Bioorganic & Medicinal Chemistry 18 (2010) 4138–4148

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design and synthesis of 2,4-disubstituted polyhydroquinolines as prospective antihyperglycemic and lipid modulating agents

Atul Kumar [a,*], Siddharth Sharma [a], Vishwa Deepak Tripathi [a], Ram Awatar Maurya [a], Swayam Prakash Srivastava [b], Gitika Bhatia [b], A. K. Tamrakar [b], Arvind Kumar Srivastava [b]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute, Council of Scientific and Industrial Research, Lucknow 226 001, India
[b] Biochemistry Division, Central Drug Research Institute, Council of Scientific and Industrial Research, Lucknow 226 001, India

ARTICLE INFO

Article history:
Received 23 October 2009
Revised 25 November 2009
Accepted 26 November 2009
Available online 6 December 2009

Keywords:
Polyhydroquinoline
Antihyperglcemic
Antidyslipidemic
PTP-1B
Glycogen phosphorylase

ABSTRACT

A series of 2,4-disubstituted polyhydroquinoline were synthesized and evaluated for their in vivo antihyperglycemic as well as antidyslipidemic activities. Several synthesized compounds have exhibited promising in vivo antihyperglycemic in SLM, STZ-S, and db/db mice model along with significant lipid and TG modulating activity. All these compounds were evaluated in various in vitro models of diabetes to know the possible mechanism of their antihyperglycemic action. Interestingly, compounds **3a–r** (diaryl substitution) have exhibited promising protein-tyrosine phosphatase 1B (PTP1B) inhibitory activity whereas, compounds **5a–d** (acid substituted) have shown significant glycogen phosphorylase activity.

1. Introduction

Recently, diabetes has again focused the world's attention as approximately 330 million will be afflicted worldwide by 2025 with a 122% overall increase.[1] Non-insulin dependent diabetes mellitus (Type-2 diabetes), is a complex metabolic disorder of heterogeneous etiology and is primarily characterized by insulin resistance and abnormal insulin secretion. These metabolic disorders cause hyperglycemia,[2,3] which is significantly responsible for diabetic complications. Complication of diabetes[4] includes neuropathy, nephropathy, retinopathy, obesity, dyslipidemia, hypertension, and other cardiovascular diseases and these are believed to be triggered by excessive protein glycation, which is due to higher levels of circulating glucose (hyperglycemia).

The increased risk of cardiovascular disease in Type 2 diabetes is commonly due to abnormalities in the quantity as well as quality of plasma lipoproteins. Besides this elevated triglyceride and high density lipoprotein cholesterol, diabetic dyslipidemia is also associated with predominance of low density lipoproteins.

Dyslipidemia is connected with insulin insensitivity and associated to increased atherosclerosis susceptibility.[4–8] Therefore, it is win to win state for antidiabetic therapy, if any chemical entity takes care of diabetes as well as dyslipidemia.

The discovery of new lead structure is important area of research in medicinal chemistry as new chemical entity is continue to fall and toxicity of the present drugs becomes a dilemma. Among various biologically active heterocyclic scaffolds, Hantzsch 1,4-dihydropyridines (1,4-DHPs) are an important class of biologically active heterocycles. The prominent biological activities associated with 1,4-dihydropyridines are as Ca^{2+} channel blockers (drugs for the treatment of cardiovascular diseases)[9] and hypertension (nifedipine, nicardipine, amlodipine, and felodipine). Beside, this dihydropyridine skeleton is common in many biological activities such as antihypertension,[10] antioxidant,[11] antiviral,[12] and anticancer activity.[13]

In continuation to our antidiabetic drug discovery program,[14] we have designed 2,4-disubstituted polyhydroquinoline derivatives based on the some recent reports that, indicate 1,4-DHPs may act as a lead in the antidiabetic drug discovery, such as glycogen phosphorylase inhibitors (**2**[15], **3**,[16] **4**,[16]), cerivastatin **5** (lipid lowering agent), and **6**[17] for the treatment of metabolic disorder (Fig. 1). A series of 2,4-disubstituted polyhydroquinoline derivatives have been synthesised and evaluated for their antidiabetic and antidyslipidemic activity in various in vivo and in vitro models.

2. Results and discussion

2.1. Chemistry

The different chalcones were synthesized by the previously reported method of Sogawa et al.[18] 2,4-Disubstituted polyhydroquinoline, were synthesized via a three-component reaction of

* Corresponding author. Tel.: +91 522 2612411; fax: +91 522 2623405.
E-mail address: dratulsax@gmail.com (A. Kumar).

0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2009.11.061

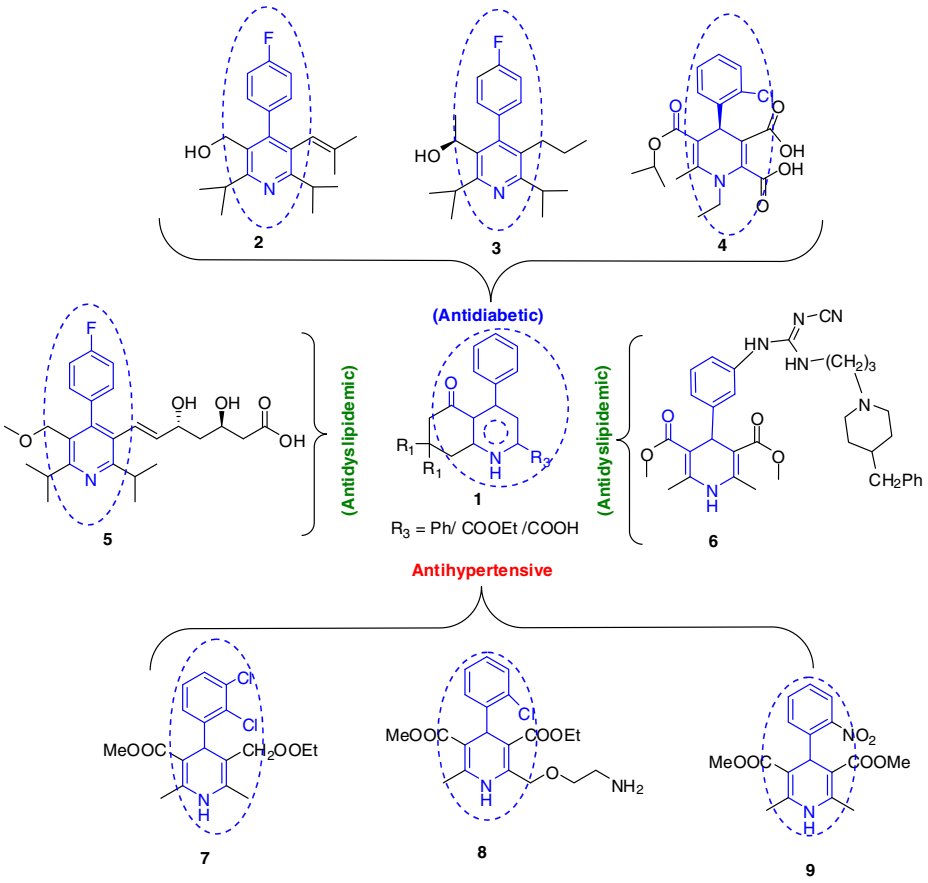


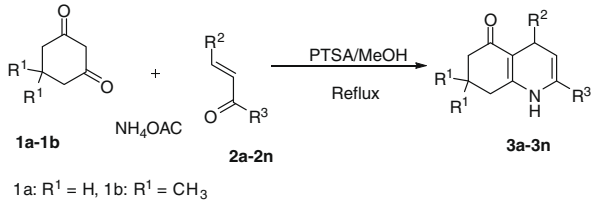
Figure 1. Design of 2,4-disubstituted polyhydroquinoline based on dihydropyridine scaffolds showing antidiabetic, antihypertensive and dyslipidemic activity.

substituted 1,3-cyclicdiketone, chalcones, and ammonium acetate (Scheme 1). Number of acidic catalysts used such as boric acid, sodium dodecyl sulfate (SDS), camphor sulfonic acid, para toluene sulfonic acid (PTSA), and tetra butyl ammonium iodide (TBAI). PTSA found to be the best among all the catalysts used. Although the reaction was sluggish in various solvents, MeOH was found to be the best among all the solvents used for the reaction. Chalcones (1 mmol), 5,5-dimethyl-1,3-cyclohexanedione (1 mmol), ammonium acetate (2 mmol) were refluxed in methanol in the presence of PTSA to afford desired product in excellent yields. All the results are summarised in Table 1.

β,γ-Unsaturated α-ketoester (**2o–s**) were synthesized in two steps procedure. Firstly, aryl aldehyde and pyruvic acid were condensed in the presence of KOH to give potassium (*E*)-2-oxo-4-phenylbut-3-enoates. Esterification of potassium (*E*)-2-oxo-4-phenylbut-3-enoates gave (*E*)-ethyl 2-oxo-4-phenylbut-3-enoates (**2o–s**) in excellent yields (Scheme 2).

Some newer ethyl 7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate derivatives **4o–s** (Scheme 3.) have been synthesized for the further diversification in our polyhydroquinoline core motif to generate ester group at C-2 position via a two step protocol. Reaction of dimedone, and β,γ-unsaturated α-ketoester in presence of acidic catalyst gave corresponding lactol derivatives (**3o–s**) in excellent yields. These lactol derivatives were transformed in to polyhydroquinoline derivatives by the reaction with ammonium acetate in quantitative yield.

Previously, it have been reported that several antidiabetic agents contain carboxylic acid group as key pharmcophores[17] (Fig. 1). Keeping this hypothesis in mind, we have hydrolyzed the ester group present at the C-2 position to generate corresponding carboxylic acid group (**5a–e**) (Scheme 4).

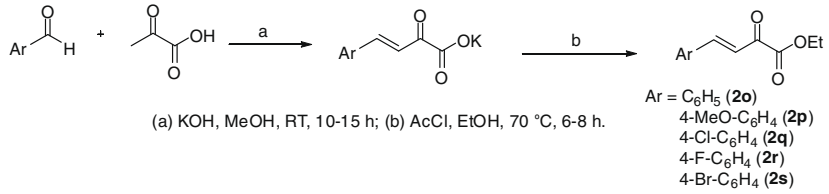


Scheme 1. Synthesis of 2,4-diaryl polyhydroquinolines.

