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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3356–3360
Bioorganic & Medicinal Chemistry Letters

6-Aryl-4-methylsulfanyl-2*H*-pyran-2-one-3-carbonitriles as PPAR-γ activators☆

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Received 16 March 2005; revised 9 May 2005; accepted 10 May 2005

Abstract—Various 6-aryl-3-cyano/methoxycarbonyl-4-methylsulfanyl-2*H*-pyran-2-ones have been synthesized as a potential substitute of 2,4-thiazolidinedione head group to express potent PPAR-γ transactivation response. Some of the screened compounds have shown promising PPAR-γ agonistic activity.
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1. Introduction

Diabetes is the fourth leading killer disease in the developed world. There are more than 200 million diabetics worldwide, accounting for a huge economic and social burden.[1,2] Thus, the development of new effective therapeutic agents is the major thrust for research and is germane to both the national and international scenario. Type 2 diabetes mellitus (T2DM) is the most important type of diabetes, as more than 80% of diabetics are of this class.

The peroxisome proliferator-activated receptors (PPARs) are legitimate molecular targets for the development of the antidiabetic agents. The reported synthetic ligands such as rosiglitazone (**I**) and pioglitazone (**II**)[3,4] had high affinity for PPAR-γ receptor, belong to the thiazolidinedione class of antidiabetic agents (Fig. 1), and have significantly improved the clinical situation of Type 2 diabetics with serious side effects of hepatotoxicity, weight gain, and edema. This situation emphasized the need to identify strategies to develop new antihyperglycemic agents that could retain the insulin sensitizing properties of TZDs through PPARγ with minimum or no adverse side effects. This necessitated a search for highly effective, safe, and orally active antihyperglycemic agents, particularly those that normalize both insulin and glucose levels.

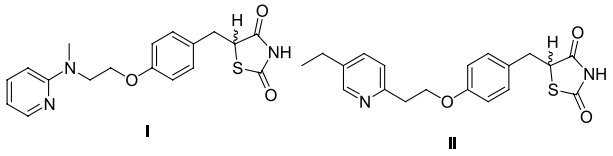

Figure 1. Thiazolidinedione class of antidiabetic drugs.

The PPARs play a significant role in regulating the storage and catabolism of dietary fats, discovered[5,6] in early 1990s. The X-ray analysis[7] of PPAR-γ bound with rosiglitazone reveals that carbonyl groups of the TZD form hydrogen bonds with two histidine residues, H323 and H449, of PPAR-γ target receptor and Y473 in the AF-2 helix forms a secondary hydrogen bond. The partially negatively charged nitrogen of the TZD head group is within hydrogen-bonding distance from the OH group of the Y473 side chain. All of these primary and secondary hydrogen bonds result in a fixed conformation of the TZD head group and of the participating amino acids.

Based on the ligand–receptor binding knowledge, the synthesis of new prototype structures **3** (Fig. 2) has been

Keywords: Diabetes; PPAR; Pyran-2-one; Transactivation.
☆ CDRI Communication No. 6724.
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doi:10.1016/j.bmcl.2005.05.031

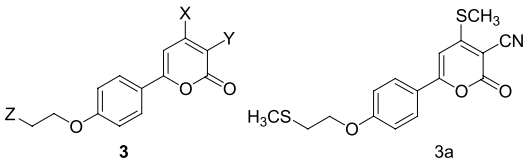


Figure 2. Chemical structure of proposed prototype molecules (**3**) and compound (**3a**).

initiated to develop a better drug candidate for treating T2DM. Prototype **3** is a pyran-2-one-based derivative and its importance is greatly realized due to its unique structural features and diverse pharmacological properties.[8–12] With careful structural analysis of pyran derivatives, it was understood that the replacement of TZD head group by pyran nucleus as a new chemical entity may retain the antihyperglycemic activity.

A flexible alignment of the synthesized compounds with rosiglitazone (**I**) was performed using the advanced program MOE-Flex Align,[13] to determine their conformational and pharmacophoric relation using **I** and **II** as a template for the (virtual) superimposition (Fig. 3) and scoring (Table 1). Each alignment is given a score that quantifies the quality of the alignment in terms of both internal strain (**U**) and overlap of molecular features (**S**).

