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Synthesis, characterization and biological evaluation of some novel 2,4-thiazolidinediones as potential cytotoxic, antimicrobial and antihyperglycemic agents

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ABSTRACT

A series of some novel 2,4-thiazolidinediones (TZDs) (**2a–x**) have been synthesized and characterized by FTIR, ^{1}H NMR, ^{13}C NMR and LC mass spectral analysis. All the synthesized compounds were evaluated for their cytotoxicity, antimicrobial and in vivo antihyperglycemic activities. Among the tested compounds for cytotoxicity using Brine Shrimp Lethality assay, compound **2t** ((*Z*)-5-(4-((E)-3-oxo-3-(thiophen-2-yl)prop-1-enyl)benzylidene)-1,3-thiazolidine-2,4-dione) exhibited significant inhibitory activity at ED_{50} value 4.00 ± 0.25 μg/mL and this level of activity was comparable to that of the reference drug podophyllotoxin with ED_{50} value 3.61 ± 0.17 μg/mL. Antimicrobial activity was screened using agar well diffusion assay method against selected Gram-positive, Gram-negative and fungal strains and the activity expressed as the minimum inhibitory concentration (MIC) in μg/mL. From the results of antimicrobial activity compound **2s** ((*Z*)-5-(4-((E)-3-(3,5-bis(benzyloxy)phenyl)-3-oxoprop-1-enyl)benzylidene)-1,3-thiazolidine-2,4-dione) was found to be the most active against all the tested strains of microorganisms with MIC value 16 μg/mL. In vivo antihyperglycemic effect of twenty four TZDs (**2a–x**) at different doses 10, 30 and 50 mg/kg b.w (oral) were assessed using percentage reduction of plasma glucose (PG) levels in streptozotocin-induced type II diabetic rat models. From the results, the novel compound **2x** ((*Z*)-5-(4-((E)-3-(9H-fluoren-2-yl)-3-oxoprop-1-enyl)benzylidene)-1,3-thiazolidine-2,4-dione) exhibited considerably potent blood glucose lowering activity than that of the standard drug rosiglitazone and it could be a remarkable starting point to evaluate structure–activity relationships and to develop new lead molecules with potential cytotoxicity, antimicrobial and antihyperglycemic activities. In addition molecular docking studies were carried out against PPARγ molecular target using Molegro Virtual Docker v 4.0 to accomplish preliminary confirmation of the observed in vivo antihyperglycemic activity.

In recent years, the chemistry of 2,4-thiazolidinediones (TZDs) captivated importance as these compounds have been found to exhibit several biological activities, such as antihyperglycemic,[1] euglycemic,[2] anti-inflammatory,[3] antimalarial,[4] antioxidant,[5] antitumor,[6] cytotoxic,[7] antimicrobial,[8] antiproliferative,[9] PPARγ agonist,[10] dual PPARα/γ activator,[11] free radical scavenger,[12] LDL oxidation inhibitor,[13] glycogen synthase kinase (GSK) 3 inhibitor,[14] aldose reductase inhibitor,[15] cholesterol esterase inhibitor,[16] 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibitor,[17] human β_3 agonist,[18] chymase inhibitor,[19] bacterial arylamine *N*-Acetyltransferases (NATs) inhibitor,[20] $P2X_7$ receptor antagonist,[21] thyroid hormone receptor antagonist,[22] PTP1B inhibitor,[23] human PTP1B and LMW-PTP inhibitor,[24] Raf/MEK/Extracellular signal regulated kinase (ERK1/2) inhibitor,[25] dual inhibitor of the Raf/MEK/ERK and the PI3K/Akt signaling pathways,[26] serine/threonine protein kinases Pim-1 and Pim-2 inhibitor,[27] G-protein coupled receptor 40 (GPR40) agonist,[28] MurD ligase inhibitor,[29] monoamine oxidase B (MAO-B) inhibitor[30] and neuroprotective.[31] Having such diverse range of pharmacological activities, these molecules have attracted medicinal chemists and consequently a number of strategies have been originated to synthesize them.

A series of TZDs (**2a–x**) synthesized in the present study were studied for their cytotoxicity, antimicrobial and antihyperglycemic activities. In fact, compounds having α,β-unsaturated ketone or 1,3-thiazolidine-2,4-dione moieties were earlier reported to

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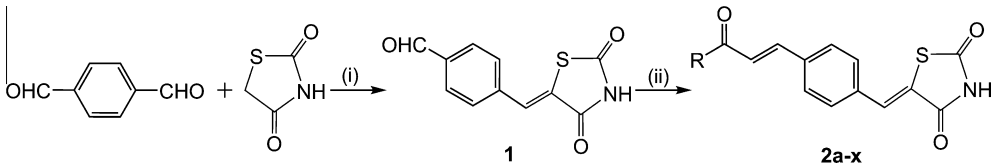
possess cytotoxicity[32–39], antimicrobial[40–47] and antihyperglycemic[48–55] activities and thus the synthesized compounds now are expected to possess synergistic effect, as both the features form part of these molecules. In the present investigation, the aim of molecular docking study of a series of TZDs (**2a–x**) against PPARγ (PDB ID: 3CS8) is to predict and compare the ligand conformation and orientation within a targeted binding site. To the best of our knowledge there is, to date, no report was published regarding the binding properties of TZDs (**2a–x**) against PPARγ 3CS8 Ligand Binding Domain (LBD).