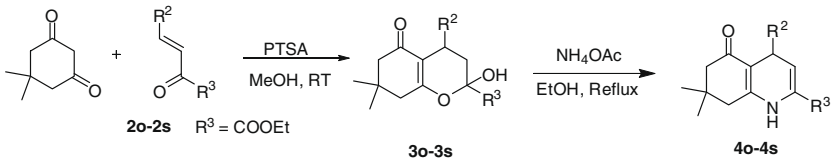
Table 1
Synthesis of 2,4-diaryl polyhydroquinoline via a multi-component reaction[a]

Entry	R^1	R^2	R^3	Time (h)	Product[b]
1	CH_3	Ph	Ph	2.5	**3a**
2	CH_3	4-NO_2 Ph	Ph	1.5	**3b**
3	CH_3	4-Cl Ph	Ph	1.7	**3c**
4	CH_3	4-F Ph	Ph	2.3	**3d**
5	CH_3	4-OMe Ph	Ph	2.7	**3e**
6	CH_3	Ph	4-OMe Ph	2.9	**3f**
7	H	Ph	Ph	2.4	**3g**
8	H	4-Cl Ph	Ph	1.7	**3h**
9	H	4-F Ph	Ph	1.5	**3i**
10	CH_3	Ph	4-CH_3 Ph	2.6	**3j**
11	CH_3	Indolyl	Ph	3.7	**3k**
12	H	Ph	4-CH_3 Ph	2.3	**3l**
13	H	4-OMe Ph	Ph	3.0	**3m**
14	CH_3	Furyl	Ph	2.5	**3n**

[a] Experimental conditions: all the reactions were performed at refluxing in PTSA in 10 mol %.
[b] Product characterized by spectroscopic (^{1}H and ^{13}C NMR) data.

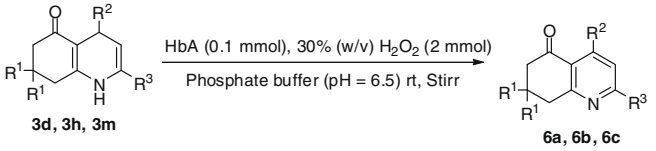


Scheme 2. Synthesis of (*E*)-ethyl 2-oxo-4-arylbut-3-enoates.



Scheme 3. Synthesis of substituted lactol and polyhydroquinoline derivatives.

Recently, we have developed Human hemoglobin (HbA) as an efficient catalyst for the oxidative aromatization of 1,4-dihydropyridines (1,4-DHPs) and pyrazolines with hydrogen peroxide in phosphate buffer.[19] In order to evaluate the effect of aromatization on antidiabetic activity of 2,4-disubstituted polyhydroquinoline were aromatized to corresponding tetrahydroquinoline ring **6a–c** in excellent yields using Human hemoglobin (HbA)/H_2O_2 catalytic system (Scheme 5).



Scheme 5. Aromatization of polyhydroquinoline with HbA/H_2O_2 catalyst system.

2.2. Biological activity

2.2.1. In vivo antihyperglycemic activity

All the synthesized compounds (**3a–s**), (**4o–s**), (**5a–e**), and (**6a–c**) were evaluated for their antihyperglycemic activity in sucrose-loaded model (SLM) and streptozotocin (STZ-S) induced β-cell damaged diabetic model of Sprague–Dawley strain male albino rats.

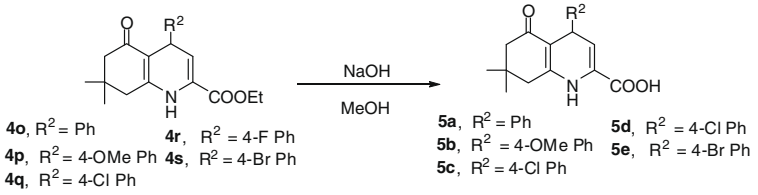
Compounds **3d** (41.3%), **3i** (34.5%), **4r** (35.9%), and **5b** (42.7%) have shown promising blood glucose lowering activity in SLM model. Compounds showing blood glucose lowering activity were further tested for antidiabetic activity in sucrose-challenged streptozotocin (STZ-S) induced diabetic rat model. The compounds **3d** (32.3%), **3i** (24.1%), **4r** (23.4%), **5b** (33.2%) exhibited comparable or higher antihyperglycemic activities to standard drugs, metformin (20.2%), and glybenclamide (28.4%) in STZ model after 24 h treatment. All the results are summarised in Table 2.

Compounds (**3d**, **5b**) exhibited 13.2% (**3d**), 17.9% (**5b**) after 5 days and 32.6% (**3d**), and 39.6% (**5b**) blood glucose lowering activity after 10 days treatment. The standard drug rosiglitazone showed 22.6% after 5 days and 52.9% blood glucose lowering activity after 10 day treatment in similar conditions at a dose of 100 mg/kg (Table 3).

2.2.2. In vitro antihyperglycemic activity

All the synthesized compounds (**3a–s**), (**4o–s**), (**5a–e**), and (**6a–c**) were evaluated for in vitro antihyperglycemic activity against glucose-6-phosphatase,[20] glycogen phosphorylase,[20] α-glucosidase, protein-tyrosine phosphatase 1B and dipeptidyl peptidase IV (DPP IV) enzymes at 100 μM concentration (Table 4) to evaluated possible mechanism of action for compounds exhibiting significant antidiabetic activity in in vivo models.

The effect of compounds on glucose-6-phosphatase was studied by the method of Hubscher and West.[21] The dipeptidyl peptidase enzyme inhibition assays were performed according to the methods of Wright et al.[22] The glycogen phosphorylase activity is measured by the modified method of Rall et al.[23] The α-glucosidase activity was determined by the method of Lebovitz et al.[24] Protein-tyrosine phosphatase 1B inhibitory activity was determined by the modified method[25] using *p*NPP as substrate. Compounds (**3a–s**) were showing poor inhibitory activities for glucose-6-phosphatase, glycogen phosphorylase, α-glucosidase, and dipeptidyl peptidase IV (DPP IV) enzymes, whereas compounds (**3b–3e, 3i, 3n, 4r**, and **4s**) have shown promising PTP 1B inhibitory activity. All the compounds with promising in vivo activity showed good PTP 1B inhibitory activity. Compound **3n** exhibited excellent PTP 1B inhibitory activity but found inactive in in vivo model. IC_{50} value of active compounds concluded that protein-tyrosine phosphatase 1B (PTP1B) may be a possible target for these antihyperglycemic compounds. Very interesting in vitro results were obtained in case of **5a–e**, as **5b** was the most active in vivo compound of the series and found to inhibit the glycogen phosphorylase more significantly instead of PTP 1B. Exact reason was not elucidated but –COOH group in **5a–e** may be playing important



Scheme 4. Hydrolysis of ester group of polyhydroquinolines.

Table 2
In vivo antihyperglycemic activity profile of compounds in SLM and STZ-S models[a]

Entry	R^1	R^2	R^3	Compounds	% Blood glucose lowering activity[b, c]			
					SLM	STZ-S		
						5 h	24 h	ED_{50}[d]
1	CH_3	Ph	Ph	**3a**	24.9	17.3	13.3	
2	CH_3	4-NO_2 Ph	Ph	**3b**	14.1	ND	ND	
3	CH_3	4-Cl Ph	Ph	**3c**	23.5	18.9	16.4	
4	CH_3	4-F Ph	Ph	**3d**	**41.3****	**29.9*****	**32.3*****	**47.3**
5	CH_3	4-OMe Ph	Ph	**3e**	17.8	8.8	10.9	
6	CH_3	Ph	4-OMe Ph	**3f**	17.3	11.9	11.7	
7	H	Ph	Ph	**3g**	19.7	15.1	13.8	
8	H	4-Cl Ph	Ph	**3h**	24.7	12.9	16.9	
9	H	4-F Ph	Ph	**3i**	**34.5****	**19.6****	**24.1****	
10	CH_3	Ph	4-CH_3 Ph	**3j**	11.4	ND	ND	
11	CH_3	Indolyl	Ph	**3k**	17.3	8.1	13.2	
12	H	Ph	4-CH_3 Ph	**3l**	13.4	ND	ND	
13	H	4-OMe Ph	Ph	**3m**	29.6	17.5	19.4	
14	CH_3	Furyl	Ph	**3n**	14.6	ND	ND	
15	CH_3	Ph	COOEt	**3o**	NA	ND	ND	
16	CH_3	4-OMe Ph	COOEt	**3p**	NA	ND	ND	
17	CH_3	4-Cl Ph	COOEt	**3q**	NA	ND	ND	
18	CH_3	4-F Ph	COOEt	**3r**	15.3	ND	ND	
19	CH_3	4-Br Ph	COOEt	**3s**	NA	ND	ND	
20	CH_3	Ph	COOEt	**4o**	16.6	7.1	11.3	
21	CH_3	4-OMe Ph	COOEt	**4p**	12.9	ND	ND	
22	CH_3	4-Cl Ph	COOEt	**4q**	11.4	ND	ND	
23	CH_3	4-F Ph	COOEt	**4r**	**35.9****	**24.8****	**23.4****	
24	CH_3	4-Br Ph	COOEt	**4s**	11.4	ND	ND	
25	CH_3	Ph	COOH	**5a**	28.9	18.3	16.7	
26	CH_3	4-OMe Ph	COOH	**5b**	**42.7****	**29.7*****	**33.2****	**48.6**
27	CH_3	4-Cl Ph	COOH	**5c**	13.7	16.6	12.2	
28	CH_3	4-F Ph	COOH	**5d**	**38.7****	**27.8*****	**23.6****	
29	CH_3	4-Br Ph	COOH	**5e**	31.7	17.8	13.6	
30	CH_3	4-F Ph	Ph	**6a**	NA	ND	ND	
31	CH_3	4-Cl Ph	Ph	**6b**	NA	ND	ND	
32	H	4-OMe Ph	Ph	**6c**	NA			
33	—	—	—	Metformin	13.5	18.0	20.2	
34	—		—	Glybenclamide	32.5	25.6	28.4	

[a] Dose = 100 mg/kg.
[b] NA = not active, ND = not determine.
[c] *p <0.05, **p <0.01 and ***p <0.001 versus vehicle treated control and Statistical analysis was made by Dunnett's test (Prism Software).
[d] mg/kg.