Figure 4 shows significant alignment indicated by superimposition of the middle phenoxy ring and similarities in the head group pyran (**3a**) and thiazolidinedione ring of rosiglitazone (**I**). The carbonyl oxygen of thiazolidinedione is considered as the most essential pharmacophore for the binding with the PPAR. Projection of pyran ring toward thiazolidinedione region of **I** as in Figure 4, prompted to visualize its importance to act as a head group.

Further, we applied a receptor-based approach to the validation of our hypothesis regarding the substitution of the pyran ring in place of thiazolidinedione. This approach essentially searches for a ligand whose orientation and conformation achieve the highest degree of

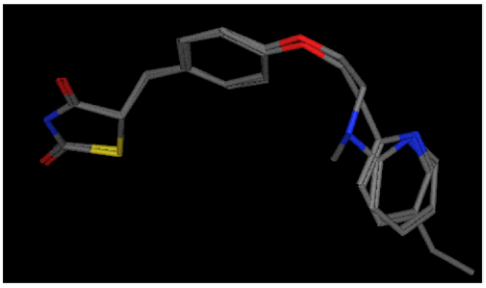


Figure 3. Flexible overlay of compounds **I** and **II** showing high similarities.

Table 1. Score for flexible alignment

Compounds	Strain energy (**U**)	Object function value (**S**)
I and **II**	30.93	169.9
I and **3a**	41.34	228.63

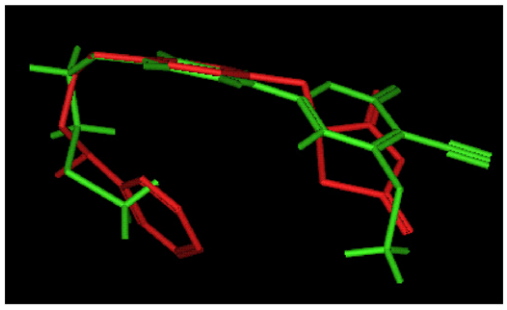


Figure 4. Flexible alignment of **I** (red) and **3a** (green) showing significant molecular alignment.

complementarity with respect to all details of the receptor's steric constraints and interaction geometries.

A successful solution to the docking problem comprises the generation of relevant binding modes ('poses') of the ligand and the correct ranking of each pose, and it is essential for the success of virtual screening approaches in structure-based drug design. It requires docking tools that are able to generate suitable configuration and conformations of a ligand within a protein binding site and energy measurements describing the quality of the interactions.

The Lamarckian genetic algorithm AutoDock 3.0.5[14] has been implemented as a more efficient alternative for docking. The X-ray conformation[7] (green) and AutoDock predicted structure (red) of rosiglitazone **I** have been superimposed in Figure 5 to define the parameters of the program. The root mean square deviation between these two conformations is ~0.8 Å, indicating that the parameter set for the AutoDock simulation is reasonable to reproduce the X-ray structure. Therefore, the AutoDock method and the parameter set could be extended to search the binding conformations for other ligands accordingly. Table 2 shows the calculated energies and docked results from the compound **3a** with the comparison of docked rosiglitazone. Figure 6 shows the 3D docked model of compound **3a** with PPAR-γ and illustrates the probable binding conformational alignment of this compound with comparison of **I** (red). H-bonding is one of the important characteristics of the ligand–receptor interaction. From these above

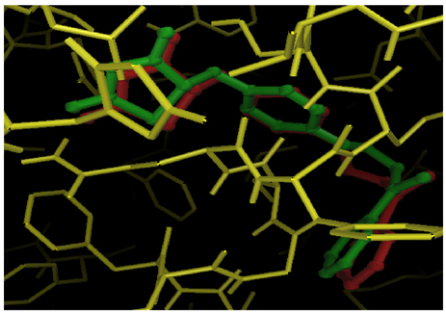


Figure 5. Superimposition of the docked **I** (red) with crystallographic rosiglitazone molecule (green) complexed in the binding cavity of PPAR-γ (gold).