The reaction sequence employed for the synthesis of title compounds (**2a–x**) is shown in Scheme 1, and their physical properties are depicted in Table 1. As described by Momose et al.[56] and Bruno et al.[57] 2,4-thiazolidinedione can undergo a Knoevenagel condensation with a variety of substituted aldehydes to produce 5-arylidene-2,4-thiazolidinediones. The key intermediate in the present study *(Z)*-4-((2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl)benzaldehyde **1** was prepared by Knoevenagel condensation reaction between terephthaldehyde and 1,3-thiazolidine-2,4-dione. Further, subsequent base catalyzed condensation of the **1** with appropriate substituted aromatic/heteroaromatic ketones in the presence of pulverized sodium hydroxide in boiling dimethylformamide (DMF) afforded a series of 2,4-thiazolidinediones (**2a–x**) in good yield.[58,59]

All the synthesized compounds as mentioned in Table 1 were characterized by CHN elemental analysis and spectroscopic methods such as FTIR, ^{1}H NMR, ^{13}C NMR and LC mass spectral analysis. The IR spectrum of **1** exhibited characteristic –C=C– (aliphatic) and –C=C– (aromatic) stretching bands at frequencies 1685 cm^{-1} and 1598 cm^{-1}, respectively. The ^{1}H NMR spectrum of **1** revealed characteristic peaks of 5-benzylidene (HC=C) and NH protons as two singlets, one at δ 7.86 ppm and the other one at δ 12.74 ppm (broad) providing evidence for formation of **1** and reconfirmed by the ^{13}C NMR peaks at δ 126.78 ppm and δ 115.14 ppm due to 5-methylidene carbon and carbon (C_5) of 1,3-thiazolidine-2,4-dione. The ESI mass spectrum (positive ion mode) of **1** revealed a $(M+H)^+$ ion at *m/z* 234. The geometry of all TZDs (**2a–x**) were assumed to be (*Z*) configuration because of its high degree of thermal stability of this isomer.[60]

The IR spectrum of all the compounds (**2a–x**) exhibited the characteristic absorptions at various frequencies correspondingly at 3300–3100 cm^{-1} and 1650–1735 cm^{-1} suggesting the presence of a secondary amine group and α,β-unsaturated carbonyl group, respectively. In the ^{1}H NMR spectra of TZDs (**2a–x**), a singlet integrating for one proton characteristic of the HC=C group was observed in between δ 7.72–8.10 ppm and a singlet integrating for one proton of the NH group was observed in between δ 12.5–13.5 ppm as a broad signal. The ^{1}H NMR spectrum of the compound **2a** exhibited characteristic peaks of H-α and H-β protons of α,β-unsaturated carbonyl group as two doublets, one at δ 8.01 ppm (H-β, J = 15.2 Hz) and the other one at δ 7.78 ppm (H-α, J = 15.2 Hz). The large J value clearly reveals the *trans* geometry at the double bond. In the ^{13}C NMR spectra, the presence of characteristic signals correspondingly at δ 123.36 ppm and δ 142.76 ppm indicating the presence of C-α and C-β carbon atoms of α,β-unsaturated carbonyl group confirmed the formation of



Scheme 1. Reagents and conditions: (i) Piperidine, ethanol, reflux, 8 h; (ii) dry dimethylformamide (DMF), pulverized NaOH, substituted aromatic/heteroaromatic ketones.

Table 1
Physical characterization and cytotoxicity data of TZDs (**2a**–**x**) produced via Scheme 1

