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Studies on some glitazones having pyridine as the linker unit☆

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Abstract—Molecular modeling on various well-known glitazones carrying a pyridine ring instead of benzene ring as the middle linker unit showed conformational rigidity as compared to their parent molecules. Blocking the lone pair of electrons on the pyridine N, made them flexible once again. A few representatives of these analogues were synthesized and their efficacy as PPARγ agonists evaluated.

1. Introduction

Type 2 diabetes (non-insulin dependent diabetes mellitus-NIDDM) is a complex metabolic disorder characterized by hyperglycemia and/or insulin resistance, and is often associated with other disorders such as obesity, hypertension and hyperlipidemia.[1–4] The type 2 diabetes is the most common type and accounts for more than 90% of the diagnosed cases of diabetes.

The discovery of nuclear receptor peroxisome proliferator activated receptors, PPARγ and PPARα as molecular targets[5,6] for antidiabetic thiazolidinediones (TZDs) has heralded a new era in the approach to understanding the patho-physiology of insulin resistance and its relationship to cardiovascular diseases.[7–9] The thiazolidinediones which are high affinity agonists for PPARγ led to the proposal that ligand-dependent modulation of gene transcription through PPARγ is an important pharmacological target for treating type 2 diabetes.[9]

A series of 5-(4-alkoxybenzyl) 2,4-thiazolidinediones were reported by Sohda et al.[10] as antihyperglycemic agents. The representative agent ciglitazone lowered elevated glucose plasma insulin and triglyceride levels in insulin resistant animals but showed no hypoglycemic effect in non-diabetic or streptozotocin induced diabetic rats.[11] Because of the several attractive features, such as improvement of insulin sensitivity and lack of hypoglycemia, the last few years have seen development of many agents of this type such as troglitazone, pioglitazone, and rosiglitazone. Although troglitazone was withdrawn in the early 2000 on the basis of idiosyncratic hepatotoxicity, several more are progressing through clinical development.[12,13] The medical needs driving this investment are the desire for greater degree of glucose lowering, and also the potential reductions in plasma triglycerides which is now known to increase not only insulin sensitivity but leptin sensitivity as well.[14]

The compounds of this class have a few essential pharmacophore elements. These comprise of an acidic group linked to a central flat ring and a large lipophilic substructure. Various structural modifications containing these essential features have been reported. But there are very few reports where the central phenyl linker

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fragment is replaced. Those reported are with thiophene,[15] naphthalene,[16] benzofuran,[17] benzoxazole[18] and pyridine.[10,19,20,21] This central linker is essentially a planar, aromatic ring and has been suggested to have hydrophobic and π–π stacking interactions while binding to the receptor.[22] It also restricts the conformations of terminal lipophilic fragment. There is some controversy regarding the antidiabetic activity when this central linker fragment is substituted by pyridine. Some TZDs having a pyridine ring instead of the benzene ring in the 4-oxyaryl position were reported to have weaker activities.[10] However, pyridyl analogues of some other thiazolidinediones,[19] 2,4-oxazolidinediones[20] and tetrazoles[23] exhibited potent glucose and lipid-lowering activities.

We carried out semi-empirical AM1 calculations to understand the electronic features, which control the conformational preferences of a series of glitazones with pyridine as the central ring. This was followed up by the synthesis of some of them and checking their PPAR agonist activity.

2. Molecular modeling and chemistry

Semi-empirical AM1 calculations[24] were performed on pioglitazone **1** (Fig. 1) to identify the most stable conformation. Complete optimizations on **1** showed that the most stable conformation has an almost planar arrangement, except that the thiazolidinedione ring (ring A) is on a slightly elevated plane to the rest of the molecule. The C1–C2–O3–C4 and C2–O3–C4–C5 torsional angles are about 180° (Fig. 1). This gas phase optimized structure is slightly different from the bioactive conformation which gives U-shape as in rosiglitazone,[25] the energy difference between the global minimum and the bioactive conformation is only about 0.2 kcal/mol at AM1 level. A U-shaped conformation is required for the drug to be an effective agonist because the PPAR homodimer and PPAR-RXR heterodimer possesses a Y-shaped active site, such that the U-shaped glitazone can fit in two arms of the Y-shaped active site.[26,27] The modeling studies reported here are based on the bioactive (U-shaped) conformation of glitazones and their derivatives.