Table 3
Antihyperglycemic activity of **3d** and **5b** in db/db mice model[c]

Entry	Compounds	n[a]	5 day[b]	10 day[b]
1	Control	5	30,730 ± 3687	32,480 ± 5098
2	**3d**	5	26,670 ± 3058 (−13.2)*	21,870 ± 3069 (−32.6)**
3	**5b**	5	25,250 ± 2220 (−17.9)*	19,630 ± 2467 (−39.6)**
4	Rosiglitazone	5	23,780 ± 2261 (−22.6)**	15,280 ± 2300 (−52.9)***

[a] No. of mice.
[b] Mean of glucose (AUC) values ± SD. *p<0.05, **p <0.01 and ***p <0.001 versus vehicle.
[c] % Lowering values are given in parentheses.

role in reversal the mode of action. Further optimization is also going on in our group to increase the glycogen phosphorylase inhibitory activity. None of the aromatized 2,4-disubstituted polyhydroquinoline **6a–c** found to inhibit any of the performed in vitro assays.

2.2.3. In vivo antidyslipidemic activity

Compounds showing significant in vivo antidiabetic activity were further evaluated for their antidyslipidemic activities in a triton model. Compound **3c**, **3d**, and **5b** showed significant lipid lowering activity in plasma level of TC, PL, and TG, respectively, while other compounds showed moderate lipid lowering activity as compared to triton. All the activity were compared with Gemfibrozil at a dose of 100 mg/kg, which showed a decrease in plasma levels of TC, PL and TG by 37.5%, 39.4%, and 36.2%, respectively (Table 5).

The SAR activity revealed interesting finding that 2,4-diaryl substituted compounds were exhibiting antidiabetic activity via inhibition of PTP-1B, whereas 2-carboxylic acid substituted compounds (**5**) were showing promising glycogen phosphorylase inhibitory activity. It is noteworthy to report that compounds having ester group at position-2- were exhibiting moderate PTP-1B and glycogen phosphorylase inhibition along with significant in vivo antidiabetic activity. Aromatization of dihydropyridine ring (**6**) resulted in complete loss of antidiabetic activity (Fig. 2). These finding indicates that dihydropyridine ring is important for hyperglycemic activity.

Table 4
In vitro antihyperglycemic activity of (**3a–s**), (**4o–s**), (**5a–e**), and (**6a–c**)

Compounds	% Inhibition[a]				
	Glucose-6-phosphatase	Glycogen phosphorylase	α-Glucosidase	DPP IV	PTP-1B
3a	NI	NI	34.32	12.0	41.8
3b	NI	NI	22.35	NI	**84.7** (6.1)
3c	7.16	5.89	12.13	NI	**84.9** (6.3)
3d	7.38	14.58	NI	NI	**88.2** (2.9)
3e	NI	NI	NI	8.6	**71.8** (14.5)
3f	8.34	18.41	22.4	NI	53.4
3g	NI	21.83	NI	NI	51.2
3h	8.21	19.34	27.9	NI	24.7
3i	21.89	17.43	NI	NI	**70.3** (12.6)
3j	NI	31.67	NI	NI	**62.3** (19.6)
3k	26.71	39.67	NI	NI	54.5
3l	27.66	NI	8.3	18.8	33.3
3m	17.28	13.81	7.3	NI	56.8
3n	11.38	18.88	NI	18.6	**82.5** (7.7)
3o	NI	NI	NI	NI	21.3
3p	NI	NI	NI	NI	13.6
3q	NI	7.6	NI	NI	16.5
3r	NI	14.9	NI	NI	5.3
3s	NI	12.3	NI	NI	4.7
4o	NI	49.45	NI	NI	43.3
4p	23.81	40.39	NI	16.47	66.7
4q	11.27	56.71	NI	12.5	53.8
4r	NI	61.23	15.8	9.8	87.2 (4.7)
4s	NI	49.3	NI	NI	65.6 (16.6)
5a	17.1	**77.3** (6.1)	4.6	14.6	28.9
5b	NI	**78.3** (5.9)	11.2	5.7	35.4
5c	NI	**63.4** (9.7)	NI	NI	25.4
5d	16.8	46.6	1.4	NI	27.0
5e	NI	**81.3** (5.3)	NI	NI	12.3
6a	11.1	20.3	NI	13.1	7.9
6b	11.3	13.8	NI	29.4	18.6
6c	NI	31.1	NI	22.0	9.1
Sod. ortho-vanadate	—	—	—	57.8	

[a] Compounds were evaluated at 100 μM concentration; NI means no inhibition. IC_{50} (μM) values are given in parentheses.

Table 5
In vivo antidyslipidemic activities of selected compounds[c]

Entry	Compounds	*n*[b]	TC[a]	PL[a]	TG[a]
1	Control	6	225 ± 12.85	227.2 ± 10.7	310 ± 12.40
2	**3a**	6	184.74 ± 10.2 (−17.89)*	183.34 ± 7.2 (−19.23)*	259.0 ± 13.2 (−16.45)*
3	**3c**	6	167.49 ± 9.7 (−25.56)**	175.99 ± 8.7 (−22.47)**	250.10 ± 14.7 (−19.32)**
4	**3d**	6	174.48 ± 10.6 (−22.45)**	184.25 ± 7.6 (−18.83)*	228.34 ± 14.6 (−26.34)**
5	**3h**	6	209.92 ± 11.8 (−6.7)	213.60 ± 6.8 (−5.9)	285.54 ± 15.8 (−7.89)
6	**3r**	6	201.82 ± 12.4 (−10.3)*	187.95 ± 6.4 (−17.2)*	284.58 ± 16.4 (−8.2)
7	**5a**	6	204.77 ± 11.3 (−8.99)	209.08 ± 7.1 (−7.89)	267.03 ± 14.6 (−13.86)*
8	**5b**	6	169.59 ± 9.8 (−24.63)**	177.46 ± 8.8 (−21.82)*	250.04 ± 13.8 (−19.34)**
9	**5c**	6	185.73 ± 8.7 (−17.45)*	192.08 ± 9.7 (−15.38)*	272.89 ± 16.7 (−11.97)*
10	Gemfibrozil	6	140.49 ± 7.4 (−37.56)**	137.49 ± 6.4 (−39.43)**	197.74 ± 10.4 (−36.21)**

TC, total cholesterol; PL, phospholipid; TG, triglyceride. Values are mean ± SD, *p <0.05, **p <0.01 and ***p <0.001 versus vehicle.
[a] mg/dL.
[b] n = no. of animals.
[c] % Lowering values are given in parentheses.

3. Conclusion

In conclusion, we have synthesized a series of 2,4-disubstituted polyhydroquinoline and evaluated them for their antihyperglycemic as well as antidyslipidemic activities. Some compounds of the series (**3d, 3i, 5b**, and **5d**) have been exhibiting antihyperglycemic activity comparable to standard drugs along with significant lipid and TG lowering activity. Interestingly, in vitro antihyperglycemic activity evaluation exhibited that compounds **3d** (diaryl substituted) (IC_{50} = 2.9 μM) and **4r** (IC_{50} = 4.7 μM) are potential PTP-1B inhibitors thereby revealing their possible mechanism of antidiabetic action. Whereas, compounds **5a–e** containing carboxylic group (–COOH) group inhibits the glycogen phosphorylase more significantly than PTP-1B and our studies shows that the acidic group is beneficial for glycogen phosphorylase inhibitory activity. Compound **3c**, **3d**, and **5b** were showing promising antidyslipidemic activity in triton induced rat model. Thus, 2,4-disubstituted polyhydroquinoline show remarkable promise for further study as antidiabetic candidates.

4. Experimental

4.1. Chemistry

Unless otherwise specified all the reagents and catalysts were purchased from Sigma–Aldrich and were used without further

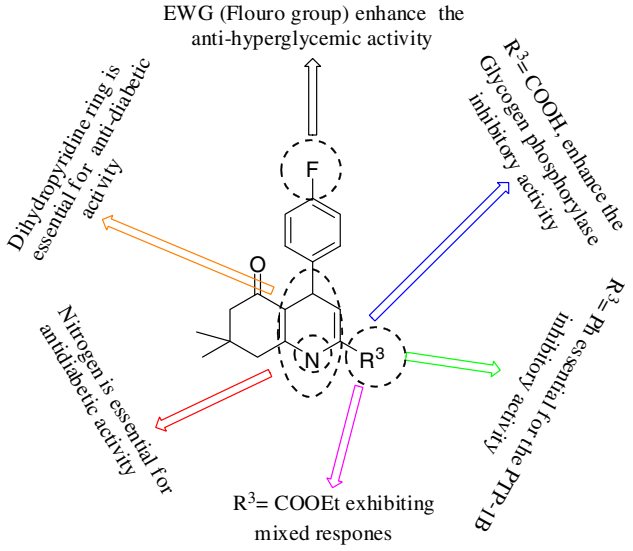


Figure 2. Structure–activity relationship and pharmacophore development of polyhydroquinoline.

any purification. The common solvents were purchased from Ranbaxy. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was accomplished using flash chromatography on 230–400 mesh silica gel. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates visualized under UV light, iodine or $KMnO_4$ staining. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 Spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hz. IR spectra were recorded on a FT IR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra (ESIMS) were obtained by Micromass Quattro II instrument.

4.1.1. Typical experimental procedure for the synthesis of β,γ-unsaturated α-ketoester (2o-s)

To a benzaldehyde (10.6 g, 0.1 mol) solution in methanol (15 mL) was added pyruvic acid (8.6 g, 0.10 mmol), and the mixture was cooled to 10 °C under the atmosphere of nitrogen. To this was added a solution of KOH (8.4 g, 0.15 mmol) in methanol dropwisely at 15–20 °C, after the addition. Then the ice-bath was removed, and the temperature of the reaction mixture increased from 20 °C to 35–40 °C. The reaction mixture was stirred at this temperature for 3 h and then maintained at 10 °C for 10 h. The solid precipitated out was filtered on a Buchner funnel under suction and washed with chilled methanol followed by diethyl ether to afford potassium 4-phenyl-2-oxo-but-3-enoate as a yellow solid. Acetyl chloride was added dropwisely to EtOH cooled in an ice bath to produce dry hydrochloric acid. Potassium benzylidene pyruvate was added at 0 °C. The reaction was warmed to room temperature and stirred for 2 h and then refluxed for 6 h. Then extracted with CH_2Cl_2, after the organic layer was separated and dried over $NaSO_4$, the solvent was evaporated, the crude product was recrystallized in ethanol. Compounds **2p–s** was prepared from the corresponding aryl aldehydes and pyruvic acid.