Compound	R	Yield[a] (%)	Molecular weight	Molecular formula	Mp (°C)	ED_{50} (μg/mL) (mean ± SEM)
2a	C_6H_5	88	335.58	$C_{19}H_{13}NO_3S$	212–215	44.73 ± 0.12
2b	4-MeC_6H_4	84	349.40	$C_{20}H_{15}NO_3S$	179–182	19.66 ± 0.25
2c	3-OMeC_6H_4	83	365.40	$C_{20}H_{15}NO_4S$	181–184	18.72 ± 0.15
2d	4-OMeC_6H_4	91	365.40	$C_{20}H_{15}NO_4S$	218–221	16.24 ± 0.52
2e	2-OHC_6H_4	91	351.38	$C_{19}H_{13}NO_4S$	189–192	41.35 ± 0.33
2f	4-OHC_6H_4	87	351.38	$C_{19}H_{13}NO_4S$	206–209	36.20 ± 0.23
2g	2,4-diOHC_6H_3	83	367.38	$C_{19}H_{13}NO_5S$	215–218	49.21 ± 0.25
2h	2,5-diOHC_6H_3	83	367.38	$C_{19}H_{13}NO_5S$	213–216	46.71 ± 0.15
2i	2-OH,5-MeC_6H_3	90	365.07	$C_{20}H_{15}NO_4S$	125–128	39.42 ± 0.52
2j	6-OH,5-MeC_6H_3	81	365.07	$C_{20}H_{15}NO_4S$	221–224	33.12 ± 0.21
2k	3-$NH_2C_6H_4$	81	350.07	$C_{19}H_{14}N_2O_3S$	211–214	33.18 ± 0.15
2l	4-$NH_2C_6H_4$	80	350.07	$C_{19}H_{14}N_2O_3S$	231–234	28.82 ± 0.12
2m	3-$NO_2C_6H_4$	86	380.05	$C_{19}H_{12}N_2O_5S$	255–258	56.06 ± 0.25
2n	4-$NO_2C_6H_4$	84	380.05	$C_{19}H_{12}N_2O_5S$	217–220	49.28 ± 0.15
2o	3-ClC_6H_4	92	369.82	$C_{19}H_{12}ClNO_3S$	219–222	24.82 ± 0.12
2p	4-ClC_6H_4	90	369.82	$C_{19}H_{12}ClNO_3S$	206–209	12.06 ± 0.25
2q	3-FC_6H_4	93	353.05	$C_{19}H_{12}FNO_3S$	212–215	9.28 ± 0.15
2r	4-FC_6H_4	89	353.05	$C_{19}H_{12}FNO_3S$	199–203	4.32 ± 0.52
2s	3,5-di$C_7H_7OC_6H_3$	94	547.62	$C_{33}H_{25}NO_5S$	229–233	26.82 ± 0.12
2t	Thiophen-2-yl	88	341.40	$C_{17}H_{11}NO_3S_2$	207–210	4.00 ± 0.25
2u	Pyridin-2-yl	87	336.06	$C_{18}H_{12}N_2O_3S$	194–197	30.28 ± 0.15
2v	Pyridin-3-yl	84	336.06	$C_{18}H_{12}N_2O_3S$	205–208	31.42 ± 0.52
2w	Naphthalen-2-yl	81	385.08	$C_{23}H_{15}NO_3S$	216–219	48.11 ± 0.33
2x	Fluoren-2-yl	79	423.48	$C_{26}H_{17}NO_3S$	218–221	39.66 ± 0.31
Standard[b]	–	–	–	–	–	3.61 ± 0.17

[a] Crystallization solvent is ethanol.
[b] Podophyllotoxin.

compound **2a**. Further, the ESI mass spectrum (positive ion mode) of compound **2a** revealed a $(M+H)^+$ ion at *m*/*z* 336. Eventually all the spectra of the new products are in keeping with the expected structures.

The cytotoxicity potential of the synthesized compounds (**2a–x**) was determined by Brine Shrimp Lethality assay as described by Meyer et al.[61] Brine Shrimp (Artemia salina) nauplii were hatched in sterile brine solution (prepared using sea water salt 38 g/L and adjusted the pH to 8.5 using 1 N NaOH) under constant aeration for 38 h. After hatching, 10 nauplii were placed in each vial and added various concentrations of drug solutions in a final volume of 5 mL, maintained at 37 °C for 24 h under light of incandescent lamps and surviving larvae were counted. Each experiment was conducted along with control (vehicle treated) at various concentrations of the test substances. The percentage lethality was determined by comparing mean surviving larvae of test and control tubes. The ED_{50} values were obtained using fenny probed analysis software.[62] The result for the test compound was compared with the positive control podophyllotoxin. The results of cytotoxicity study are given in Table 1.

The investigation of cytotoxicity screening data of all the synthesized TZDs (**2a–x**) revealed that the compounds **2t**, **2r** and **2q** demonstrated comparatively the most potent cytotoxicity, with ED_{50} values of 4.00 ± 0.25, 4.32 ± 0.52 and 9.28 ± 0.15 μg/mL, respectively (Table 1). It is interesting to note that the compounds **2p**, **2d**, **2c** and **2b** also showed appreciable cytotoxicity with ED_{50} values of 12.06 ± 0.25, 16.24 ± 0.52, 18.72 ± 0.15 and 19.66 ± 0.25 μg/mL, respectively. The other compounds such as **2o**, **2s**, **2l** and **2u** showed moderate level of activity at concentrations ranging from 24.82 ± 0.12 – 30.28 ± 0.15 μg/mL. The compounds **2v**, **2j**, **2k**, **2f**, **2i**, **2x**, **2e**, **2a**, **2h**, **2w**, **2g**, **2n** and **2m** exhibited comparatively less activity with ED_{50} values ranging from 31.42 ± 0.52 – 56.06 ± 0.25 μg/mL in comparison with the standard drug (podophyllotoxin, ED_{50}: 3.61 ± 0.17 μg/mL).

