1.9±0.3	4.6±0.3	103.2±12.6	1.03±0.07	133.3±11.2	3.1±1.7	53.2±4.9	42.3±9.3

[a] Values are Means ±SEM (n=6).
[b] Bid, oral (po) dosing.

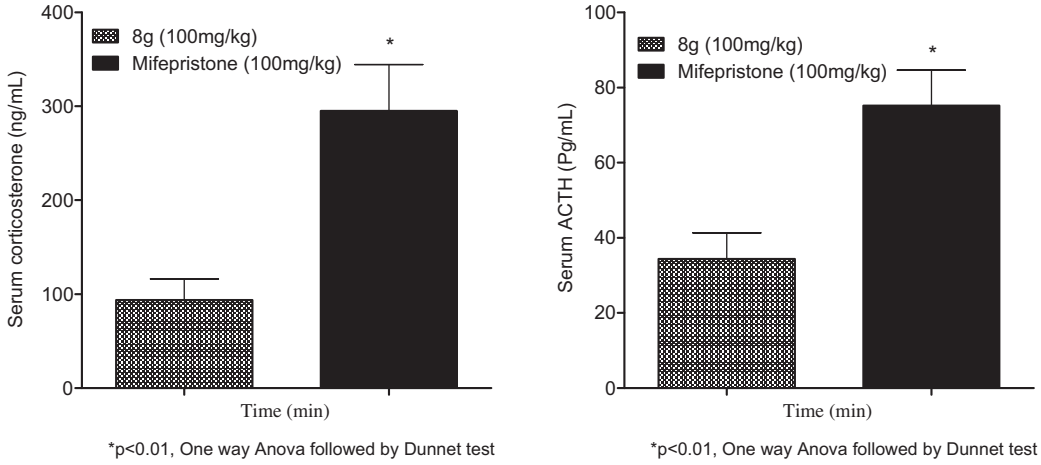


Figure 3. Effect of Compound **8g** on HPA axis in male Sprague–Dawley rats. A single oral dose (100 mpk) of **8g** is given to normal Sprague–Dawley rats ($N = 10$/group); measurements of ACTH and corticosterone levels in serum were done 2 h post-dosing. Mifepristone (100 mg/kg) was taken as a positive control.

Antidiabetic activity of **8g** was assessed in ob/ob mice (sub-chronic diabetic animal models).[23] Compound **8g** was given to ob/ob mice orally bid for 28 days at 10 and 20 mpk doses. After repeated dosing, **8g** dose dependently lowered postprandial blood glucose levels (32% and 42% decrease in AUC glucose at 10 and 20 mpk dose, respectively; Fig. 2). The insulin levels remain unchanged, despite considerable changes in glucose levels, which indicate a significant improvement in insulin sensitivity (Table 4). Decrease in HbA1c (2.6% and 3.3% at 10 and 20 mpk, respectively), TG, FFA and cholesterol levels was observed, which confirms oral antidiabetic and antihyperlipidemic effects of **8g,** without affecting liver functions (no change in AST/ALT levels) and body weight gain.

One of the primary side effect concerns associated with GR antagonism is activation of the HPA axis. To determine the potential impact of a liver selective and passive GR antagonist on HPA axis, **8g** was administered to male Sprague Dawley (SD) Rats, at 100 mpk.[24] 2 h later, plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone levels were analyzed (Fig. 3). No change relative to vehicle was observed with **8g**, indicating no impact upon the HPA axis under unstressed conditions.

To gain insight into the structural aspect for the observed potent antagonistic behavior of **8g**, we docked the RU486 (**1**) and **8g** into the GR-Ligand Binding Domain (GR-LBD) binding pocket, using the co-complex X-ray structure of RU486 (Fig. 4a-b).[25,26] In our docking studies, both the compounds (**1** and **8g**) get docked and overlay into the GR binding pocket. The docking study shows that **8g** and RU486 occupies a similar space, wherein the 2,4-difluoro phenoxy benzyl moiety, dibromo-phenyl acetic-acid group and diflurobenzyl ring of **8g** overlays with the C11-dimethylaniline, C3-ketone and C17β-hydroxyl groups of RU486, respectively (Fig. 4a). In hydrophobic binding pocket, C3-ketone and C17β-hydroxyl groups of RU486 interacts with Gln_{570}, Arg_{611} and Gln_{642} of GR binding pocket, respectively.[25] Compound **8g** shows potential interactions/hydrogen bondings (carbonyl group of dibromo-phenyl acetic-acid with Asn_{564}, diflurobenzyl ring with Gln_{642} and 2,4-difluoro phenoxy benzyl moiety with the side chain of Gln_{570} and Arg_{611}), Figure 1 in Supplementary data.