Table 2. Docking results

Compound	Cluster rank	Final intermolecular energy (kcal/mol)	Final docked energy (kcal/mol)
I (rosiglitazone)	I	−13.42	−13.89
3a	I	−11.39	−10.72

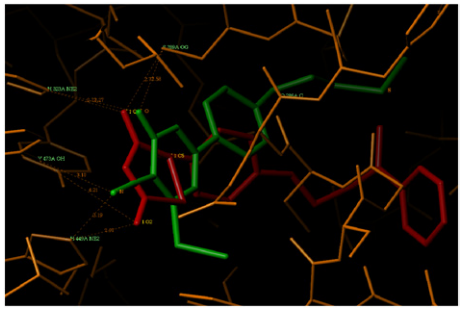


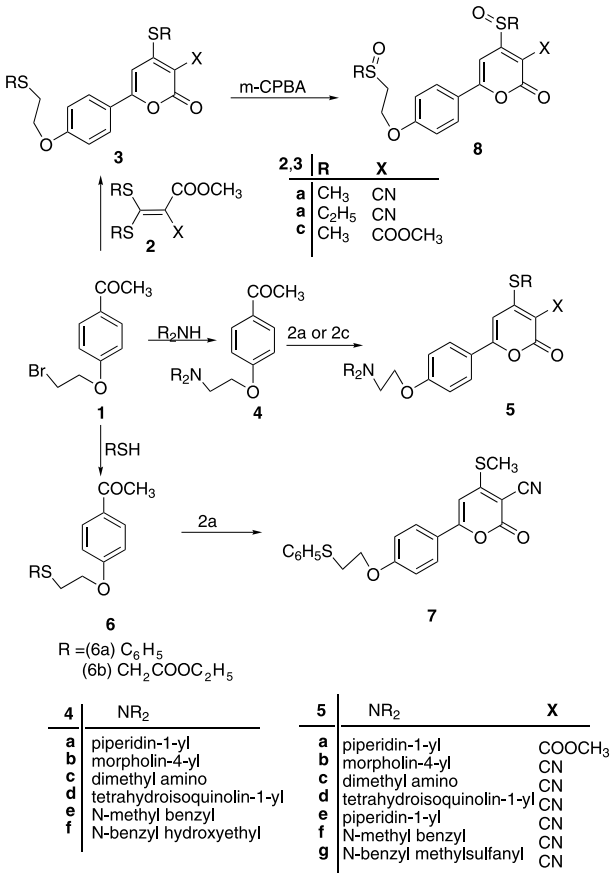
Figure 6. Docked compound **3a** (green) with rosiglitazone (**I**) (red) for comparison in the binding cavity of PPAR, showing interaction with neighboring residues through H-bonding.

silico analyses, we may consider pyran as a head group in lieu of thiazolidinedione.

2. Chemistry

The ketene dithioacetals[15,16] (**2a**–**c**) have been used as synthon for the synthesis of various pyran derivatives by reaction with aryl methyl ketones. Numerous highly functionalized 2*H*-pyran-2-ones have been prepared from 4-hydroxy acetophenone. The reaction of 1,2-dibromoethane with 4-hydroxy acetophenone in the presence of K_2CO_3 provided 1-[4-(2-bromoethoxy)phenyl]ethanone (**1**) as a major product. Reaction of ketone **1** with methyl 2-substituted-3,3-bis-methylsulfanylacrylate (**2**) in the presence of potassium hydroxide provided the corresponding 2*H*-pyran-2-one derivatives (**3**).[17] The intermediate **4**, used for the preparation of **5**, was obtained from the reaction of **1** with secondary amine in DMF. Thus, various 3,6-disubstituted-4-methylsulfanyl-2*H*-pyran-2-ones (**5**) were synthesized by base-catalyzed condensation–cyclization of aryl methyl ketone (**4**) with **2**[17] (Scheme 1).