A close look at the SAR (Structure–Activity Relationship) of these compounds clearly revealed the inherent cytotoxicity associated with the basic nucleus consisting of 2,4-thiazolidinedione and α,β-unsaturated ketone moieties as seen in case of the unsubstituted compound **2a** with ED_{50} value of 44.73 ± 0.12 μg/mL, which in some cases was enhanced by the influence of some substituents and decreased by some other substituents. For example, the compounds **2r**(*p*-F, ED_{50}: 4.32 ± 0.52 μg/mL) > **2q**(*m*-F, ED_{50}: 9.28 ± 0.15 μg/mL) > **2p**(*p*-Cl, ED_{50}: 12.06 ± 0.25 μg/mL) having halogen substituents either at *meta* or *para* positions significantly enhanced the activity. A reduction in the activity was observed when the substituted phenyl ring was replaced by a naphthalene moiety, as seen in the case of compound **2w** with ED_{50} value 48.11 ± 0.33 μg/mL. The presence of a fluorene ring in compound **2x** (ED_{50}: 39.66 ± 0.31 μg/mL) in the place of substituted phenyl ring of α,β-unsaturated carbonyl system enhanced the activity compared to the one possessing naphthalene moiety, but less than that of the one having substituted phenyl ring. It is also interesting to see the presence of pyridine ring in the place of substituted phenyl ring contributing to an increase in activity compared to the one possessing fluorene moiety, as seen in the case of compounds **2u** and **2v** with ED_{50} values 30.28 ± 0.15 and 31.42 ± 0.52 μg/mL, respectively. It is interesting to note that an enhanced level of activity was observed when the thiophene ring is introduced in the place of substituted phenyl ring of α,β-unsaturated carbonyl system, as seen in case of compound **2t**, with ED_{50} value 4.00 ± 0.25 μg/mL.

However, it was revealed that various aromatic/heteroaromatic rings substituted at position 3 of α,β-unsaturated carbonyl system followed its activity order as thiophen-2-yl > pyridine-2-yl > pyridine-3-yl > fluoren-2-yl > phenyl > naphthalene-2-yl moieties, respectively. It is also reported that the cytotoxicity of compounds **2b–2d** and **2i–2l**, substituted with electron releasing groups was found to be biologically relevant and the activity order was (**2d** (4-OCH_3, ED_{50}: 16.24 ± 0.52 μg/mL) > **2c** (3-OCH_3, ED_{50}: 18.72 ± 0.15) > **2b** (4-CH_3, ED_{50}: 19.66 ± 0.25 μg/mL) > **2l** (4-NH_2, ED_{50}: 28.82 ± 0.12 μg/mL) > **2k** (3-NH_2, ED_{50}: 33.18 ± 0.15 μg/mL), respectively. It is important that less activity was observed when the hydroxyl groups are substituted at different positions on the phenyl ring as seen in the case of compounds **2e**–**2h** and the order of activity was **2f** (4-OH, ED_{50}: 36.20 ± 0.23 μg/mL) > **2e** (2-OH, ED_{50}: 41.35 ± 0.33) > **2h** (2,5-diOH, ED_{50}: 46.71 ± 0.15 μg/mL) > **2g** (2,4-diOH, ED_{50}: 49.21 ± 0.25 μg/mL), respectively. It is notable that loss of activity observed when the nitro groups are introduced on the phenyl ring of α,β-unsaturated carbonyl system as seen in the case of compounds **2m** and **2n** with ED_{50} values 56.06 ± 0.25 and 49.28 ± 0.15 μg/mL. The compounds **2j** (ED_{50}: 33.12 ± 0.21 μg/mL) and **2i** (ED_{50}: 33.12 ± 0.21 μg/mL) having the methyl group substitution on the phenyl ring at position 5 along with the hydroxyl group substitution at 6 (**2j**) and 2 (**2i**) positions, respectively, showed enhanced level of cytotoxicity when compared with that of the compounds (**2e–2h**) possessing only hydroxyl group substitution. The compound **2s** (ED_{50}: 26.82 ± 0.12 μg/mL) having dibenzyloxy substitution on the phenyl ring at 3 and 5 positions is also relevant for enhancing the cytotoxicity.