Helix-12 (H-12) of GR-LBD plays a key role as a molecular switch that modulates the functional response to ligand binding (agonist/antagonist).[4] In the case of RU486 (passive antagonist), the H-12 is displaced into activation factor-2 (AF-2) domain.[3] The displaced H-12 blocks the recruitment of coactivator and provides a molecular basis for its passive GR antagonism. Particularly, the C-11 dimethylaniline group of RU486 protrudes from this binding pocket, which displaces H-12 from its characteristic agonist position (close conformation/capping the binding cavity) to antagonist position (open conformation, where H-12 stacks into the N-terminal part of the coactivator pocket), in receptor/ligand complex

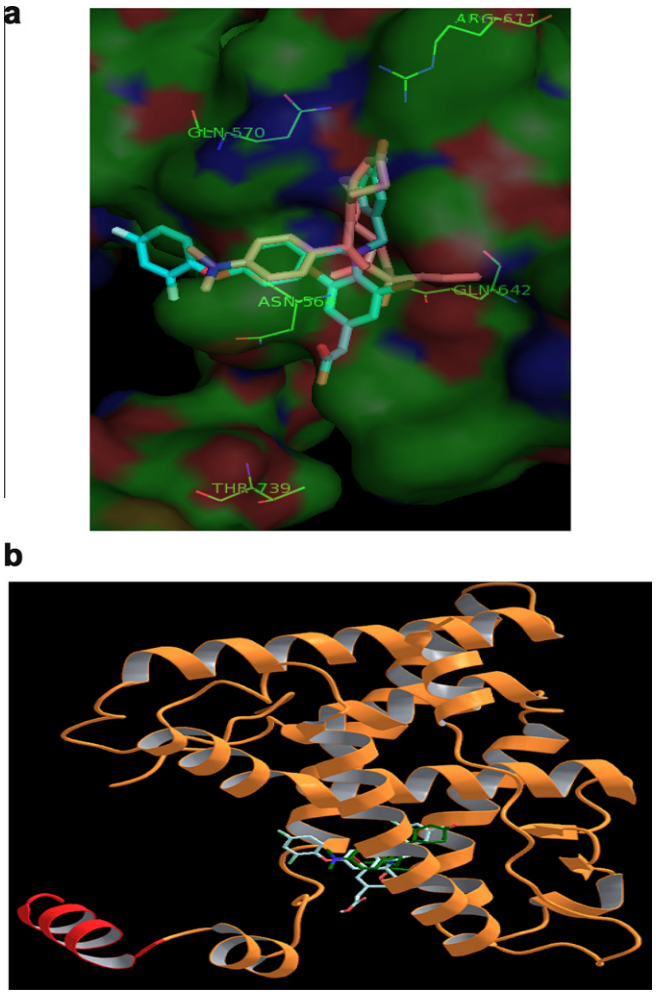


Figure 4. a–b Key interactions of compound **8g** and RU486 with GR binding site. **(a)** Binding pose of compound **8g** (Torquoise) and RU486 (orange) in the GR binding site is indicated (Surface view: Green), wherein **8g** overlay on RU486 and interact closely with key residues of binding site. (**b**) Binding pose of compound **8g** (Torquoise) and RU486 (green) in the GR, wherein both compounds displaces H-12.

(Fig. 4b). Binding of compound **8g** to GR binding pocket induces similar conformational changes, wherein the 2,4-difluoro phenoxy benzyl moiety of **8g** overlays with the C11-dimethylaniline of RU486 and compare to C11-dimethylaniline group, 2,4-difluoro phenoxy group of **8g** protrude-out more from the binding pocket and strongly displaces H-12 to antagonist position, which may account for its strong passive in vitro antagonistic activity (both in GRAF and TAT functional assays). Together, docking studies results illustrate favorable interactions of compound **8g** with key residues of GR binding pocket, which supports its potent in vitro binding affinity and functional antagonistic activity.