All the synthesized compounds were characterized by their spectroscopic and elemental analyses.[20] The IR spectrum of one of the compound **3b** showed two bands at ν 1706 and 2209 cm^{-1} due to CO and CN groups, respectively. ^1H NMR spectrum of **3b** showed two triplets at δ 1.27 and 1.46 ppm due to two methyl groups and two quartets at δ 2.61 and 3.19 for two methylene protons of SCH_2CH_3 substituents. Two methylene groups of linker resonated at δ 2.91 and δ 4.19 ppm as triplet for SCH_2 and OCH_2, respectively. A singlet of pyran ring proton appears at 6.6 ppm while peaks at δ 6.97–7.9 ppm were attributed to the aromatic protons. The FAB mass peak at 362 supported the proposed structure. Finally, the structure of **3b** was confirmed by single crystal X-ray analysis.[18]



2,3	R	X
a	CH_3	CN
a	C_2H_5	CN
c	CH_3	$COOCH_3$

4	NR_2
a	piperidin-1-yl
b	morpholin-4-yl
c	dimethyl amino
d	tetrahydroisoquinolin-1-yl
e	N-methyl benzyl
f	N-benzyl hydroxyethyl

5	NR_2	X
a	piperidin-1-yl	$COOCH_3$
b	morpholin-4-yl	CN
c	dimethyl amino	CN
d	tetrahydroisoquinolin-1-yl	CN
e	piperidin-1-yl	CN
f	N-methyl benzyl	CN
g	N-benzyl methylsulfanyl	CN

Scheme 1. Synthetic scheme for the preparation of ketones **4**, **6**, and various pyrans **3**, **5**, **7**, and **8**. Formation of **3** indicates the in situ substitution of bromine atom by thioalkyl group.

The conformation of **3b** along with the atom numbering scheme is shown in Figure 7. X-ray crystal structure showed that one molecule is in the asymmetric unit. The co-planarity was found between pyran and phenoxy ring; phenoxy ring is twisted with 7.88 (1)° from the mean plane of the pyran ring. As expected, this planarity can be attributed to weak intramolecular H-bond [C13–H13···O1; H···A = 2.38 Å, D···A = 2.72 Å (1) and ⟨(DHA) = 100.9°].

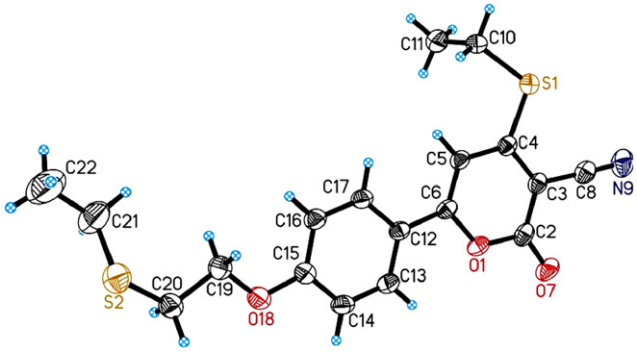


Figure 7. ORTEP diagram of **3b** showing the X-ray molecular structure in 50% probability level.

Table 3. In vitro PPAR-γ transactivation assay[19] for some synthesized compounds of prototype **I**

Compound	Fold activation of PPAR-γ[a]		
	10 nM	100 nM	1000 nM
3a	12	22	60
3b	7	11	30
3c	9	13	44
5a	3	3	17
5b	3	4	5
5c	4	6	7
5d	4	5	19
5e	3	2	16
5f	5	4	7
5g	4	6	8
7	3	5	14
8	9	14	27
Rosiglitazone	9	28	176

[a] Compounds were tested in quadruple at concentrations ranging from 10 to 1000 nM. Each compound was tested in at least two separate experiments. The activity of a compound is calculated as fold induction compared to an untreated sample.