With respect to the antimicrobial activity, the standard strains were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. The antimicrobial activity of the synthesized compounds (**2a–x**) was determined by agar well diffusion method as recommended by the National Committee for Clinical Laboratory Standards, (NCCLS).[63–65] The compounds were evaluated for antimicrobial activity against bacteria viz. *Bacillus subtilis* (NCIM 2063), *Bacillus pumilus* (NCIM 2327), *Staphylococcus aureus* (NCIM 2079), *Micrococcus luteus* (NCIM 2155), *Pseudomonas aeruginosa* (NCIM 2036), *Klebsiella pneumonia* (NCIM 5082), *Escherichia coli* (NCIM 2065), *Proteus vulgaris* (NCIM 2813), *Candida albicans* (NCIM 3102), *Aspergillus niger* (NCIM 548), *Aspergillus oryzae* (NCIM 643) and *Penicillium chrysogenum* (NCIM 738). Serial solutions of compounds (**2a–x**) were diluted in dimethyl sulfoxide (1% DMSO) to give a final concentration ranging from 16 to 512 μg/mL used for determining MIC value. The Minimum inhibitory concentration (MIC) was defined as the lowest concentration of compound required for a complete inhibition of the bacterial and fungal growth after incubation time. For antibacterial activity nutrient agar was used seeded with 0.1 mL of the respective bacterial culture strains suspension prepared in a sterile saline (0.85%) of 10^5 CFU/mL dilution. For antifungal activity, different fungal spore suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 CFU/mL. An inoculum of 0.1 mL spore suspension of each fungus was spread on Potato-Dextrose-Agar (PDA) plates. The wells of 6 mm diameter were filled with 0.1 mL of each compound having different concentrations separately for each test of bacterial and fungi strain. The DMSO (1%) alone was used as a control. The antibiotic chloramphenicol (16 μg/mL) and ketoconazole (16 μg/mL) are used as reference antibacterial and antifungal agents, respectively, for comparison. Inoculated plates in triplicate were then incubated at 37 ± 0.5 °C for antibacterial activity for 24 h and 48 h at 28 ± 0.2 °C for antifungal activity. After incubation, the minimum inhibitory concentrations (MICs) were noted. The results of antimicrobial activity studies are given in Table 2.

The results of antimicrobial activity of the synthesized compounds (**2a–x**) against selected Gram-positive, Gram-negative bacteria and fungi are illustrated in Table 2. The compounds **2r** and **2s** were found to be more active than other compounds with MIC 16–32 μg/mL against all tested microorganisms. Compounds **2a**, **2e–2h**, **2k**, **2m**, **2n**, **2w** and **2x** showed comparatively less antimi-

Table 2
Antimicrobial activity of TZDs (**2a–x**)

Compound	R	Minimum inhibitory concentration (MIC) (μg/mL)											
		Gram positive bacteria				Gram negative bacteria				Fungi			
		Bs	Bp	Sa	Ml	Pa	Kp	Ec	Pv	Ca	An	Ao	Pc
2a	C_6H_5	256	256	512	512	256	512	256	512	512	512	512	512
2b	4-MeC_6H_4	32	32	32	64	32	64	32	64	256	256	64	64
2c	3-$OMeC_6H_4$	128	128	128	256	128	128	128	256	128	64	256	256
2d	4-$OMeC_6H_4$	64	64	32	64	64	64	32	64	128	128	128	128
2e	2-OHC_6H_4	512	512	256	512	256	512	256	512	512	512	512	512
2f	4-OHC_6H_4	256	128	128	256	512	128	128	512	512	256	256	512
2g	2,4-diOHC_6H_3	256	256	256	512	256	256	256	256	256	256	256	256
2h	2,5-diOHC_6H_3	512	256	256	512	512	256	256	512	128	256	256	128
2i	2-OH,5-MeC_6H_3	128	256	256	512	256	128	128	512	128	128	64	64
2j	6-OH,5-MeC_6H_3	64	128	128	512	64	64	64	32	64	128	128	256
2k	3-$NH_2C_6H_4$	256	256	256	256	128	256	64	256	256	64	128	256
2l	4-$NH_2C_6H_4$	128	128	128	256	64	128	128	256	128	128	128	256
2m	3-$NO_2C_6H_4$	256	256	128	256	128	256	128	512	512	256	128	256
2n	4-$NO_2C_6H_4$	256	512	512	512	256	512	512	512	256	512	512	256
2o	3-ClC_6H_4	64	64	64	64	32	64	64	32	128	64	64	64
2p	4-ClC_6H_4	32	16	32	64	32	16	32	32	64	64	64	64
2q	3-FC_6H_4	32	32	32	64	32	32	32	64	64	64	64	64
2r	4-FC_6H_4	16	16	32	32	16	16	16	32	32	32	32	16
2s	3,5-di$C_7H_7OC_6H_3$	16	16	16	16	16	16	32	32	16	16	16	16
2t	Thiophen-2-yl	32	16	32	32	32	16	32	64	32	32	32	32
2u	Pyridin-2-yl	128	64	64	32	128	64	64	64	64	64	64	128
2v	Pyridin-3-yl	128	128	128	128	128	128	128	256	128	256	128	256
2w	Naphthalen-2-yl	512	512	512	512	512	512	512	512	512	512	512	512
2x	Fluoren-2-yl	256	256	256	512	128	256	128	512	256	256	256	512
Standard[a]	—	16	16	16	16	16	16	16	16	—	—	—	—
Standard[b]	—	—	—	—	—	—	—	—	—	16	16	16	16
DMSO (1%)	—	—	—	—	—	—	—	—	—	—	—	—	—

Bs: *Bacillus subtilis* (NCIM 2063), Bp: *Bacillus pumilus* (NCIM 2327), Sa: *Staphylococcus aureus* (NCIM 2079), Ml: *Micrococcus luteus* (NCIM 2155), Pa: *Pseudomonas aeruginosa* (NCIM 2036), Kp: *Klebsiella pneumonia* (NCIM 5082), Ec: *Escherichia coli* (NCIM 2065), Pv: *Proteus vulgaris* (NCIM 2813), Ca: *Candida albicans* (NCIM 3102), An: *Aspergillus niger* (NCIM 548), Ao: *Aspergillus oryzae* (NCIM 643) and Pc: *Penicillium chrysogenum* (NCIM 738).