AM1 calculations were then carried out on **2**, where the central benzene ring of 1 is replaced by pyridine. Complete optimizations of **2** lead to a local minimum structure, conformational analysis on **2** showed that **2a**, which is more stable by about 4.62 kcal/mol, is the global minimum on the potential energy (PE) surface of **2**. The differences in the structures **2** and **2a** are due to the torsional angle N1–C2–O3–C4 being 180° in **2** and 0° in **2a**. This change in the conformation of the most stable structure of **2** indicates that the replacement of benzene with pyridine in **1** leads to some instability, which can be attributed to the repulsive interactions between the lone pairs of electrons on N1 and O3 in **2**. The AM1 calculated rotation barrier for the rotation across the C2–O3 bond in **2** is ~0.32 kcal/mol and that in **2a** is 4.94 kcal/mol. This C2–O3 rotational process indicates that **2a** conformation should be the preferred conformation of the pyridine derivative of pioglitazone. A Boltzman population estimate indicates that **2a** structure is much more populated than **2**, in the ratio 1:2353 (Table 1). Similar rotational process in **1** shows an energy difference of only 0.04 kcal/mol with a barrier of only 1.77 kcal/mol at AM1 level. This suggests that there is an inherent conformational rigidity to the pyridine derivative of pioglitazone, originating because of the lone-pair repulsions on N and O. This observation was also confirmed by the *ab initio*[28] HF/3-21G*//AM1 calculations performed on **2** and **2a**, which show an energy difference (ΔE) of 5.17 kcal/mol and a rotational barrier of about 6.50 kcal/mol. This is further supported by the same study in the solvent phase using the Self-consistent Reaction Field (SCRF)[29] with water (ε = 78.39) as solvent, which shows ΔE value of 5.07 kcal/mol and rotational barrier of 6.89 kcal/mol. Fig. 2a and 2b shows the 3-D structures of **1** and **2a**.

The AM1 studies on the pyridine derivatives of rosiglitazone **6**, troglitazone **7**, bromo derivative of rosiglitazone **8**, and indole derivative of glitazone **9** (Fig. 1), showed large rotational barriers, 4.84, 5.30, 4.93 and 5.40 kcal/mol respectively, which amounts the Boltzman ratios of 1:3404, 1:2016, 1:3946 and 1:4606 respectively. This suggests that the replacement of the central benzene ring in well-known glitazones with a pyridine ring

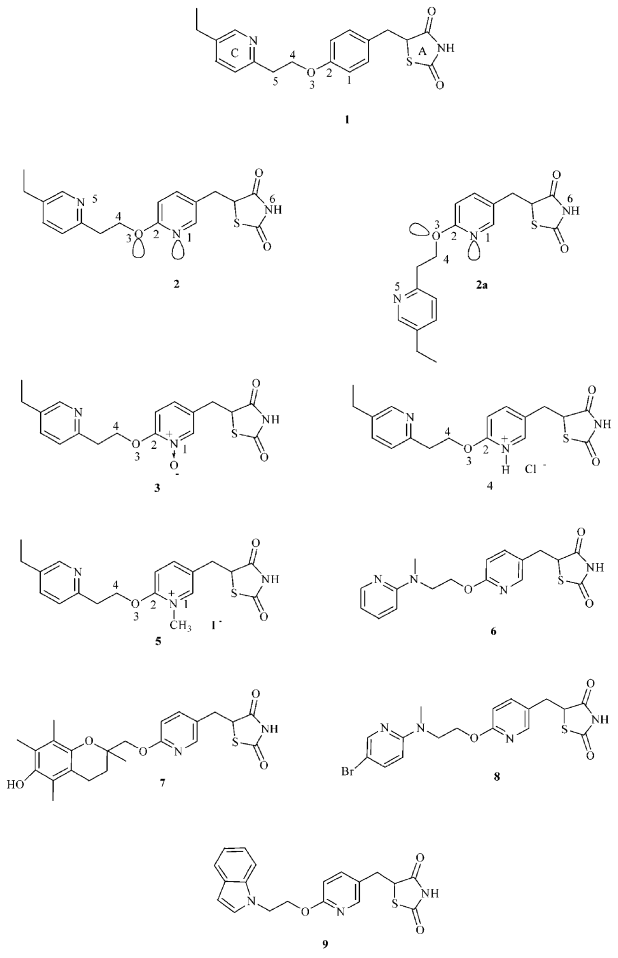


Figure 1. Structures of pyridine derivatives of some glitazones.

Table 1. Heats of formation (H_f) and relative energuies (kcal/mol) of various conformers across C2–O3 rotational path of Pioglitazone and i9ts derivatives

Compd	H_f-180°	H_f-0°	H_f-TS	ΔH_f	Rotational barrier	Population ratio
1	−57.77	−57.73	−55.99	0.04	1.77	1:1
2	−42.44	−47.06	−42.12	4.62	4.94	1:2353
3	−35.11	−35.06	−34.73	0.05	0.38	1:1
4	102.16	103.08	106.70	0.45	3.86	2:1
5	106.16	107.06	109.70	1.00	3.53	5:1

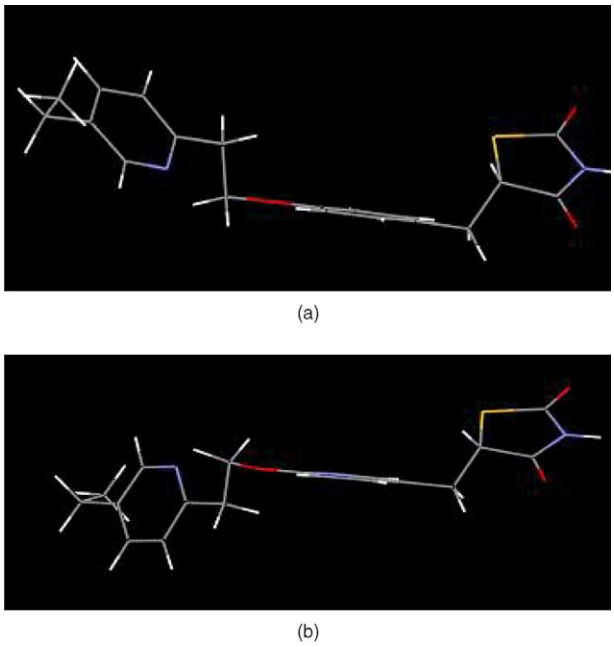


Figure 2. (a) 3-D structure of **1**; (b) 3-D structure of **2a**.