3. Results and discussion

Our objective was to design pyran-based PPAR-γ ligands to identify lead structures through an in silico approach to evaluate their transactivation response. It is evident from the first series of designed and synthesized compounds **3a**–**c** that the compounds displayed (Table 3) significant increases in activation response. Oxidation of both the methylsulfanyl groups in **3a** to its corresponding sulfoxide **8** reduced the activity profile of the compound. Exchange of methylsulfanyl group by highly hydrophobic phenylsulfanyl substituent in **7** drastically reduced the activity. In attempts to obtain more active compounds, *sec*-amino groups were introduced in lieu of SCH_3 in the linker. A series of compounds **5a**–**g** were prepared and evaluated for their agonistic property but none of them displayed any significant activity.

4. Biological activity

Of various compounds evaluated in in vitro PPAR-transactivation assay, only three compounds, **3a**–**c**, demonstrated significant transactivation responses. This study made it possible to modify the structure of **3a** to obtain more compounds as potent PPAR-activators. Thus, it was imperative to generate information through computational analyses including docking and molecular simulations with iterative synthesis and biological screening.

Acknowledgments

V.J.R. and R.P.S. are thankful to ICMR for financial support of the project. A.S. thanks CSIR for Senior Research Fellowship. Authors are thankful to SAIF, CDRI, Lucknow for providing spectroscopic data and elemental analyses.

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18. Crystal data of **3b**: $C_{18}H_{19}NO_3S_2$, M = 361.46, monoclinic, space group $P2(1)/n$, a = 5.419 (0), b = 22.523 (1), c = 14.833 (2) Å, V = 1804.9(3) Å^3, T = 293 K, Z = 4, D_c = 1.330 g cm^{-1}, μ(Mo-Kα) = 0.31 mm^{-1}, $F(000)$ = 760, yellow rectangular crystal, size 0.42 × 0.22 × 0.20 mm, 4496 reflections measured, 3187 unique, R_w = 0.15 for all data, conventional R = 0.05 for 2031 $F_o > 4\sigma(F_o)$ and 0.0922 for all 3187 data, S = 1.004 for all data and 219 parameters. Unit cell determination and intensity data collection (2θ = 50°) were performed on a Bruker P4 diffractometer at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2. Programs: *XSCANS* [(Siemens Analytical X-ray Instruments: Madison, Wisconsin, USA 1996) used for data collection and data processing], *SHELXTL-NT* [(Bruker AXS: Madison, Wisconsin, USA 1997) used for structure determination, refinements and molecular graphics]. (CCDC No of **3b**: 273004).
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20. Spectroscopic data of 4-methylsulfanyl-6-substituted-phenyl-2-oxo-2*H*-pyran-3-carbonitrile **3a**: yield: 48%;

mp: 165–168 °C; MS (FAB): 334 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.23 (s, 3H, SCH_3), 2.70 (s, 3H, SCH_3), 2.91 (t, 2H, $J = 5.8$ Hz, CH_2), 4.24 (t, 2H, $J = 5.8$ Hz, CH_2), 6.60 (s, 1H, ArH), 7.0 (d, 2H, $J = 8.8$ Hz, ArH), 7.83 (d, 2H, $J = 8.8$ Hz, ArH); Anal. Calcd for $C_{16}H_{15}NO_3S_2$: C, 57.64; H, 4.53; N, 4.20. Found: C, 57.67; H, 4.63; N, 4.32. **3b**: yield: 46%; mp: 181 °C; MS (FAB): 362 ($M^+ + 1$); IR : 1706.6 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.31 (t, 3H, $J = 7.4$ Hz, CH_3), 1.49 (t, 3H, $J = 7.4$ Hz, CH_3), 2.61–2.68 (q, 2H, $J = 7.3$ Hz, CH_3), 2.94 (t, 2H, $J = 6.7$ Hz, CH_2), 3.19–3.24 (q, 3H, $J = 7.5$ Hz, CH_2), 4.22 (t, 2H, $J = 6.8$ Hz, CH_2), 6.60 (s, 1H, ArH), 7.0 (d, 2H, $J = 8.9$ Hz, ArH), 7.81 (d, 2H, $J = 8.9$ Hz, ArH); Anal. Calcd for $C_{18}H_{19}NO_3S_2$: C, 59.81; H, 5.30; N, 3.87. Found: C, 59.91; H, 5.35; N, 3.89.