[a] Chloramphenicol.

[b] Ketoconazole.

crobial activity with MIC 256–512 μg/mL against all tested microorganisms. From the results of antibacterial activity, compound **2s** was found to be more active against all Gram-positive bacteria with MIC value 16 μg/mL. Among all the tested compounds, compound **2r** showed significant inhibition against all Gram-negative bacteria with MIC 16 μg/mL except a moderate potency (32 μg/mL) against *Proteus vulgaris*. A broad spectrum of antifungal activity of the compound **2s** was obtained against all the fungi with MIC 16 μg/mL, while other compounds displayed less antifungal activity.

From the obtained data, the following conclusions on antimicrobial activity can be made: the compounds substituted with halogens on the phenyl ring at *meta* and *para* positions enhanced the antibacterial activity (F > Cl) as seen in the case of compounds **2o–2r**. It is noteworthy that compounds **2b–2d**, **2k** and **2l** having electron donating substituents (4-CH_3 > 4-OCH_3 > 3-OCH_3 > 4-NH_2 > 3-NH_2) on the phenyl ring at *meta* and *para* positions was found to enhance the antibacterial activity. It is also interesting to observe that various aromatic/heteroaromatic ring substituted at position 3 of α,β-unsaturated carbonyl system was found to be biologically relevant and followed its activity order as thiophen-2-yl (**2t**) > pyridine-2-yl (**2u**), pyridine-3-yl (**2v**) > phenyl (**2a**), fluoren-2-yl (**2x**) > naphthalen-2-yl (**2w**) moieties, respectively. A decrease in the antimicrobial activity is attributed to the presence of hydroxyl group on the phenyl ring at *ortho, meta* and *para* positions as seen in the case of compounds **2e–2h**. The compounds **2j** and **2k** having the methyl group substitution on the phenyl ring at position 5 along with the hydroxyl group substitution on at 6 (**2j**) and 2 (**2i**) positions, respectively, showed enhanced level of antimicrobial activity when compared with that of the compounds (**2e–2h**) possessing only hydroxyl group substitution. The compounds **2m** and **2n** having nitro group either at *meta* or *para* position of the phenyl ring is not relevant for enhancing the antimicrobial activity. An increase in the antifungal activity was also observed for the compounds having halogen substitution but the level of activity in many cases is found to be less than that of the antibacterial activity. Similarly, the presence of dibenzyloxy group at position 3 and 5 of the phenyl ring as seen in case of compound **2s** also improved the antibacterial activity but the level of activity is much enhanced in case of antifungal activity.

As a general rule, the acute toxicological studies of TZDs (**2a–x**) were performed as per OECD 425 guidelines in mice. All the tested compounds were found to be safe upto dose (median lethal dose) 2000 mg/kg b.w. No changes in any of vital functions were observed throughout the study period.

With respect to the antihyperglycemic activity, Wistar albino rats of either sex weighing 150–200 g were used for this study. All animals were maintained under 12 h light and 12 h dark cycle at 25 ± 1 °C. All animals were fed with standard pellet diet and water ad libitum. Animals were fasted for 16 h prior to drug administration, allowing access only to water and were deprived of both food and water during the experiment. The animal housing and handling were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC) (Proceedings No. 516/01/A/CPCSEA). To induce type II diabetes, the acclimatized animals were fasted over night before injecting with streptozotocin (STZ). STZ was dissolved in citrate buffer pH 4.5 and a dose of 65 mg/kg was given after the administration of 150 mg/kg dose of Nicotinamide which is helpful to produce partial destruction of pancreas. The blood glucose levels were estimated after 48 h

for the confirmation of diabetes induction. The diabetic rats were divided into four groups of six rats each. Control animals received distilled water only (Group I), diabetic animals received STZ injection (Group II), diabetic animals orally fed with rosiglitazone (as 0.25% sodium CMC suspension) at doses 10, 30 and 50 mg/kg (Group III) and the diabetic rats orally fed with synthesized TZDs (**2a–x**) (as 0.25% sodium CMC suspension) at doses 10, 30 and 50 mg/kg (Group IV). For single dose study (acute study) the fasting blood glucose samples were taken before the administration of the test compounds at doses 10, 30 and 50 mg/kg for one day, the blood samples were collected from the retro orbital plexus periodically at 1, 2, 4, 6, 8, 12 and 24 h and were analyzed for plasma glucose (PG) content. Levels of plasmatic glucose were analyzed using commercially available glucometer kits (ACCU-CHEK ACTIVE), based on enzymatic methods. All the data were statistically analyzed for variance and significance, by one way ANOVA followed by Student's *t*-test and Dunett's test. All results are expressed as Mean ± SEM and observed P value is <0.05. The results of antihyperglycemic activity studies are given in Table 3.[66]