In summary, we report series of benzyl-phenoxybenzyl amino-phenyl acid derivatives (**8a**–**q**) as non-steroidal GR antagonist. Compound **8g** showed excellent h-GR binding, selectivity over closely associated NR and potent antagonistic activity (in-vitro). VP16 assay and docking studies confirms passive antagonistic activity of **8g**. The lead compounds **8g** showed no impact upon the HPA axis, exhibited significant oral antidiabetic and antihyperlipidemic effects (in vivo), along with liver selectivity. Thus preliminary study results confirm discovery of potent and liver selective GR antagonist, which could be viable approach for the safe and effective treatment of T2DM.

Acknowledgments

Authors are thankful to management of Zydus Group for encouragement and analytical department for analytical support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.078.

References and notes

1. Geldren, T.; Kym, P. R.; Tu, N.; Link, J. T.; Jae, H.; Lai, C.; Apelqvist, T.; Rhonnstad, P.; Grynfarb, M.; Sandberg, J.; Osterlund, M.; Barkhem, T.; Hoglund, M.; Fung, S.; Nguyen, P.; Jakob, C.; Hutchins, C.; Kauppi, B.; Ohman, L.; Koehler, K.; Wang, J.; Jacobson, P.; Hutchins, C.; Goos-Nilsson, A.; Farnegardh, M. *J. Med. Chem.* **2004**, *47*, 4213.
2. (a) Lee, K. C. *Trends Endocrinol. Metab.* **2001**, *12*, 191; (b) McKenna, N. J.; O'Malley, B. W. *J. Steroid Biochem. Mol. Biol.* **2000**, *74*, 351; (c) Whitefield, G. K.; Jurutka, P. W.; Haussler, C. A.; Haussler, M. R. J. Cell. Biochem. 1999, Suppl. 32/33, 110.; (d) Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. *Cell* **1995**, *83*, 835; (e) Schacke, H.; Rehwinkel, H. *Curr. Opin. Invest. Drugs.* **2004**, *5*, 524; (f) Beato, M.; Herrlich, P.; Schutz, G. *Cell* **1995**, *83*, 851; (g) Reichardt, H. M.; Tornche, F.; Berger, S.; Kellendonk, C.; Schutz, G. *Adv. Pharmacol.* **2000**, *47*, 1.
3. (a) McMaster, A.; William, D. *Exp. Physiol.* **2007**, *92*(2), 29; (b) Barker, M.; Clackers, M.; Demaine, D. a; Humphreys, D.; Johnston, M. J.; Jones, H. T.; Pacquet, F.; Pritchard, J. M.; Salter, M.; Shanahan, S. E.; Skone, P. A.; Vinader, V. M.; Uings, I.; Mclay, I. M.; Macdonald, S. J *J. Med. Chem.* **2005**, *48*, 4507.
4. (a) Horner, C.; Nam, K.; Fink, C.; Marshall, P.; Ksander, G.; Chatelain, R. E.; Cornell, W.; Steele, R.; Schweitzer, R.; Schumacher, C. *Mol. Pharmacol.* **2003**, *63*, **1012**; (b) Bledsoe, R. K.; Montana, V. g.; Stewart, E. L.; Xu, H. E. EP1375517 A1, 2004.; (c) Bledsoe, R. K.; Montana, V. G.; Stanley, T. B.; Delves, C. J.; Apolito, C. J.; Mckee, D. D.; Consler, T. G.; Parks, D. J.; Stewart, E. L.; Willson, T. M.; Lambert, M. H.; Moore, J. T.; Pearce, K. H.; Xu, H. E. *Cell* **2002**, *110*, 93.
5. Takahhashi, H.; Razavi, H.; Thomson, D. *Curr. Top. Med. Chem.* **2008**, *8*, 521.
6. (a) Clark, R. D. *Curr. Top. Med. Chem.* **2008**, *8*, 813; (b) McMaster, A.; Ray, D. W. *Nat. Clin. Pract. Endocrinol. Metab.* **2008**, *4*, 91; (c) Kym, P. R.; Lane, B. C.; Pratt, J. K.; Von, G. T.; Winn, M.; Brenneman, J.; Patel, J. R.; Arendsen, D. L.; Akritopoulou-zanze, I.; Ashworth, K. L.; US 20010041802.
7. Baxter, J. D. *Pharmacol. Ther [B].* **1976**, *2*(3), 605.