4.1.1.1. (*E*)-Ethyl 2-oxo-4-phenylbut-3-enoate (2o). Yield (85.09%), ESIMS (m/z) = 205 $(M+H)^+$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.40 (t, J = 7.2 Hz, 3H), 4.37 (q, J = 7.2 Hz, 2H), 7.36 (d, J = 16.0 Hz, 1H), 7.40–7.62 (m, 5H), 7.83 (d, J = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$,75 MHz,) δ: 13.8, 62.2, 120.3, 128.77, 128.83, 131.4, 133.7, 148.1, 161.9, 182.6.

4.1.1.2. (*E*)-Ethyl 4-(4-methoxyphenyl)-2-oxobut-3-enoate (2p). Yield (92.56%), ESIMS (m/z) = 235 $(M+H)^+$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.41 (t, J = 7.2 Hz, 3H), 3.86 (s, 3H), 4.39 (q, J = 7.2 Hz, 2H), 6.93–7.61 (m, 4H), 7.25 (d, J = 16.0 Hz, 1H), 7.84 (d, J = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.2, 55.6, 62.5, 114.8, 118.4, 126.9, 131.2, 148.4, 162.7, 162.8, 182.9.

4.1.1.3. (*E*)-Ethyl 4-(4-chlorophenyl)-2-oxobut-3-enoate (2q). Yield (87.38%), ESIMS (m/z) = 238 $(M+H)^+$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.42 (t, J = 7.2 Hz, 3H), 4.40 (q, J = 7.2 Hz, 2H), 7.35 (d, J = 16.0 Hz, 1H), 7.39–7.58 (m, 4H), 7.81 (d, J = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 62.5, 120.8, 129.3, 130.1, 132.4, 137.5, 146.6, 161.9, 182.4.

4.1.1.4. (*E*)-Ethyl 4-(4-fluorophenyl)-2-oxobut-3-enoate (2r). Yield (85.50%), ESIMS (m/z) = 223 $(M+H)^+$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.42 (t, J = 7.2 Hz, 3H), 4.40 (q, J = 7.2 Hz, 2H), 7.10–7.67 (m, 5H), 7.84 (d, J = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ:14.0, 62.5, 120.8, 129.3, 130.1, 132.4, 137.5, 146.6, 161.9, 182.4.

4.1.1.5. (*E*)-Ethyl 4-(4-bromophenyl)-2-oxobut-3-enoate (2s). Yield (88.89%), ESIMS (m/z) = 282, 284 $(M+H)^+$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.42 (t, J = 7.2 Hz, 3H), 4.40 (q, J = 7.2 Hz, 2H), 7.37 (d, J = 16.0 Hz, 1H), 7.49–7.58 (m, 4H), 7.79 (d, J = 16.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.7, 62.2, 120.7, 125.7, 130.0, 132.0, 132.6, 146.3, 161.6, 182.1.

4.1.2. Typical experimental procedure for the synthesis of 2,4-Substituted polyhydroquinoline (3a–n)[27]

Mixture of 5,5-dimethyl-cyclohexane-1,3-dione (1.2 mmol) and 1,3-diphenyl-propenone (1 mmol), ammonium acetate (2 mmol), PTSA (20 mol %) were taken in 50 mL round bottem flask, was added to MeOH. The reaction mixture was stirred at 80 °C for 2.5 h. After completion of the reaction as indicated by TLC, then treated with EtOAc followed by water and brine solution and dried with anhydrous Na_2SO_4. The solution was concentrated in vacuum to give the crude solid. The crude solid was purified by recrystallization from methanol. Although in case of nitro substituent, crude was purified by column chromatography with EtOH/hexane 1:5 as elutant. Compounds **3b–n** were prepared from the corresponding chalcone following similar procedure.

4.1.2.1. 7,7-Dimethyl-2,4-diphenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3a). Yield (82.78%), mp 204–205 °C. ESIMS (m/z) = 330.2 $(M+H)^+$. IR (KBr) 3283.1, 3077.5, 2959.1, 1657.1, 1621.5, 1590.4, 1241.3, 762.6, 698.0 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.90 (s, 3H), 0.97 (s, 3H), 2.01–2.30 (m, 4H), 4.61 (d, J = 5.22 Hz, 1H), 5.16 (dd, J = 5.33 Hz, 1.7 Hz, 1H), 5.85 (s, 1H), 6.99–7.27 (m, 10H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 26.78, 28.89, 31.65, 37.27, 39.43, 50.35, 106.18, 106.42, 125.14, 125.32, 127.11, 127.52, 127.71, 127.82, 134.19, 135.37, 147.85, 152.14, 194.43. Anal. Calcd for $C_{23}H_{23}NO$: C, 83.85; H, 7.04; N, 4.25. Found: C, 83.81; H, 7.01; N, 4.13.

4.1.2.2. 7,7-Dimethyl-4-(4-nitrophenyl)-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3b). Yield (85.6%), mp 189–190 °C. ESIMS (m/z) = 375.2 $(M+H)^+$. IR (KBr); 3319.9, 3063.7, 1660.1, 1591.6, 838.4 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.99 (s, 3H), 1.10 (m, 3H), 2.24–2.31 (m, 2H), 2.39–2.49 (m, 2H), 4.60 (d, J = 5.01 1H), 5.04 (dd, J = 5.01 Hz, J = 1.53, 1H), 6.76–6.82 (m, 2H), 7.15–7.33 (m, 7H), 7.53 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 26.37, 28.49, 31.21, 37.22, 39.65, 49.7, 103.74, 104.92, 122.37, 125.69, 126.46, 127.39, 127.55, 127.63, 128.07, 134.56, 135.03, 144.8, 152.13, 193.47. Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.82, H, 5.62, N, 7.39.

4.1.2.3. 4-(4-Chlorophenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3c). Yield (85.6%), mp 207–209 °C. ESIMS (m/z) = 375.2 $(M+H)^+$. IR (KBr): 3286.7, 3076.1, 1654.8,

1567.0, 1210.3, 749.6 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.00 (s, 3H), 1.10 (s, 3H), 2.00–2.15 (m, 2H), 2.52–2.56 (m, 2H) 4.61 (d, J = 5.15 1H), 5.08 (dd, J = 5.15 Hz, J = 1.50, 1H), 7.19–7.97 (m, 9H), 8.32 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 28.18, 31.40, 35.13, 40.21, 43.77, 54.33, 109.75, 110.06, 118.09, 129.11, 131.97, 132.00, 132.61, 132.79, 136.54, 138.78, 147.45, 156.53, 198.31. Anal. Calcd for $C_{23}H_{22}ClNO$: C, 75.92; H, 6.09; N, 3.85. Found: C, 75.99; H, 6.14; N, 3.81.

4.1.2.4. 4-(4-Fluorophenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3d). Yield (92.7%), ESIMS (m/z) = 347.2 $(M+H)^+$. IR (KBr); 3275.7, 3076.5, 1658.8, 1593.0, 1222.3, 760.6 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ: 0.99 (s, 3H), 1.08 (s, 3H), 2.03–2.48 (m, 4H), 4.63 (d, J = 5.22 Hz 1H), 5.15 (dd, J = 5.22 Hz, J = 1.44 Hz 1H), 6.93–7.50 (m, 9H), 8.6 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 30.81, 33.01, 35.73, 40.57, 44.26, 54.33, 109.81, 110.13, 118.10, 129.46, 132.01, 132.08, 132.73, 132.83, 138.57, 139.16, 148.15, 156.58, 198.36. Anal. Calcd for $C_{23}H_{22}FNO$: C, 79.51; H, 6.38; N, 4.03. Found: C, 79.49, H, 6.42, N, 4.12.

4.1.2.5. 4-(4-Methoxyphenyl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3e). Yield (81.0%), mp 212–214 °C. ESIMS (m/z) = 360.2 $(M+H)^+$. IR (KBr): 3434.3, 3020.0, 1605.5, 1216.2, 760.5, 670.0 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.92 (s, 3H), 1.00 (s, 3H), 2.08–2.26 (m, 4H), 3.66 (s, 3H), 4.58 (d, J = 5.21 Hz, 1H), 5.18 (dd, J = 5.21 Hz, J = 1.56 Hz, 1H), 6.05 (s, 1H), 6.70–7.36 (m, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 26.36, 28.38, 31.45, 35.81, 40.97, 49.76, 54.17, 106.21, 107.59, 112.64, 124.13, 127.50, 127.79, 132.93, 134.83, 139.21, 149.65, 156.83, 194.61. Anal. Calcd for $C_{24}H_{25}NO_2$: C, 80.19; H, 7.01; N, 3.90. Found: C, 80.13; H, 6.95; N, 3.91.

4.1.2.6. 2-(4-Methoxyphenyl)-7,7-dimethyl-4-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3f). Yield (78.7%), mp 214–216 °C. ESIMS (m/z) = 360.0 $(M+H)^+$. IR (KBr): 3274.7, 3076.5, 1668.8, 1573.0, 1221.3, 760.6 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.00 (s, 3H), 1.08 (s, 3H), 1.99–2.43 (m, 4H), 3.72 (s, 3H), 4.54 (d, J = 5.22 Hz, 1H), 5.10 (dd, J = 5.22 Hz, J = 1.28 Hz, 1H), 6.71 (d, J = 8.55 2H), 7.15 (d, J = 8.55, 2H), 7.29–7.48 (m, 5H), 8.35 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 26.11, 28.31, 30.94, 35.6, 37.5, 49.64, 53.75, 105.58, 105.8, 112.2, 124.68, 126.94, 127.09, 127.34, 133.36, 134.72, 139.72, 151.11, 156.33, 193.14. Anal. Calcd for $C_{24}H_{25}NO_2$: C, 80.19; H, 7.01; N, 3.90. Found: C, 80.18; H, 6.91; N, 3.98.