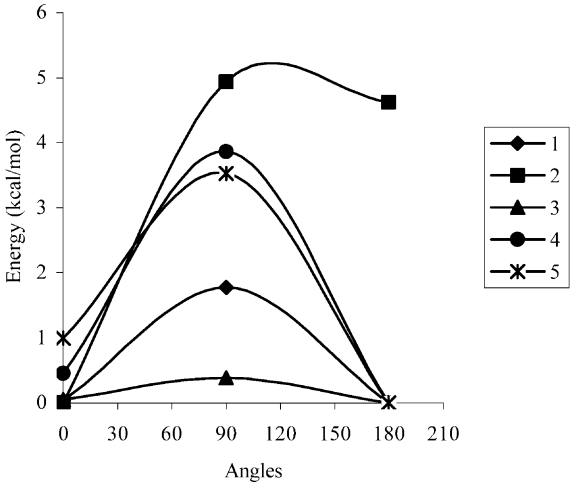


Figure 3. The PE surface for the rotation across C2–O3 bond in the derivatives of pioglitazone.

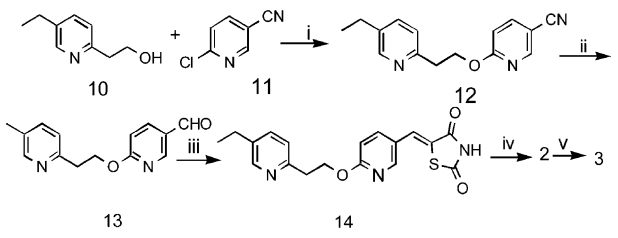
would lead to an increase in the conformational rigidity of these systems, and the preferred conformation in these systems is opposite to that of the parent glitazones.

Methods to eliminate the conformational rigidity in **2** in order to make the pyridine derivatives as flexible as pioglitazone, were explored. Ideally, it can be done by converting **2** to its HCl salt. We carried out AM1 calculations on **2** with protonation at various possibilities and found that the most favored protonation site is N1 (Fig. 1) hence the HCl salt of **2** is expected to have a structure **4**. The C2–O3 rotational barrier in **4** is 3.86 kcal/mol and the ΔE between the rotamers is 0.45 kcal/mol. Boltzman population analysis suggests that the ratio of population of **4** and **4a** are 2:1, indicating that the relative preferences are much less pronounced and conformational rigidity disappears in the HCl salt of **2**. Similarly the N-oxide derivative **3** and the methoiodide salt **5** of **2**, also showed a much reduced conformational rigidity. Comparison of the relative energies and the rotation barriers of the various conformations of these derivatives is given in Table 1 and Figure 3. Similar results have been observed in the protonated salts, N-oxides and methoiodide salts of **6**, **7**, **8** and **9.**

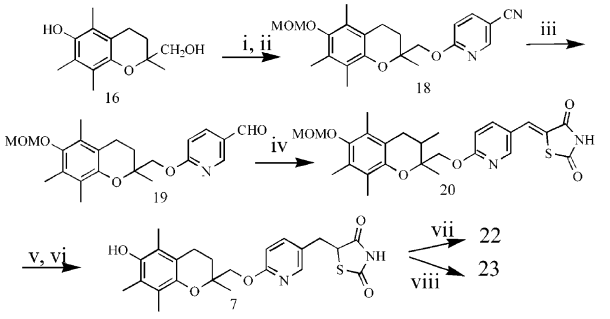
The above molecular modeling studies indicate that substitution of benzene ring in glitazones with pyridine as a linker unit brings in conformational rigidity in glitazones (Fig. 2b), which may be removed by converting them to their corresponding N-oxides, protonated salts, and methoiodide salts.

To explore the effect of these novel pyridine analogues on their biological action, we synthesized the pyridine analogue of pioglitazone, **2**; its HCl salt, **4** and N-oxide, **3** and N-oxide as its HCl salt, **3a** (Scheme 1). We also made pyridine analogue of troglitazone, **7**, its N-oxide **22** and HCl salt **23** (Scheme 2). To our knowledge, compounds having N-oxides have never been tested for antidiabetic activity.