The results of in vivo antihyperglycemic activity of the synthesized TZDs (**2a–x**) showed that all the compounds were found to possess moderate to potential ability to reduce blood glucose levels in streptozotocin- induced type II diabetic rats. We consequently found that the% PG reduction ability of the titled compounds (**2a–x**) was followed a dose-dependent manner. Among all the tested compounds, compound **2x** showed significant antihyperglycemic activity at various doses such as 10, 30 and 50 mg/kg b.w with% PG reduction values 39.83 ± 0.29, 44.62 ± 0.32 and 52.81 ± 0.32 mg/dL, respectively. Correspondingly, compounds **2j**, **2d** and **2h** also showed promising antihyperglycemic activity. In the same way, compounds such as **2u**, **2i**, **2g**, **2f**, **2w**, **2p**, **2c**, **2e**, **2r**, **2v** and **2s** exhibited moderate range of antihyperglycemic activity at all the doses. The other compounds such as **2l**, **2b**, **2n**, **2o**, **2k**, **2q**, **2m**, **2a** and **2t** showed lower level of antihyperglycemic activity when compared to that of the reference drug rosiglitazone (% PG reduction values at doses: 10 mg/kg b.w (38.57 ± 0.25 mg/dL), 30 mg/kg b.w (14.83 ± 0.18 mg/dL) and 50 mg/kg b.w. (12.74 ± 0.16 mg/dL), respectively). The results of antihyperglycemic activity are summarized in Table 3.

From the results described in Table 3, we could establish the structure-activity relationships for antihyperglycemic activity of TZDs (**2a–x**) synthesized in the present study. It is clear from the results of antihyperglycemic activity that the structural requirements for retaining (or enhancing) antihyperglycemic property in TZDs (**2a–x**) are not rigid. As such, the observations supported the following general conclusions: The α,β-unsaturated ketone

Table 3
Dose-response effect of the TZDs (**2a**–**x**) on blood glucose levels in streptozotocin-induced type II diabetic rats

Compound	R	Dose (mg/kg b.w) (oral)	% Reduction, PG (mg/dL) (mean ± SEM)	Compound	R	Dose (mg/kg b.w) (oral)	% Reduction, PG (mg/dL) (mean ± SEM)
2a	C_6H_5	10	NS	**2o**	3-ClC_6H_4	10	11.71 ± 0.23
		30	11.51 ± 0.61			30	15.42 ± 0.78
		50	15.61 ± 0.22			50	21.83 ± 0.43
2b	4-MeC_6H_4	10	17.66 ± 0.34	**2p**	4-ClC_6H_4	10	26.74 ± 0.22
		30	21.81 ± 0.22			30	32.38 ± 0.41
		50	28.72 ± 0.32			50	36.17 ± 0.21
2c	3-$OMeC_6H_4$	10	16.41 ± 0.65	**2q**	3-FC_6H_4	10	12.08 ± 0.56
		30	28.73 ± 0.75			30	16.87 ± 0.27
		50	34.61 ± 0.22			50	20.66 ± 0.66
2d	4-$OMeC_6H_4$	10	33.88 ± 0.21	**2r**	4-FC_6H_4	10	22.74 ± 0.34
		30	37.04 ± 0.55			30	25.67 ± 0.21
		50	48.71 ± 0.13			50	33.12 ± 0.28
2e	2-OHC_6H_4	10	NS	**2s**	3,5-di$C_7H_7OC_6H_3$	10	21.76 ± 0.34
		30	NS			30	26.87 ± 0.32
		50	14.44 ± 0.25			50	30.12 ± 0.48
2f	4-OHC_6H_4	10	21.83 ± 0.22	**2t**	Thiophen-2-yl	10	NS
		30	30.21 ± 0.61			30	11.66 ± 0.78
		50	37.12 ± 0.75			50	13.71 ± 0.74
2g	2,4-diOHC_6H_3	10	26.86 ± 0.12	**2u**	Pyridin-2-yl	10	22.78 ± 0.12
		30	33.72 ± 0.22			30	34.68 ± 0.16
		50	41.67 ± 0.43			50	43.72 ± 0.10
2h	2,5-diOHC_6H_3	10	30.74 ± 0.22	**2v**	Pyridin-3-yl	10	19.04 ± 0.19
		30	38.86 ± 0.34			30	27.86 ± 0.31
		50	47.73 ± 0.57			50	31.81 ± 0.17
2i	2-OH,5-MeC_6H_3	10	31.76 ± 0.34	**2w**	Naphthalen-2-yl	10	23.75 ± 0.18
		30	37.03 ± 0.25			30	31.84 ± 0.22
		50	42.13 ± 0.32			50	36.93 ± 0.18
2j	6-OH,5-MeC_6H_3	10	36.76 ± 0.66	**2x**	Fluoren-2-yl	10	39.83 ± 0.29
		30	43.14 ± 0.35			30	44.62 ± 0.32
		50	49.99 ± 0.62			50	52.81 ± 0.32
2k	3-$NH_2C_6H_4$	10	12.14 ± 0.32	Group-I	-	-	2.78 ± 2.41
		30	15.36 ± 0.57				
		50	21.66 ± 0.21				
2l	4-$NH_2C_6H_4$	10	21.34 ± 0.35	Group-II	-	-	1.78 ± 6.32
		30	24.72 ± 0.28				
		50	29.87 ± 0.26				
2m	3-$NO_2C_6H_4$	10	11.18 ± 0.32	Group-III	-	10	38.57 ± 0.25
		30	14.72 ± 0.45			30	14.83 ± 0.18
		50	16.56 ± 0.27			50	12.74 ± 0.16
2n	4-$NO_2C_6H_4$	10	13.63 ± 0.32				
		30	16.74 ± 0.22				
		50	22.73 ± 0.54				