4.1.2.7. 2,4-Diphenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3g). Yield (81.5%), mp 206–209 °C. ESIMS (m/z) = 302.2 $(M+H)^+$. IR (KBr): 3247.3, 3063.5, 1653.3, 1586.5, 1488.9, 1272.2, 756.2, 693.0 cm^{-1}. ^1H NMR ($CDCl_3$ 200 MHz) δ: 1.91–2.00 (m, 2H), 2.25–2.32 (m, 2H), 2.57–2.67 (m, 2H), 4.66 (d, J = 5.32 1H), 5.14 (dd, J = 5.32 Hz, J = 1.57, 1H), 8.43 (s, 1H), 7.09–7.45 (m, 10H). Anal. Calcd for $C_{21}H_{19}NO$: C, 83.69; H, 6.35; N, 4.65. Found: C, 83.78; H, 6.29; N, 4.61.

4.1.2.8. 4-(4-Chlorophenyl)-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3h). Yield (88.5%), mp 210 °C. ESIMS (m/z) = 320.1 $(M+H)^+$. IR (KBr): 3221.5, 1663.6, 1584.0, 757.6 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.85–1.24 (m, 2H), 2.22–2.28 (m, 2H), 2.54–2.60 (m, 2H), 4.63 (d, J = 5.26 1H), 5.07 (dd, J = 5.26 Hz, J = 1.5 Hz, 1H), 7.14–8.05 (m, 9H), 8.46 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 20.16, 26.35, 36.04, 36.14, 104.74, 106.71, 124.95, 126.35, 127.40, 127.58, 128.04, 128.86, 129.83, 131.82, 134.9, 136.82, 141.79, 153.23, 193.75. Anal. Calcd for $C_{21}H_{18}ClNO$: C, 75.11; H, 5.40; N, 4.17. Found: C, 75.14; H, 5.34; N, 4.12.

4.1.2.9. 4-(4-Fluorophenyl)-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3i). Yield (74.5%), mp 202–204 °C. ESIMS (m/z) = 320.1 $(M+H)^+$. IR (KBr): 3319.9, 3063.7, 1660.1, 1591.6, 838.4 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ: 1.79–1.86 (m, 2H), 2.18–2.20 (m, 2H), 2.39–2.49 (m, 2H), 4.60 (d, J = 5.01 1H), 5.04 (dd, J = 5.01 Hz, J = 1.53, 1H), 6.76–6.82 (m, 2H), 7.15–7.33 (m, 7H), 7.53 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 21.12, 27.69, 36.82, 37.15, 106.40, 108.58, 125.75, 128.35, 128.43, 129.10, 129.21, 134.78, 135.82, 143.94, 143.98, 153.76, 162.63, 195.72. Anal. Calcd for $C_{21}H_{18}FNO$: C, 78.98; H, 5.68; N, 4.39. Found: C, 79.06; H, 5.71; N, 4.43.

4.1.2.10. 7,7-Dimethyl-2-phenyl-4-*p*-tolyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3j). Yield (79.4%), mp 206–208 °C. ESIMS (m/z) = 320.1 $(M+H)^+$. IR (KBr): 3339.9, 3064.2, 1656.1, 1588.6, 838.4 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ: 0.96 (s, 3H), 1.03 (s, 3H), 1.91- 2.20 (m, 4H), 2.30 (s, 3H), 4.55 (d, J = 5.28 Hz, 1H), 5.10 (dd, J = 5.28 Hz, 2H), 6.94–7.49 (m, 9H), 8.54 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 21.18, 27.37, 29.68, 32.35, 37.68, 40.72, 50.89, 105.70, 106.48, 125.87, 126.00, 127.80, 128.48, 129.42, 132.81, 134.79, 138.14, 148.99, 152.88, 194.27. Anal. Calcd for $C_{24}H_{25}NO$: C, 83.93; H, 7.34; N, 4.08: Found: C, 83.91; H, 7.48; N, 4.13.

4.1.2.11. 4-(1*H*-Indol-3-yl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3k). Yield (79.4%), mp 240–242 °C. ESIMS (m/z) = 369.2 $(M+H)^+$. IR (KBr): 3381, 3284.1, 3076.4, 2956.2, 1655.6, 1623.2, 1591.3, 1241.3, 762.6, 698.1 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.90 (s, 3H), 0.97 (S, 3H), 2.03–2.33 (m, 4H), 4.59 (d, J = 5.22 Hz, 1H), 5.14 (dd, J = 5.33 Hz, 1.7 Hz, 1H), 5.85 (s, 1H), 6.65–7.27 (m, 10H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 26.81, 28.89, 31.63, 37.26, 39.41, 50.34, 106.12, 106.41, 123.32, 125.14, 125.32, 127.11, 127.38, 127.52, 127.72, 127.83, 134.19, 135.38, 147.83, 152.11, 194.49. Anal. Calcd for $C_{25}H_{24}N_2O$: C, 81.49; H, 6.57; N, 7.60. Found: C, 81.53; H, 6.51; N, 7.53.

4.1.2.12. 2-Phenyl-4-*p*-tolyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3l). Yield (77.9%), mp 203–205 °C. ESIMS (m/z) = 320.1 $(M+H)^+$. IR (KBr): 3331.9, 3068.7, 1662.1, 1582.1, 843.5 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.87–2.09 (m, 2H), 2.14–2.30 (m, 2H), 2.37 (s, 3H), 2.49–2.69 (m, 2H), 4.58 (d, J = 5.40 Hz, 1H), 5.18 (d, J = 5.40, 1H), 7.07–7.46 (m, 9H), 8.61 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 21.17, 21.41, 27.37, 37.32, 37.38 105.60, 107.52, 125.89, 125.96, 127.74, 128.26, 128.53, 129.41, 132.79, 134.78, 138.16, 148.84, 154.77, 194.77. Anal. Calcd for $C_{22}H_{21}NO$: C, 83.78; H, 6.71; N, 4.44. Found: C, 83.85; H, 6.62; N, 4.48.

4.1.2.13. 4-(4-Methoxyphenyl)-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3m). Yield (87.6%), mp 207–208 °C. ESIMS (m/z) = 332.3 $(M+H)^+$. IR (KBr): 3275.7, 3074.5, 1658.8, 1593.0, 1222.3, 760.6 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.93–2.19 (m, 2H), 2.19–2.25 (m, 2H), 2.57–2.60 (m, 2H), 3.70 (s, 3H), 4.55 (d, J = 5.34, 1H), 5.10 (dd, J = 5.34 Hz, J = 1.65, 1H), 7.13–7.84 (m, 9H), 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 19.78, 25.91, 35.06, 35.72, 53.53, 105.17, 106.72, 124.46, 126.70, 126.87, 127.10, 127.57, 128.11, 133.15, 134.43, 139.43, 156.12, 193.07. Anal. Calcd for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.39; N, 4.23. Found: C, 79.61; H, 6.42; N, 4.28.

4.1.2.14. 4-(Furan-2-yl)-7,7-dimethyl-2-phenyl-4,6,7,8-tetrahydroquinolin-5(1*H*)-one (3n). Yield (84.3%), mp 167–169 °C. ESIMS (m/z) = 320 $(M+H)^+$. IR (KBr): 3325.4, 3289.1, 3046.5, 2934.2, 1655.6, 1623.2 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.00 (s, 3H), 1.08 (s, 3H), 1.99–2.43 (m, 4H), 4.34 (d, J = 5.16 Hz, 1H), 5.06 (dd, J = 5.16 Hz, J = 1.28 Hz, 1H), 6.21 (d, J = 6.34, 1H), 7.29–7.58 (m, 7H), 8.35 (s, 1H). Anal. Calcd for $C_{21}H_{21}NO_2$: C, 78.97; H, 6.63; N, 4.39. Found: C, 78.49; H, 6.75; N, 4.48.

4.1.3. Typical experimental procedure for the synthesis of lactol derivatives (3o–s)

Dimedone (1 mmol) and ethyl 4-phenyl-2-oxo-3-butenoate (**2o**) at 0 °C under nitrogen atmosphere was added PTSA 20 mol % in THF. After 24 h the solvents were removed in vacuo. The residue was purified by silica gel chromatography to afford corresponding lactol derivatives as colorless oil of **3o** in 78.5% yield.

4.1.3.1. Ethyl-2-hydroxy-7,7-dimethyl-5-oxo-4-phenyl-3,4,5,6,7,8-hexahydro-2*H*-chromene-2-carboxylate (3o). Yield (78.5%), ESIMS (m/z) = 345 $(M+H)^+$. IR (KBr): 2965.2, 1745.3, 1625.6, 1265.4, 1069.6 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.09 (s, 3H), 1.11 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H), 2.21–2.61 (m, 6H), 3.89 (t, J = 8.4 Hz, 1H), 4.17 (q, J = 7.2 Hz, 2H), 4.44 (s, 1H), 7.16–7.20 (m, 3H), 7.23–7.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 27.83, 28.79, 31.76, 36.27, 38.31, 42.55, 50.86, 63.08, 95.04, 112.19, 126.14, 127.18, 143.20, 167.40, 167.91, 168.97, 196.55, 196.90. Anal. Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.59; H, 7.18.

4.1.3.2. Ethyl-2-hydroxy-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-3,4,5,6,7,8-hexahydro-2H-chromene-2-carboxylate (3p). Yield (78.0%), ESIMS (m/z) = 375 $(M+H)^+$. IR (KBr): 3456, 2960, 1745, 1623, 1070 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.08 (s, 3H), 1.11 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H), 2.18–2.56 (m, 6H), 3.75 (s, 3H), 3.82–4.01 (m, 1H), 4.11–4.33 (m, 3H), 6.78 (d, J = 6.82, 2H), 7.07 (d, J = 6.82, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.03, 27.80, 29.42, 31.25, 36.04, 38.44, 42.54, 51.06, 55.26, 63.11, 95.89, 112.38, 114.33, 138.39, 135.06, 157.94, 166.94, 167.58, 169.11, 196.47, 196.92. Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.43; H, 6.87.