The thiazolidinediones represented by formulas **2** and **7** were synthesized according to the Schemes 1 and 2. The pioglitazone analogue 5-[2-[2-(5-ethyl-2-pyridyl) ethoxy] pyridyl methyl]-2,4-thiazolidinedione **2** was synthesized by known procedures, starting from 5-ethyl-2-(2-hydroxy-ethyl) pyridine, **10**.[30] Base mediated coupling with 2-chloro-5-cyanopyridine, **11** followed by treatment with Raney-nickel alloy in aqueous formic acid furnished the aldehyde **13**. Knoevenagel condensation between **13** and thiazolidine-2,4-dione in the presence of piperidinium acetate catalyst afforded pyridyl methylene thiazolidinedione analogue **14**. The olefinic bond in **14** was hydrogenated in 1,4-dioxane using 10% Pd-C in equivalent amounts to give **2**. In an alternative method to prepare **2**, **10** was coupled with 2-chloro-5-nitropyridine, followed by hydrogenation to afford the amine



Scheme 1. Reagents: (i) NaH, THF (ii) Raney Ni, HCOOH (iii) 2,4-TZD, piperidinium acetate toluene (iv) 10% Pd/C (v) MCPBA.



Scheme 2. Reagents: (i) NaH, MOMCl, DMF; (ii) 11, NaH, THF; (iii) Raney Ni, HCOOH; (iv) TZD, Piperidinium acetate, toluene; (v) 10% Pd/C, dioxane; (vi) gl. acetic acid, 10% H_2SO_4; (vii) MCPBA; (viii) HCl.

derivative. The product of Meerwein arylation of methyl acrylate and amine in the presence of cuprous oxide could not be isolated in pure form. Oxidation of **2** with m-chloroperbenzoic acid gave N-oxide, **3**; that the pyridine ring of the linker fragment is the one to get oxidized was confirmed by NMR studies. We failed to obtain methoiodide salt in pure form.

The troglitazone analogue **7** was synthesized according to Scheme 2. Reduction of 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid **15** gave the corresponding alcohol **16**. The phenolic group of **16** was protected by the methoxymethyl (MOM) group and coupled with 2-chloro-5-cyanopyridine to afford **18**. The other synthetic steps after this coupling were similar to the pioglitazone analogue **2**.

3. Biological screening

3.1. PPAR transactivation

The conformational insights of some of these novel pyridine analogues (**2, 7**) though interesting, will be difficult to test in actual biological systems, be it in binding affinity or functional assays since these assays are done in buffered system.

However, we tested their PPAR agonist competence by assaying their cells in cells transfected with a GAL4-PPAR ligand binding domain chimer expression vector and a luciferase reporter plasmid as described by Chakrabarti et al.[31] The results of the PPARγ transcriptional activity by the compounds given in Table 2. As seen from the study, **4**[32], **7**, **22** and **23** show significant but less activity as compared to the known PPARγ activator, rosiglitazone. Activity of pioglitazone derivatives, **2** and **3** (both non-salt forms) could not be assessed due to solubility problem. However, the N-oxide as its HCl salt, **3a** showed significant activity.

Table 2. Activation of PPARχ. HEK 293T cells were transfected with Gal4-PPARχ1LBD, pGL2(Gal 4×5)-SV 40-Luc reporter and pAdvantage constructs. These values are an average of three experiments conducted in triplicate

PPARγ—Fold Activation								
		Compd						
Conc.	Rosiglitazone	**2**	**3**	**3a**	**4**	**7**	**22**	**23**
1 μM	21.7	1.6	0.6	1.8	2.3	3.0	4.0	1.3
10 μM	28.3	3.3	2.7	10.1	10.2	18.8	19.8	9.6
50 μM	34.7	*	*	17.8	20.1	23.7	25.4	28.1

*Compounds precipitated

None of these compounds show any PPARα activation even at 100 μM concentration, when tested with respective plasmid. This is in keeping with the fact that glitazones are selective for PPARγ over PPARα.

4. Conclusions

Molecular modeling studies on some well-known glitazones containing pyridine ring as the linker unit were carried out. These studies showed that the pyridine analogues by themselves suffer from restricted rotation, which could be set right by suitable derivatization. This led to the synthesis of some novel N-oxide glitazones. Functional assay showed that they are PPARγ agonists.

5. Experimental

5.1. General experimental details

Semi-empirical AM1 calculations have been performed using Gaussian 98 package[33] on pioglitazone, rosiglitazone, troglitazone, bromo derivative of rosiglitazone and indole glitazone and their pyridine derivatives. Complete optimisations have been performed on all conformations considered in this work, at AM1 level. Heats of formation values of various conformations have been compared to estimate the relative energies of the various conformations along with their rotation barriers. The rotational barriers in **2** in gas phase and solution phase have been estimated using absolute energy estimates at HF/3-21G* level using AM1 optimised geometries.

Thin layer chromatography analyses were performed on precoated silica gel plates (F254, Merck). Chromatography was performed on flash silica gel (230–400 mesh). Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet Impact-410 FTIR spectrometer. Proton magnetic resonance (NMR) spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solution.

The chemical shifts are reported in δ (ppm) relative to internal standard tetramethylsilane (TMS) and coupling constants J are given in Hz. Mass spectroscopy was conducted using Shimadzu QP5000 mass spectrometer.