8. (a) Hamann, L. G.; Farmer, L. J.; Johnson, M. G.; Bender, S. L.; Mais, D. E.; Wang, M. W.; Crombie, D.; Goldman, M. E.; Jones, T. K. *J. Med. Chem.* **1996**, *39*, 1778; (b) Friedman, J. E.; Sun, Y.; Ishizuka, T.; Farrell, C. J.; McCormack, S. E.; Herron, L. M.; Hakimi, P.; Lechner, P.; Yun, J. S. *J. Bio. Chem.* **1997**, *272*, 31475.
9. Link, J. T.; Sorensen, B.; Patel, J.; Emercy, M.; Grynfarb, M.; Goos-Nilsson, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2209.
10. Lamberss, S. W.; Koper, J. W.; Jong, F. H. *J. Clin. Endocrinol. Metab.* **1991**, *73*, 187.
11. Li, Q.; Zhang, M.; Hallis, T. M.; DeRosier, T. A.; Yue, J.; Ye, Y.; Mais, D. E.; Wang, M. *Biochem. Biophy. Res. Comm.* **2010**, *391*, 1531.
12. Link, J. T.; Sorensen, B.; Patel, J.; Nguyen, P.; Fung, S.; Goos-Nilsson, A.; Wang, J.; Geldren, T.; Wilcox, D.; Wang, J.; Schmidt, J. M.; Rotert, G.; Hickman, B.; Lane, B.; Jacobson, P. B.; Rotert, G. *J. Med. Chem.* **2005**, *48*, 5295.
13. (a) Apelqvist, T.; Efendic, S. PCT Int. App. WO 199963976.; (b) Apelqvist, T.; Gillner, M.; Gustavsson, A.; Hagberg, L.; Koch, E.; Lindberg, M.; Pelcman, B.; Jinchang, W.; Kym, P. R. PCT Int. App. WO 2001047859.
14. (a) Wagner, B. L.; Pollio, G.; Giangrande, P.; Webster, J. C.; Breslin, M.; Mars, D. E.; Cook, C. E.; Vedeckis, W. V. *Endocrinology* **1999**, *140*, 1449; (b) Schulz, M.; Eggert, M.; Baniahmad, A.; Dostert, A.; Heinzel, T.; Renkawitz, R. *J. Bio. Chem.* **2002**, *277*, 26238; (c) Zhang, S.; Jonklaas, J.; Danielsen, M. *Steroids* **2007**, *72*, 600.
15. Chu, J. W.; Matthias, D. F.; Belanoff, J.; Schatzberg, A.; Hoffmann, A. R.; Feldman, D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3568.
16. (a) Watts, L. M.; Manchem, V. P.; Leedom, T. A.; Rivard, A. L.; Mckay, R. A.; Bao, D.; Neroladakis, T.; Monia, B. P.; Boden, D. M.; Cao, J. C.; Zhang, H. Y.; Cox, A. L.; Jacob, S. J.; Michael, M. D.; Sloop, K. W.; Bhanot, S. *Diabetes* **1846**, *2005*, 54; (b) Apelqvist, T.; Wu, J.; Hoehler, K. F. PCT Int. App. WO 2000058337.; (c) Sorensen, B.; Link, J. T.; Geldren, T.; Emercy, M.; Wang, J.; Hickman, B.; Grynfarb, M.; Goos-Nilsson, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2307; (d) Jacobson, P. B.; Geldern, T. W.; Ohman, L.; Osterland, M.; Wang, J.; Zinker, B.; Wilcox, D.; Nguyen, B.; Mika, A.; Fung, S.; Fey, T.; Goos-Nilsson, A.; Opgenorth, T. J. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 191; (e) Zinker, B.; Mika, A.; Nguyen, P.; Wilcox, D.; Oehman, L.; Von, G.; Thomas, W.; Opgenorth, T.; Jacobson, P. *Clinical and Experimental* **2007**, *56*, 380.
17. Link, J. T.; Sorensen, B.; Lai, C.; Wang, J.; Fung, S.; Deng, D.; Emercy, M.; Carroll, S.; Grynfarb, M.; Goos-Nilsson, A.; Geldern, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4173.
18. NR binding activities of test compounds were assessed using radioligand binding assays. For h-GR binding assay, [^{3}H]-dexamethasone (^{3}H-dex; Sigma) was used as a radioligand. The h-GR was extracted from Sf9 cells and IC_{50} values were determined for test compounds. Similar protocols were employed to measure affinity/selectivity of test compounds over other NR (PR, MR, AR, ER (α/β) and TR (α/β)).The reporter gene of GRAF cells expressing h-GR, encodes a secreted form of alkaline phosphatase (ALP). Antagonists are evaluated by their inhibition of dexamethasone-induced ALP expression in these cells. Compounds are also tested in freshly isolated rat hepatocytes for their effects on dexamethasone induced expression of the GR regulated enzyme tyrosine. In both the functional assays, IC_{50} values were determined.