4.1.3.3. Ethyl-4-(4-chlorophenyl)-2-hydroxy-7,7-dimethyl-5-oxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-2-carboxylate (3q). Yield (79.4%), ESIMS (m/z) = 379 $(M+H)^+$. IR (KBr): 3462, 2963, 1743, 1629, 1070 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.08 (s, 3H), 1.11 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H), 2.16–2.67 (m, 6H), 3.82–4.01 (m, 1H), 4.19–4.42 (m, 3H), 7.12 (d, J = 7.13, 2H), 7.43 (d, J = 7.13, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.09, 28.49, 29.43, 32.27, 38.13, 42.59, 51.13, 63.59, 94.88, 95.62, 129.14, 131.48, 143.69, 167.51, 168.2, 196.48, 197.95. Anal. Calcd for $C_{20}H_{23}ClO_5$: C, 63.41; H, 6.12: Found: C, 63.38; H, 6.19.

4.1.3.4. Ethyl-4-(4-fluorophenyl)-2-hydroxy-7,7-dimethyl-5-oxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-2-carboxylate (3r). Yield (84.8%), ESIMS (m/z) = 363 $(M+H)^+$. IR (KBr): 3468, 2969, 1745, 1637, 1076 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.08 (s, 3H), 1.11 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H), 2.19–2.69 (m, 6H), 3.86–4.09 (m, 1H), 4.19–4.42 (m, 3H), 7.17–7.59 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.09, 28.50, 29.43, 32.29, 38.18, 42.61, 51.15, 63.59, 94.88, 95.62, 120.21, 131.79, 143.84, 167.62, 168.37, 196.53, 197.99. Anal. Calcd for $C_{20}H_{23}FO_5$: C, 66.29; H, 6.40. Found: C, 66.38; H, 6.29.

4.1.3.5. Ethyl-4-(4-bromophenyl)-2-hydroxy-7,7-dimethyl-5-oxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-2-carboxylate (3s). Yield (92.1%), ESIMS (m/z) = 423 $(M+H)^+$. IR (KBr). 3440, 3053, 1745, 1070 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.08 (s, 3H), 1.11 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H), 2.14–2.62 (m, 6H), 3.82–4.01 (m, 1H), 4.17–4.39 (m, 3H), 7.03 (d, J = 6.95, 2H), 7.34 (d, J = 6.95, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.08, 27.81, 29.44, 31.72, 33.23, 35.54, 38.13, 42,56, 50.96, 63.39, 94.84, 111.84, 113.95, 119.88, 128.95, 131.30, 142.48, 143.56, 167.34, 167.96, 169.00, 196.35, 196.81. Anal. Calcd for $C_{20}H_{23}BrO_5$: C, 56.75; H, 5.48. Found: C, 56.70; H, 5.86.

4.1.4. Typical experimental procedure for the synthesis of ethyl 7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-2-carboxylates derivatives (4o–s)

A solution of **3o** (1 mmol) and ammonium acetate (2 mmol) in MeOH (20 mL) was refluxed for 1 h. The solvent was evaporated and solid was filtered and washed with cold ethanol to provide **4o** as a yellow solid (97% yield).

4.1.4.1. Ethyl 7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate (4o). Yield (97.0%), ESIMS (m/z) = 356 $(M+H)^+$. IR (KBr): 3331.7, 1686, 1662.3, 845.3 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.02 (s, 3H), 1.08 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H), 2.19–2.35 (m, 4H), 4.25 (m, 2H), 4.72 (d, J = 5.6 Hz, 1H), 6.18 (d, J = 5.6 Hz, 1H), 6.45 (s, 1H), 6.93–7.48 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.25, 27.64, 29.33, 32.68, 37.11, 41.81, 50.83, 55.33, 61.94, 107.54, 113.97, 117.47, 127.65, 132.23, 139.78, 152.01, 159.23, 165.9, 195.98. Anal. Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.91; H, 7.25; N, 4.17.

4.1.4.2. Ethyl-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate (4p). Yield (95.2%), ESIMS (m/z) = 356 $(M+H)^+$. IR (KBr cm^{-1}); 3328.4, 1683, 1662.1, 1581.1, 845.7. ^1H NMR ($CDCl_3$, 300 MHz) δ = 1.02 (s, 3H), 1.08 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H), 2.18 (d, J = 16.4 Hz, 1H), 2.21 (d, J = 16.4 Hz, 1H), 2.30 (d, J = 16.4 Hz, 1H), 2.35 (d, J = 16.4 Hz, 1H), 3.76 (s, 3H), 4.25 (m, 2H), 4.68 (d, J = 5.6 Hz, 1H), 6.11 (d, J = 5.6 Hz, 1H), 6.44 (br s, 1H), 6.81 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 14.24, 27.64, 29.33, 32.68, 37.06, 41.79, 50.83, 55.32, 61.93, 107.53, 113.95, 117.46, 125.77, 129.01, 138.78, 150.01, 158.33, 163.18, 195.50. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.99; H, 7.17; N, 4.08.

4.1.4.3. Ethyl 4-(4-chlorophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate (4q). Yield (95.2%), ESIMS (m/z) = 360 $(M+H)^+$. IR (KBr): 3265.78, 1683.7, 1662.1, 1583.2 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ:1.02 (s, 3H), 1.08 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H), 2.23–2.41 (m, 4H), 4.26 (m, 2H), 4.74 (d, J = 5.6 Hz, 1H), 6.19 (d, J = 5.6 Hz, 1H), 6.51 (s, 1H), 7.19 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H). Anal. Calcd for $C_{20}H_{22}ClNO_3$: C, 66.75; H, 6.16; N, 3.89. Found: C, 66.45; H, 6.84; N, 4.12.

4.1.4.4. Ethyl-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate (4r). Yield (87.8%), ESIMS (m/z) = 344 $(M+H)^+$. IR (KBr): 3275.7, 3076.2, 1661.4, 1594.3 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.02 (s, 3H), 1.08 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H), 2.13–2.48 (m, 4H), 4.28 (m, 2H), 4.79 (d, J = 5.6 Hz, 1H), 6.23 (d, J = 5.6 Hz, 1H), 6.53 (s, 1H), 7.21 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H). Anal. Calcd for $C_{20}H_{22}FNO_3$: C, 69.95; H, 6.46; N, 4.08. Found: C, 70.12; H, 6.58; N, 3.97.

4.1.4.5. Ethyl-4-(4-bromophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylate (4s). Yield (92.8%), ESIMS (m/z) = 404 $(M+H)^+$. IR (KBr): 3254.3, 3073.5, 1661.6 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.02 (s, 3H), 1.08 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H), 2.12–2.43 (m, 4H), 4.28 (m, 2H), 4.75 (d, J = 5.6 Hz, 1H), 6.20 (d, J = 5.6 Hz, 1H), 6.53 (s, 1H), 7.16 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H). Anal. Calcd for $C_{20}H_{22}BrNO_3$: C, 59.42; H, 5.48; N, 3.46. Found: C, 59.19; H, 5.49; N, 3.32.

4.1.5. Typical experimental procedure for the synthesis of 7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid derivatives (5a–e)

Compound **4o** (1 mmol) was dissolved in EtOH (10 mL and taken in to the 50 mL R.B. flask and cool to 0 °C. 20% sodium carbon-

ate solution of EtOH was added dropwise to compound **4o** and stirred for 20 h. After the solid appeared was filtered and washed with cold ethanol to give **5a** in 96.7% yield.

4.1.5.1. 7,7-Dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid (5a). Yield (96.7%), ESIMS (m/z) = 298 $(M+H)^+$. IR (KBr): 3456–3113.7, 1686, 1659.3, 845.3 cm^{-1}. ^1H NMR (CD_3OD, 300 MHz) δ: 1.00 (s, 3H), 1.06 (s, 3H), 2.19–2.35 (m, 4H), 4.69 (d, J = 5.6 Hz, 1H), 6.17 (d, J = 5.6 Hz, 1H), 6.45 (s, 1H), 6.89–7.42 (m, 5H), 12.34 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 27.64, 29.33, 32.65, 37.11, 41.81, 50.83, 55.33, 107.51, 113.96, 117.47, 127.66, 130.21, 138.56, 152.01, 159.23, 161.67, 194.78. Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.71; H, 6.44; N, 4.71.

4.1.5.2. 4-(4-Methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid (5b). Yield (92.8%), ESIMS (m/z) = 328 $(M+H)^+$. IR (KBr): 3412–3132.4, 1684.5, 1656.1, 1576.9 cm^{-1}. ^1H NMR (CD_3OD, 300 MHz) δ: 1.00 (s, 3H), 1.06 (s, 3H), 2.16 (d, J = 16.4 Hz, 1H), 2.21 (d, J = 16.4 Hz, 1H), 2.30 (d, J = 16.4 Hz, 1H), 2.35 (d, J = 16.4 Hz, 1H), 3.89 (s, 3H), 4.68 (d, J = 5.6 Hz, 1H), 6.11 (d, J = 5.6 Hz, 1H), 6.79 (d, J = 8.1 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 10.24 (s, 1H), 12.89 (s, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ: 27.63, 29.33, 32.65, 37.06, 41.77, 50.78, 55.65, 107.57, 113.75, 117.18, 125.77, 128.88, 138.78, 150.01, 158.32, 159.39, 195.24. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.62; H, 6.38; N, 4.11.

4.1.5.3. 4-(4-Chlorophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid (5c). Yield (96.2%), ESIMS (m/z) = 332 $(M+H)^+$. IR (KBr cm^{-1}); 3417.8–3119.6, 1683.7, 1656.2, 1585.9. ^1H NMR (CD_3OD, 300 MHz) δ: 1.00 (s, 3H), 1.06 (s, 3H), 2.19–2.36 (m, 4H), 4.73 (d, J = 5.45 Hz, 1H), 6.13 (d, J = 5.45 Hz, 1H), 7.14 (d, J = 8.39 Hz, 2H), 7.33 (d, J = 8.39 Hz, 2H). 10.45 (s, 1H), 12.68 (s, 1H). Anal. Calcd for $C_{18}H_{18}ClNO_3$: C, 65.16; H, 5.47; N, 4.22. Found: C, 65.04; H, 5.12; N, 4.06.