5.2. Biological study

The response element (UASGAL4X5) was cloned upstream of the Pgl2-sv 40-Luc reporter (Promega, Madison. WI, USA), which contains the Simian virus early promoter for luciferase assay. GAL4 fusions were made by fusing human PPARγ1 or PPARα ligand-binding domain (amino acids: 174–475) to the C-terminal end of the yeast GAL4 DNA- binding domain (amino acids: 1–147) of the pM1 vector. pAdVantage (Promega, Madison, WI, USA) vector was used to enhance luciferase expression.

HEK 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum (DMEM-FBS) at 37 °C in 5% CO_2.. At 1 day prior to transfection, cells were plated to 50–60% confluence in DMEM containing 10% delipidated FBS (DMEM-DFBS). Cells were transfected by Superfect as per the manufacturer's protocol. At 3 h after transfection, the reagent was removed and cells maintained in DMEM-DFBS. At 42 h after transfection, the cells were placed in phenol red-free DMEM-DFBS, and treated for 18 h with the test compounds or vehicle alone. The cells were lysed and assayed for luciferase activity. Luciferase activity was determined by using Luclite kit (Packard, CT, USA) in a Packard Top-count and expressed as fold activation relative to untreated cells.

5.2.1. 2-Chloro-5-cyano pyridine (11). A mixture of 6-chloro nicotinic acid (10 g, 0.064 mol) and thionyl chloride (9.26 mL, 0.127 mol) were refluxed for 4 h. The excess thionyl chloride removed by adding toluene and distilling under vacuum. The resulting gum was dissolved in ether and dry ammonia gas was passed into the solution; a white solid begins to precipitate. The solution was filtered and the precipitate was leached with hot acetone. The acetone layer was collected, distilled under vacuum to give 9 g (90.5%) of 6-chloronicotinamide. (mp 208 °C).

The amide was refluxed with phosphrous oxychloride (21.4 mL, 0.23 mol) and chloroform (60 mL) for 2.5 h. The reaction mixture was concentrated and then poured into ice water. The reaction mixture was extracted with dichloromethane, washed with saturated sodium bicarbonate solution, water, dried and concentrated to give 7 g (88%) of **11** as white solid (mp 117–118 °C). [lit. mp 117–118 °C].

5.2.2. 5-[2-(5-ethyl-2-pyridyl) ethoxy] 5-pyridonitrile (12). To a stirred suspension of sodium hydride (5.45 g, 227.4 mol, 60% w/w dispersion) in dry THF was added 5-ethyl-2-(2-hydroxyethyl) pyridine, **10** (4 g, 75.8 mol) in dry THF (20 mL), and the mixture was stirred for 30 min. A solution of **11** (10.5 g, 75.8 mol) in dry THF (20 mL) was added dropwise at 0 °C. The reaction was stirred at 0 °C for 2 h and then poured into ice water and extracted with ethyl acetate. The organic extract was washed with water, dried over sodium sulphate and evaporated in vacuum to give 17.6g (92%) of crude 5-[2-(5-ethyl-2-pyridyl) ethoxy] pyridonitrile, **12**. The crude product was purified by column chromatography to yield 12.32 g (64.5%) of pure **12** as white solid (mp 88–90 °C). IR (cm^{-1}) 3430, 2223, 1608, 1562, 1491, 1300, 996. MS $(M+1)^+$ 254. ^1H NMR ($CDCl_3$) δ 8.47 (1H, d, $J=1.9$ Hz), 8.40 (1H, d, $J=1.63$ Hz), 7.75 (1H, dd, $J=2.3$, 6.4 Hz), 7.45 (1H, dd, $J=2.1$, 5.8 Hz), 7.15 (1H, d, $J=7.85$ Hz), 6.78 (1H, d, $J=8.7$ Hz), 4.75 (2H, t, $J=6.78$ Hz), 3.24 (2H, t, $J=6.77$ Hz), 2.63 (2H, q, $J=7.57$ Hz), 1.24 (3H, t, $J=7.60$ Hz). Anal. calcd for $C_{15}H_{15}N_3O$: C, 71.15; H, 5.93; N, 16.60. found C, 69.79; H, 5.89; N, 16.06.

5.2.3. 5-[2-(5-ethyl-2-pyridyl) ethoxy] pyridoxaldehyde (13). A mixture of 5-[2-(5-ethyl-2-pyridyl) ethoxy] 5-pyridonitrile **12** (8 g, 31.6 mol), Raney-Ni alloy (16 g) and formic acid (100 mL, 98-100%) was refluxed for 5 h. After filtration, the filtrate was diluted with water, made alkaline with 4N KOH and extracted with diethyl ether. The organic extract was washed with water, dried over sodium sulphate and concentrated in vacuum to give 5.48 g (68%) of **13.** The crude product was purified by chromatography using ethyl acetate/hexane (20/80 v/v) to yield 2.85 g (35%) of **13** as white crystalline solid. (mp 64–67 °C). IR (cm^{-1}) 2967, 1683, 1605, 1565, 1493, 1357, 1292. MS $(M+1)^+$ 257. ^1H NMR ($CDCl_3$) δ 9.94 (1H, s), 8.61 (1H, s), 8.41 (1H, s,), 8.04 (1H, dd, $J=2.26$, 6.4 Hz), 7.45 (1H, dd, $J=2.0$, 5.9 Hz) 7.16 (1H, d, $J=7.89$ Hz), 6.8 (1H, d, $J=8.66$ Hz), 4.79 (2H, t, $J=6.78$ Hz), 3.26 (2H, t, $J=6.78$ Hz) 2.63 (2H, q, $J=7.59$ Hz) 1.24 (3H, t, $J=7.60$ Hz) Anal. calcd for $C_{15}H_{16}N_2O_2$: C, 70.31H, 6.25; N, 10.94; found C, 69.10; H, 6.46; N, 10.11.