19. Link, J. T.; Sorensen, B.; Patel, J.; Arendsen, D.; Li, G.; Swanson, S.; Nguyen, B.; Emercy, M.; Grynfarb, M.; Goos-Nilsson, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4169.
20. Wang, J.; Harvey, R. G. *Tetrahedron* **2002**, *58*, 5927.
21. A third functional assay (VP16) is used to assess the active or passive nature of test compound. VP16 is a transcriptional activation domain that if present in the nucleus, activates GRE's. Human liver hepatoma (HuH7) cells were transiently transfected with VP16-GR fusion protein expression plasmid and a reporter (GRE-Lue). In this assay passive antagonist show little or no response while active antagonists robustly stimulate luciferase expression.
22. For single dose PK study, compound **8g** was administered orally/iv on a body weight basis (20 mpk) to fasted male SD rats ($n = 6$). Serial blood samples were collected and the plasma concentrations of **8g** were determined by the LC-MS/MS. To assess liver selectivity of **8g**, at Tmax (0.5 h), liver concentrations of **8g** were determined and compared with its Cmax plasma concentration.
23. Antidiabetic and antihyperlipidemic activities of **8g** were assessed in ob/ob mice. Compound **8g** was given to ob/ob mice orally bid for 28 days at 10 and 20 mpk doses. HbA1c, serum glucose, insulin, ACTH levels and rest of the biochemical parameters (free fatty acids (FFA), triglyceride (TG), cholesterol and ALT/AST) were estimated.
24. Effect of **8g** on Hypothalamic-Pituitary-Adrenal (HPA) axis activation was assessed in male Sprague-Dawley rats. Briefly, non-fasted male Sprague-Dawley rats ($n = 10$), weighing approximately 150 g, were dosed with vehicle, Mifepristone (RU-486; **1**) or **8g** (100 mpk, po). Two hours post-dosing, mice were euthanized, bled and the plasma was analyzed for corticosterone by mass spectroscopy and for adrenocorticotropic hormone (ACTH) levels by ELISA.
25. Kauppi, B.; Jakob, C.; Farnegardh, M.; Yang, J.; Ahola, H.; Alarcon, M.; Calles, K.; Engstrom, O.; Harlan, J.; Muchmore, S.; Ramqvist, A.; Thorell, S.; Ohman, L.; Greer, J.; Gustafsson, J.; Carlstedt-Duke, J.; Carlquist, M. *J. Biol. Chem.* **2003**, *278*, 22748.
26. (a) The molecular docking analysis of RU486 (**1**) and **8g** was carried out using extra precision (XP) glide version 5.6 docking software of Schrodinger, to understand their critical interactions with GR crystal structure. The three-dimensional protein structure of GR (pdb id: 1NHZ) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box. For docking study, the ligand structures (**1** and **8g**) were prepared using the Ligprep module of Schrodinger to generate energy-minimized correct 3D ligand structures. (b) Schrodinger Suite 2010, Glide version 5.6, Prime version 2.2; Schrodinger, LLC: *New York*, **2010**.