4.1.5.4. 4-(4-Fluorophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid (5d). Yield (79.8%), ESIMS (m/z) = 316 $(M+H)^+$. IR (KBr): 3424.6–3123.9, 3278.5, 3079.3, 1654.9 cm^{-1}. ^1H NMR (CD_3OD, 300 MHz) δ: 1.00 (s, 3H), 1.06 (s, 3H), 2.13–2.48 (m, 4H), 4.74 (d, J = 5.58 Hz, 1H), 6.21 (d, J = 5.58 Hz, 1H), 7.20 (d, J = 8.59 Hz, 2H), 7.41 (d, J = 8.59 Hz, 2H), 10.38 (s, 1H), 12.56 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 27.69, 29.34, 32.69, 36.99, 41.84, 50.83, 113.98, 117.46, 125.77, 128.76, 138.69, 149.82, 158.31, 156.11, 195.17. Anal. Calcd for $C_{18}H_{18}FNO_3$: C, 68.56; H, 5.75; N, 4.44. Found: C, 68.49; H, 5.83; N, 4.11.

4.1.5.5. 4-(4-Bromophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-2-carboxylic acid (5e). Yield (82.3%), ESIMS (m/z) = 404 $(M+H)^+$. IR (KBr cm^{-1}); 3425.7–3124.3, 3279.3, 3081.9, 1658.1, 1555.8. ^1H NMR (CD_3OD, 300 MHz) δ: 1.00 (s, 3H), 1.06 (s, 3H), 2.15–2.41 (m, 4H), 4.74 (d, J = 5.57 Hz, 1H), 6.19 (d, J = 5.57 Hz, 1H), 7.19 (d, J = 8.74 Hz, 2H), 7.69 (d, J = 8.74 Hz, 2H), 10.29 (s, 1H), 12.63 (s,1H). Anal. Calcd for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72. Found: C, 57.49; H, 4.75; N, 3.81.

4.1.6. Typical experimental procedure for the aromatization of polyhydroquinoline (6a–c)

Polyhydroquinoline **3d** (1 mmol), HbA (0.1 mmol), H_2O_2 (30% w/v, 2 mmol), 15% acetonitrile in phosphate buffer (pH 6.5, 3 mL was taken in to the 50 mL R.B. flask and stirred at room temperature for 12 h. After the completion of the reaction acetonitrile was evaporated and diluted with DCM (125 mL) and washed with water (3 × 50 mL). Obtained crude product was purified by silica gel column chromatography in 82% yield.

4.1.6.1. 4-(4-Fluorophenyl)-7,7-dimethyl-2-phenyl-7,8-dihydroquinolin-5(6*H*)-one (6a). Yield (82.3%), mp 176–178 °C. ESIMS (m/z) = 346 $(M+H)^+$. IR (KBr): 3074.6, 1656.2, 1591.5 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.01 (s, 3H), 1.08 (s, 3H), 2.04- 2.43 (m, 4H), 7.08–7.56 (m, 7H), 7.62–7.96 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 24.23, 24.37, 32.55, 43.35, 49.48, 112.32, 113.46, 118.84, 124.10, 124.21, 126.18, 127.35, 132.31, 133.21, 138.29, 144.26, 146.33, 152.17, 159.47, 198.29. Anal. Calcd for $C_{23}H_{20}FNO$: C, 79.98; H, 5.84; N, 4.06. Found: C, 79.74; H, 5.69; N, 4.22.

4.1.6.2. 4-(4-Chlorophenyl)-7,7-dimethyl-2-phenyl-7,8-dihydroquinolin-5(6*H*)-one (6b). Yield (82.3%), mp 188–189 °C. ESIMS (m/z) = 362 $(M+H)^+$. IR (KBr): 3074.2, 1655.4, 1588.2, 956, 742 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.01 (s, 3H), 1.08 (s, 3H), 2.03–2.43 (m, 4H), 6.93–7.42 (m, 7H), 7.39–7.61 (m, 2H), 7.79 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 24.23, 24.35, 32.55, 43.31, 49.48, 114.24, 115.16, 118.81, 124.23, 125.67, 128.18, 132.31, 137.24, 138.37, 144.73, 147.33, 153.21, 159.47, 198.29. Anal. Calcd for $C_{23}H_{20}ClNO$: C, 76.34; H, 5.57; N, 3.87. Found: C, 76.42; H, 5.64; N, 4.01.

4.1.6.3. 4-(4-Methoxyphenyl)-7,7-dimethyl-2-phenyl-7,8-dihydroquinolin-5(6*H*)-one (6c). Yield (82.3%), mp 220–222 °C. ESIMS (m/z) = 358 $(M+H)^+$. IR (KBr): 3070.3, 1654.2, 1588.5 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.01 (s, 3H), 1.08 (s, 3H), 2.03–2.39 (m, 4H), 3.66 (s, 3H), 6.57–7.47 (m, 7H), 7.39–7.58 (m, 2H), 7.77 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 24.22, 24.33, 32.55, 43.32, 49.48, 54.12, 111.23, 114.78, 118.65, 124.13, 125.62, 128.11, 131.89, 137.23, 138.33, 142.39, 144.73, 147.26, 153.21, 159.47, 197.86. Anal. Calcd for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 76.42; H, 5.64; N, 4.01.

4.2. Pharmacology

4.2.1. Sucrose loaded rat model (SLM)

Male albino rats of Charles Foster/Wistar strain of average body weight 160 ± 20 g were selected for this study. The blood glucose level of each animal was checked by glucometer using glucostrips (Boehringer Mannheim) after 16 h starvation. Animals showing blood glucose levels between 3.33 and 4.44 mM (60–80 mg/dl) were divided into groups of five to six animals in each. Animals of experimental group were administered suspension of the desired synthetic compound orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load (10.0 g/kg) was given to each animal orally exactly after 30 min post administration of the test sample/vehicle. Blood glucose profile of each rat was again determined at 30, 60, 90, and 120 min post administration of sucrose by glucometer Food but not water was withheld from the cages during the course of experimentation. Quantitative glucose tolerance of each animal was calculated by Area Under Curve (AUC) method (Prism Software). Comparing the AUC of experimental and control groups determined the percentage anti-hyperglycemic activity.

4.2.2. Sucrose-challenged streptozotocin-induced diabetic rat model (STZ-S)

Male albino rats of Sprague Dawley strain of body weight 160 ± 20 g were selected for this study. Streptozotocin (Sigma, USA) was dissolved in 100 mM citrate buffer pH 4.5 and calculated amount of the fresh solution was injected to overnight fasted rats (60 mg/kg) intraperitoneally. Blood glucose level was checked 48 h later by glucostrips and animals showing blood glucose values between 144 and 270 mg/dl (8–15 mM) were included in the experiment and considered as diabetic. The diabetic animals were divided into groups consisting of five to six animals in each group.

Animals of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg body weight was given after 30 min of compound administration. After 30 min of post sucrose load blood glucose level was again checked by glucostrips at 30, 60, 90, 120, 180, 240, 300 min, and at 24 h, respectively. Animals not found diabetic after 24 h post treatment of the test sample were not considered and omitted from the calculations and termed as non-responders. The animals, which did not show any fall in blood glucose profile in a group while the others in that group, showed fall in blood glucose profile were also considered as non-responders. Food but not water was withheld from the cages during the experimentation. Comparing the AUC of experimental and control groups determined the percent antihyperglycemic activity.

4.2.3. Antihyperglycemic activity in db/db mice

The background for the db/db mouse is the C57BL/Ks strain. The major deficiency of the C57BL/KsBom-db mouse (db/db) is lack of a functional leptin receptor. This leads to defective leptin signaling and a complete lack of feedback from leptin. Both hypothalamic Neuropeptide Y (NPY) content and secretion are consequently elevated, and this result in hyperphagia and decreased energy expenditure, obesity, insulin-resistance, hyperinsulinemia, hyperglycaemia and dyslipidemia. The db/db mouse develops non insulin dependent diabetes mellitus (NIDDM) from around week 10. The disease is stable until week 20, where destruction of pancreatic β-cells can be recognized clinically as decreasing levels of plasma insulin and very severe hyperglycaemia. The db/db mouse has a maximal life span of 9–12 months. The male mice are more diabetic than, and will normally die earlier than the females. The advantage of using male mice for experimental purposes is that the fluctuations in plasma parameters are less than in the females where the oestrogen cycle affects the clinical diabetes. The optimal age of db/db mice used for experiments is from week 12 to 18 when they have developed non insulin dependent diabetes mellitus (NIDDM) with diabetic dyslipidemia but still have functional β-cells in the pancreas. C57BL/KsBom-db mice 12–18 weeks, 40–50 g bred in the animal house of CDRI, Lucknow. Ten mice (5 males and 5 females) were used in the experiments. The mice were housed in groups of 5 (same sex) in a room controlled for temperature (23 ± 2.0 °C) and 12/12 h light/dark cycle (lights on at 6.00 am). Body weight was measured daily from day 1 to day 10. All animals had free access to fresh water and to normal chow except on the days of the postprandial protocol day 6 and during the overnight fast before the OGTT on day 10. The animals always had access to water during experimental periods. Blood glucose was checked every morning up till day 5. On day 6 postprandial protocol was employed, in this method blood glucose was checked at −0.5 h and 0 h. Test drugs were given to the treatment group whereas vehicle received only gum acacia (1.0%); the blood glucose was again checked at 1, 2, 3, 4, and 6 h post test drug treatment. On day 10 an oral glucose tolerance test (OGTT) was performed after an overnight fasting. Blood glucose was measured at −30.0 min and test drugs were administered. The blood glucose was again measured at 0.0 min post treatment and at this juncture glucose solution was given at a dose of 3 gm/kg to all the groups including vehicle. The blood glucose levels were checked at 30 min, 60 min, 90 min, and 120 min post glucose administration. At the end of the experiment blood has been withdrawn from the retro-orbital plexus of mice for the estimation of serum insulin. Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method (Prism software). Comparing the AUC of experimental and control groups determined the %antihyperglycemic activity. Statistical comparison was made by Dunnett's test.