5.2.4. 5-[2-[2-(5-ethyl-2-pyridyl) ethoxy] pyridylmethylene]-2,4 thiazolidinedione (14). A solution of pyridoxaldehyde **13** (2.5 g, 9.8 mmol) and 2,4-thiazolidinedione (1.26 g, 10.74 mmol) in toluene containing piperidinium acetate (0.620 g, 4.9 mmol) was boiled under reflux in a Dean–Stark water trap for 5 h. The solution was cooled and filtered. The precipitate was washed with toluene. The toluene layer was concentrated and cooled to give 1.38 g (40%) of **14** as a yellow solid. (mp 165–168 °C). IR (cm^{-1}) 3433, 2960, 1702, 1596. MS 355.7. ^1H NMR (DMSO-d_6) δ 8.47 (1H,d), 8.36 (1H, d), 7.85 (1H, dd, $J=2.45$, 6.3 Hz), 7.76 (1H, s), 7.56 (1H, dd, $J=2.05$, 5.82 Hz), 7.25 (1H, d), 6.92 (1H, d), 4.68 (2H, t, $J=6.66$ Hz), 3.16 (2H, t, $J=6.63$ Hz), 2.58 (2H, q, $J=7.54$ Hz), 1.17 (3H, t, $J=7.58$ Hz). Anal. calcd for $C_{18}H_{17}N_3O_3S$: C, 60.85; H, 4.79; N, 11.83; S, 9.02; found C, 59.96; H, 4.58; N, 11.45; S, 9.0.

5.2.5. 5-[2-[2-(5 ethyl-2-pyridyl) ethoxy] pyridyl methyl]-2-4, thiazolidinedione (2). A mixture of **14** (1.35 g, 3.8 mmol), 10% palladium on carbon (1.35 g) in 1,4-dioxane (30 mL) was hydrogenated under 50 kg/cm^2 at 50 °C for 24 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuum to give 1.29 g (95%) crystals of **2** (mp 128–131 °C). IR (cm^{-1}) 3430, 2965, 1739, 1706, 1605. MS $(M+1)^+$ 358. ^1H NMR (DMSO-d_6) δ 8.28 (1H, s), 7.93 (1H,s), 7.49 (2H,

d), 7.17 (1H,d), 6.65 (1H, d), 4.83 (1H, m), 4.50 (2H, t, $J=6.65$ Hz), 3.05 (4H, m), 2.52 (2H, q, $J=7.5$ Hz), 1.13 (3H, t, $J=7.48$ Hz) Anal. calcd for $C_{18}H_{19}N_3O_3S$: C, 60.59; H, 5.32; N, 11.76; S, 8.96; found C, 60.45; H, 5.10; N, 11.54; S, 8.90.

5.2.6. 5-[2-[2-(5-ethyl-2-pyridyl) ethoxy pyridyl N-oxide methyl]-2,4 thiazolidinedione (3). To a mixture of **2** (0.100 g, 0.28 mmol) in chloroform (1 mL) and methanol (0.5 mL) was added m-chloroperbenzoic acid (60%, 0.056 g, 0.32 mmol) in one portion. The mixture was allowed to stir overnight at room temperature and then worked up by washing subsequently with saturated aqueous $NaHCO_3$, aqueous sodium sulfite and with brine. The organic layer was dried and concentrated in vacuum to afford the product as an oil. IR (cm^{-1}) 3345, 2967, 1752, 1689, 788. MS 373. ^1H NMR (DMSO-d_6) δ 8.37 (1H, d), 8.0 (1H, s), 7.41 (2H, d), 7.16 (1H, d) 6.66 (1H, d), 4.35 (1H, m), 3.95 (2H, t, $J=7.22$ Hz), 3.05 (4H, m), 2.62 (2H, q, $J=7.5$ Hz), 1.22 (3H, t, $J=7.5$ Hz). Anal. calcd for $C_{18}H_{19}N_3O_4S$; C, 57.91; H, 5.09; N, 11.26; S,8.58; found C, 57.55; H, 4.90; N, 11.01; S, 8.5.

5.2.7. 6-hydroxy-2,5,7,8-tetramethylchroman-2-yl methanol[40] (16). A mixture of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid **15** (5 g, 0.02 mol), absolute ethanol (25 mL) and concd sulfuric acid (1 mL) refluxed for 5 h. The excess ethanol was concentrated, residue was diluted with water and extracted with diethyl ether. The ether layer washed with brine, dried and concentrated to give 5.56 g (100%) of the ethyl ester. IR (cm^{-1}) 3527, 2986, 1734, 1448, 1187. To a well stirred mixture of lithium aluminium hydride (2.28 g, 0.06 mol in dry THF (100 mL) was added this ethyl ester (5.56 g, 0.02 mol) in THF (25 mL) dropwise. After stirring for 2 h at room temperature, the reaction mixture was poured into ice water, acidified with diluted HCl and extracted with diethyl ether. The extract was dried and concentrated to give 6-hydroxy-2,5,7,8-tetra-methyl chroman-2-yl methanol **16** as an oil (4.48 g, 95%). IR (cm^{-1}) 3379, 2926, 1461, 1416, 1259, 1058. MS 236. ^1H NMR ($CDCl_3$) δ 3.61 (2H, t), 2.67 (2H, brt), 2.17 (3H, s), 2.12 (3H, d), 1.94 (2H, m), 1.59 (3H, s), 1.22 (3H, s).

5.2.8. [6-(methoxymethoxy)-2,5,7,8-tetramethylchroman-2-yl] methanol (17). Compound **16** (2 g, 8.5 mmol) was dissolved in dry DMF (20 mL) and cooled to 5–10 °C. Sodium hydride (0.610 g, 25.4 mmol) was added gradually to the resulting solution with stirring at 5–10 °C. The mixture was reacted for 1 h at room temperature and then cooled to 3–5 °C, and 0.682 g (8.5 mmol) of chloro-methylmethyl ether dissolved in dry benzene (20 mL) was added drop wise. After the addition was completed the solution was stirred for 1 h at room temperature. The reaction mixture was then poured into ice water and extracted with cyclohexane. The extract was washed with 5% aqueous NaOH solution and then with water. It was then dried (Na_2SO_4) and the solvent was evaporated, giving 1.97 g (83%) of **17** as a white solid. (mp 56–59 °C). IR (cm^{-1}) 3435, 2924, 1593, 1455, 1396, 1055, MS 280. ^1H NMR ($CDCl_3$) δ 5.32 (2H, s), 4.87 (2H, s), 3.61 (3H, s), 2.61 (2H, brt), 2.18 (3H, d), 2.09 (3H, s), 1.73 (2H, m), 1.59 (3H, s), 1.22 (3H, s). Anal. calcd for $C_{16}H_{24}O_4$: C, 68.57; H, 8.57; found C, 67.88; H, 8.45.

5.2.9. [2-(6-(methoxymethoxy)-2,5,7,8-tetramethyl)-5-pyrido-nitrile] methoxychroman (18). To a well stirred and cooled mixture of 17 (1.5 g, 5.36 mmol) and 2-chloro-5-cyano pyridine 11 (0.742 g, 5.36 mmol) in dry THF (30 mL), sodium hydride 0.386 g (16.07 mmol) was added gradually. The resultant mixture was stirred for 2 h at room temperature, and then poured into ice water and extracted with ethyl acetate. The extract was washed with water, dried and concentrated to give 1.84 g (90%) of **18** as an oil. IR (cm^{-1}) 3019, 1602, 1486, 1396, 1292, 1254, 1215. MS $(M+1)^+$ 383. ^1H NMR ($CDCl_3$) δ 8.45 (1H, s), 7.78 (1H, d) $J=2.3$, 6.4 Hz), 6.84 (1H, d), 4.85 (2H, s), 4.40 (2H, q), 3.61 (3H, s), 2.64 (2H, brt), 2.17 (3H, d), 2.02 (3H, s), 1.88 (2H, m), 1.38 (3H, s), 1.26 (3H, s). Anal. calcd for $C_{22}H_{26}N_2O_4$: C, 69.11; H, 6.81; N, 7.33; found C, 68.5; H, 6.5; N, 7.00.

5.2.10. [2-(6-(methoxymethoxy)-2,5,7,8-tetramethyl)-5-pyridox -aldehyde] methoxychroman (19). A mixture of **18** (1.5 g, 3.9 mmol), Raney-Ni alloy (39 g) and formic acid (20 mL, 98–100%) were refluxed for 5 h. After removal of the alloy by filtration, the filtrate was diluted with water, made alkaline with 4N KOH and extracted with diethyl ether. The extract was washed with water, dried and concentrated to give 1.36 g (90%) of crude product which was purified by column chromatography to give 0.272 g (20%) of pure aldehyde **19** as a viscous oil. MS $(M+1)^+$ 386. ^1H NMR ($CDCl_3$) δ 9.95 (1H, s), 8.59 (1H, d), 8.07 (1H, dd, $J=2.2$, 6.5 Hz), 6.84 (1H, d), 5.30 (2H, s), 4.48 (2H, q), 4.28 (3H, s), 2.67 (2H, brt), 2.13 (3H, d), 2.06 (3H, s), 1.88 (2H, m), 1.58 (3H, s), 1.40 (3H, s). Anal. calcd for $C_{22}H_{27}NO_5$: C, 68.57; H, 7.01; N, 3.64; found C, 67.4; H, 6.5; N, 3.5.