4.2.4. Protein-tyrosine phosphatase 1B inhibitory assay

The test compounds were pre-incubated for 10 min with the enzyme in the absence and presence of 0.01% Triton X–100. Assay was performed in final volume of 1.0 mL in test mixture containing 10 mM of pNPP in 50 mM HEPES buffer (pH 7.0) with 1 mM DTT and 2 mM EDTA. The reaction was stopped after 30 min of incubation at 37 °C by addition of 500 μl of 0.1 N NaOH and the absorbance was determined at 410 nm. A molar extinction coefficient of $1.78 \times 10^4\ M^{-1}\ cm^{-1}$ was utilized to calculate the concentration of *p*-nitrophenolate ion generated in the reaction mixture. Sodium orthovanadate was taken as standard in enzyme assay. The IC_{50} of the compounds were determined by constructing a dose–response curve and examining the effect of different concentrations of compounds.

4.2.5. Glucose-6-phosphatase inhibitory activity assay

The 1.0 mL assay system contained 0.3 M citrate buffer (pH 6.0), 28 mM EDTA, 14 mM NaF, 200 mM glucose-6-phosphate, and enzyme protein. The mixture was incubated at 37 °C for 30 min after which 1.0 mL of 10% TCA was added. Glucose-6-phosphatase activity was defined as micromole Pi released per minute per milligram protein.

4.2.6. Glycogen phosphorylase inhibitory activity assay

Mixture A contained 57 mg glycogen (substrate), 188 mg glucose-1-phosphate, 42 mg sodium fluoride, 138.8 mg 5′-AMP (4 mM) dissolved in 10 mL water. The reaction mixture containing 0.2 mL Mixture-A, 0.1 mL enzyme is incubated for 30 min at 37 °C and reaction terminated by adding 0.1 mL TCA (10%) and 0.4 mL of 0.1 M sodium acetate. The mixture is kept overnight at 4 °C and the Pi released is estimated.

4.2.7. α-Glucosidase inhibitory activity assay

α-Glucosidase inhibitory activity was determined in 1 mL reaction system in 67 mM sodium phosphate buffer (pH 6.8) containing 1.0 mg/mL glutathione in the presence of 0.1 mg/mL purified α-glucosidase. The reaction was started by adding 3.0 mg/mL pNPG to the reaction mixture. The reaction was followed for 3 min at 405 nm at the interval of 30 s.

4.2.8. DPP IV enzyme inhibition assay

Enzymatic activity was determined at 37 °C by the cleavage rate of a substrate, Gly-Pro-AMC (30 lM) (Sigma–Aldrich, USA). Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μL Gly-Pro-AMC, 10 μL of 500 mM Tris–HCl (pH 7.4), 20 μL of distilled water, and 10 μL of a test compound. The change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-IV enzyme activity was calculated over the first 15 min of the reaction, with units/mL being defined as the rate of increase in the fluorescence intensity (arbitrary units) under these conditions.

4.2.9. Antidyslipidemic activity

Adult male Charles Foster rats (200 ± 225 g) bred in the animal house of the institute were used for the lipid lowering activity. Rats were divided in control, triton induced, triton plus compounds and Gemfibrozil (100 mg/kg) treated groups containing five rats in each. Hyperlipidemia was developed by administration of Triton WR-1339 (Sigma chemical co., St. Louis, USA) at a dose of 400 mg/kg body wt intraperitoneally to animals of all groups except the control. Compounds were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton at a dose of 100 mg/kg po to the animals of treated groups. Animals of the control and triton group without treatment with test com-

pounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment (50 mg/kg b. wt) 1.0 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated eppendorf tube (3.0 mg/mL blood). The blood was centrifuged (at 2500*g*) at 4 °C for 10 min and the plasma was separated. Plasma was diluted with normal saline (ratio 1:3) and used for analysis of total cholesterol (TC), phospholipids (PL), and triglycerides (TG) by standard enzymatic procedures.

Acknowledgements

S.S, V.D.T, R.A.M, and S.P.S are thankful to CSIR New Delhi for the award of fellowship. The authors also acknowledge CSIR networking project NWP-032 for financial support and highly thankful to SAIF for providing the spectral data.

References and notes

1. (a) Tassoni, E.; Giannessi, F.; Brunetti, T.; Pessotto, P.; Renzulli, M.; Travagli, M.; Rajamäki, S.; Prati, S.; Dottori, S.; Corelli, F.; Cabri, W.; Carminati, P.; Botta, M. *J. Med. Chem.* **2008**, *51*, 3073; (b) Ross, S. A.; Gulve, E. A.; Wang, M. *Chem. Rev.* **2004**, *104*, 1255; (c) Rakowitz, D.; Maccri, R.; Ottana, R.; Vigorita, M. G. *Bioorg. Med. Chem. Lett.* **2006**, *14*, 567.
2. King, H.; Aubert, R. E.; Herman, W. H. *Diabetes Care* **1998**, *21*, 1414.
3. Boyle, J. P.; Honeycutt, A. A.; Narayan, V. K. M.; Hoerger, T. J.; Geiss, L. S.; Chen, H.; Thompson, T. J. *Diabetes Care* **2001**, *24*, 1936.
4. Tackling the threat to health of diabetes mellitus (Editorial). *Lancet* **2006**, *398*, 1624.
5. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047.
6. Zimmet, P. Z.; Alberti, K. G. M. M.; Shaw, J. *Nature* **2001**, *414*, 782.
7. American Diabetes Association *Diabetes Care* **2003**, *26*, 917–932.
8. Pyorala, K.; Pedersen, T. R.; Kjekshus, J.; Fraergeman, O.; Olsson, A. G.; Thorgeirsson, G. *Diabetes Care* **1997**, *20*, 614.
9. (a) Bossert, F.; Meyer, H.; Wehinger, E. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 762; (b) Nakayama, H.; Kasoaka, Y. *Heterocycles* **1996**, *42*, 901.
10. Triggle, D. J.; Langs, D. D.; Janis, R. A. *Med. Res. Rev.* **1989**, *9*, 123.
11. (a) Mason, R. P.; Mak, I. T.; Trumbore, M. W.; Mason, P. E. *Am. J. Cardiol.* **1999**, *84*, 16–22; (b) Aruoma, O.; Smith, C.; Cecchini, R.; Evans, P.; Halliwell, B. *Biochem. Pharmacol.* **1991**, 42.
12. Hilgeroth, A. *Mini-Rev. Med. Chem.* **2002**, *2*, 235.
13. Kawase, M.; Shah, A.; Gaveriya, H.; Motohashi, N.; Sakagami, H.; Varga, A.; Molnar *J. Bioorg. Med. Chem.* **2002**, *10*, 1051.
14. (a) Kumar, A.; Maurya, R. A.; Sharma, S.; Ahmad, P.; Singh, A. B.; Tamrakar, A. K.; Srivastava, A. K. *Bioorg. Med. Chem.* **2009**, *17*, 5285–5292; (b) Kumar, A.; Ahmad, P.; Maurya, R. A.; Singh, A. B.; Tamrakar, A. K.; Srivastava, A. K. *Eur. J. Med. Chem.* **2009**, *44*, 109; Kumar, A.; Maurya, R. A.; Sharma, S.; Ahmad, P.; Singh, A. B.; Bhatia, G.; Srivastava, A. K. *Bioorg. Med. Chem. Lett.* **2009**. doi:10.1016/j.bmcl.2009.09.031.
15. (a) Ladouceur, G. H.; Cook, J. H.; Doherty, E. M.; Schoen, W. R.; MacDougall, M. L.; Livingston, J. N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 461; (b) Schoen, W. R.; Ladouceur, G. H.; Cook, J. H.; Lease, T. G.; Wolanin, D. J.; Kramss, R. H.; Hertzog, D. L.; Osterhout, M. H. U.S. Patent No. 6218,431, 2001.
16. (a) Goldmann, S.; Ahr, H.-J.; Puls, W.; Bischoff, H.; Petzinna, D.; Schlossman, K.; Bender, J. U.S. Patent, 4786,641, 1988; (b) Oikonomakos, N. G.; Tsitsanou, K. E.; Zographos, S. E.; Skamnaki, V. T.; Goldmann, S.; Bischoff, H. *Protein Sci.* **1999**, *8*, 1930; (c) Bergans, N.; Stalmans, W.; Goldmann, S.; Vanstapel, F. *Diabetes* **2000**, *49*, 1419.
17. Poindexter Swann, G. S.; Swann, R. T.; Bruce, M. A.; Morton, M. A.; Huang, Y.; Sit, S.; Breitenbucher, J. G. WO/1998/054136, 1998.
18. Sogawa, S.; Nihro, Y.; Ueda, H.; Izumi, A.; Miki, T.; Matsumoto, H.; Satoh, T. *J. Med. Chem.* **1993**, *36*, 3904.
19. Kumar, A.; Maurya, R. A.; Sharma, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4432.
20. Taussky, H. H.; Shorr, E. A. *J. Biol. Chem.* **1953**, *202*, 675.
21. Hubscher, G.; West, G. R. *Nature* **1965**, *205*, 799.
22. Wright, S. W.; Ammirati, M. J.; Andrews, K. M.; Brodeur, A. M.; Danley, D. E.; Doran, S. D.; Lillquist, J. S.; McClure, L. D.; McPherson, R. K.; Orena, S. J.; Parker, J. C.; Polivkova, J.; Qiu, X.; Soeller, W. C.; Soglia, C. B.; Treadway, J. L.; VanVolkenberg, M. A.; Wang, H.; Wilder, D. C.; Olson, T. V. *J. Med. Chem.* **2006**, *49*, 3068.
23. Rall, T. W.; Sutherland, E. W.; Barthet, J. *J. Biol. Chem.* **1957**, *244*, 463.
24. Lebovitz, H. E. *Endocrinol. Metab. Clin. North Am.* **1997**, *26*, 539–551.
25. Goldstein, B. J.; Bittner-Kowalezyk, A.; White, M. F.; Harbeck, M. *J. Biol. Chem.* **2000**, *275*, 4283.
27. Kumar, A.; Maurya, R. A. *Tetrahedron* **2007**, *64*, 3477.

Further reading

26. (a) Schurr, P. E.; Schultz, J. R.; Parkinson, T. M. *Lipids* **1972**, *7*, 68; (b) Deeg, R.; Ziegehorn *J. Clin. Chem.* **1983**, *29*, 1798.