5.2.11. 5-[2-(2-(6-(methoxymethoxy)-2,5,7,8-tetramethyl-chroman-2-yl)methoxy)-2-pyridylmethylene]-2,4-thiazolidinedione (20). A solution of **19** (0.250 g, 0.65 mmol) and 2,4-thiazolidinedione (0.076 g, 0.65 mmol) in toluene containing piperidinium acetate (0.042 g, 0.33 mmol) was boiled under reflux in a Dean–Stark water trap for 5 h. The solution was cooled and filtered. The filtrate was concentrated to give 0.30 g (95%) of yellow oil, purified by column chromatography to give 0.075 g (25%) of pure **20**. MS 484. ^1H NMR (DMSO-d_6) δ 8.43 (1H, d), 8.01 (1H, d), 7.76 (1H, S), 6.86 (1H, d), 4.40 (2H, s), 4.28 (3H, s), 4.02 (2H, q), 2.57 (2H, brt), 2.02 (3H, d), 1.99 (3H, s), 1.88 (2H,m), 1.70 (3H, s), 1.30 (3H, s). Anal. calcd for $C_{25}H_{28}N_2O_6S$: C, 61.98; H, 5.79; N, 5.79; S, 6.61; found C, 60.55; H, 5.00; N, 5.01; S, 6.0.

5.2.12. 5-[2-(2(6-(methoxymethoxy)-2,5,7,8-tetramethyl-chroman -2-yl) methoxy)-2-pyridylmethyl]-2,4-thiazolidinedione (21). A mixture of **20** (0.500 g, 10.33 mmol), 10% palladium on carbon (0.500 g) in 1,4-dioxane (10 mL) was hydrogenated under 50 kg/cm^2 at 50 °C for 20 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuum to get 0.502 g (95%) of the oily hydrogenated product. ^1H NMR ($CDCl_3$) δ 8.03 (1H,d), 7.58 (1H,d), 6.85 (1H, d) 4.62 (2H, s), 4.50 (1H, m), 4.30 (2H, q), 3.18 (2H, m), 2.67 (2H, brt), 2.13

(3H, d), 2.06 (3H, s), 1.88 (2H, m), 1.62 (3H, s), 1.38 (3H, s), 3.45 (3H, brs). MS 486. Anal. calcd for $C_{25}H_{30}N_2O_6S$: C, 61.73; H, 6.17; N, 5.76; S, 6.58; found; C, 60.5; H, 5.50; N, 5.5; S, 6.5.

5.2.13. 5-[2-[2-(6-hydroxy-2,5,7,8-tetramethyl chroman-2-yl) methoxy]-2 pyridyl methyl]-2,4-thiazolidinedione (7). The product **21** was dissolved in gl. acetic acid (15 mL) containing 10% H_2SO_4 (30 mL) and the mixture heated at 90 °C for 20 h. The reaction mixture cooled and poured into a mixture of cold aq $NaHCO_3$ solution and extracted with diethyl ether. The extract was washed with water, dried and concentrated to give 0.456 g (85%) of **7** as an oil. MS $(M+1)^+$ 443. ^1H NMR ($CDCl_3$) δ 7.99 (1H, s), 7.45 (1H, dd), 6.73 (1H, d), 4.51 (1H, m), 4,30 (2H, q), 3.18 (2H, m), 2.64 (2H, brt), 2.18 (3H, d), 2.07 (3H, s), 1.88 (2H, m), 1.38 (3H, s), 1.25 (3H, s). Anal. calcd for $C_{23}H_{26}N_2O_5S$: C, 62.44; H, 5.88; N, 6.33; S, 7.24; found C, 61.40; H, 5.00; N, 6.20; S, 7.20.

5.2.14. 5-[2-[2-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl) methoxy]-2-pyridyl *N*-oxide methyl]-2,4-thiazolidinedione (22). To a mixture of **7**(0.055 g, 0.12 mmol)in chloroform (1 mL) and methanol (0.5 mL) was added *m*-chloroperbenzonic acid (0.025 g, 0.143 mmol) in one portion. The mixture was allowed to stir overnight at room temperature and then worked up. Extracted with methylene chloride and washed subsequently with saturated aqueous $NaHCO_3$, sodium sulfite and brine. The organic layer dried and concentrated to give an oily product **22**. ^1H NMR ($CDCl_3$) δ 8.05 (1H, d), 7.53 (1H, dd), 6.75 (1H, d), 4.52 (1H, m), 4.30 (2H, q), 3.18 (2H, m), 2.64 (2H, brt), 2.20 (3H, d), 2.07 (3H, s), 1.87 (2H, m), 1.38 (3H, s), 1.25 (3H, s). Anal. calcd for $C_{23}H_{26}N_2O_6S$: C, 60.26; H, 5.68; N, 6.11; S, 6.99;. found; C, 60.00; H, 5.00; N, 6.0; S, 7.00.

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