NS: Not significant; $n = 6$; $P < 0.05$

and 2,4-thiazolidinedione moieties are critical for enhancing the antihyperglycemic activity. The substitution of phenyl ring of α,β-unsaturated carbonyl system with a fluorene ring as in compound **2x** exhibited significant increase in activity compared to the compounds possessing other aromatic or heteroaromatic ring systems, the order of activity was **2u** (pyridin-2-yl) > **2w** (naphthalen-2-yl) > **2v** (pyridin-3- yl) > **2a** (phenyl) > **2t** (thiophen-2-yl), respectively. It is noteworthy that enhanced level of activity was observed when the phenyl ring of α,β-unsaturated carbonyl system is substituted with different functional groups and decreased by some other substituents. The order of activity was **2j** (6-OH,5-CH_3) > **2d** (4-OCH_3) > **2h** (2,5-diOH) > **2i** (2-OH,5-CH_3) > **2g** (2,4-diOH) > **2f** (4-OH) > **2p** (4-Cl) > **2c** (3-OCH_3) > **2e** (2-OH) > **2r** (4-F) > **2s** (3,5-dibenzyloxy) > **2l** (4-NH_2) > **2b** (4-CH_3) > **2n** (4-NO_2) > **2o** (3-Cl) > **2k** (3-NH_2) > **2q** (3-F) > **2m** (3-NO_2), respectively. An *ortho, meta* and *para* substitution on the phenyl ring of α,β-unsaturated carbonyl system with hydroxyl group may increase the activity. A *para*-substitution on the phenyl ring of α,β-unsaturated carbonyl system with electron-donating groups possibly will increase the activity. A *para*-substitution on the phenyl ring of α,β-unsaturated carbonyl system with less electronegative halogens compared to fluorine, such as chloro- may increase the activity. On the other hand, molecular docking studies were performed on a series of TZDs (**2a–x**) against PPARγ target protein 3CS8 to predict and compare the ligand conformation and orientation of binding properties of TZDs (**2a–x**) with that of the compound proven by experimental studies within the targeted binding site region. The binding mode analyses of the compounds TZDs (**2a–x**) with the active site residues provided important information of the catalytic site.

With respect to the molecular modeling study, software Molegro Virtual Docker (MVD) v 4.0.0 (www.molegro.com) along with Graphical User Interface (GUI), MVD tools was utilized to generate grid, calculate dock score and evaluate conformers. The structures of title compounds (**2a**–**x)** were drawn using Chemdraw ultra v 10.0 (Chemical Structure Drawing Standard; Cambridge Soft corporation, USA), copied to Chem3D ultra v 10.0 to create a 3D model and, finally subjected to energy minimization using molecular mechanics (MM_2). The minimization was executed until the root mean square gradient value reached a value smaller than 0.001 kcal/mol. Such energy minimized structures are considered for molecular docking studies. However, corresponding pdb files were prepared using Chem3D ultra v 10.0 integral option (save as /Protein Data Bank (pdb)). The selection of protein for molecular docking studies is based upon several factors, that is, structure should be determined by X-ray diffraction, and resolution should be between 2.5–3.0 A°, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. On the other hand, we considered Ramachandran plot statistics as the important filter for protein selection with none of the residues present in disallowed region. Finally the resultant protein target was prepared for molecular docking simulation in such a way that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc) were removed. Kollmann charges were assigned. Among all the entries of PPARγ proteins deposited in RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), 3CS8 was selected for molecular docking analysis, where the residues were bonded more closely to the rosiglitazone agonist, co-crystallized with PPARγ. In this crystal structure, the Ligand Binding Domain (LBD) forms a homodimer in which both monomers have nearly identical Cα conformations. The structure of the 'A' monomer of the LBD homodimer was selected for docking studies. Molecular docking was performed using MolDock[67] docking engine of software. The scoring function used by MolDock is derived from the Piecewise Linear Potential (PLP) scoring functions.[68] The active binding site region was defined as a spherical region which encompasses all protein within 15.0 A° of bound crystallographic ligand atom with a size of X: 18.89 A°, Y: 2.16 A° and Z: 30.05 A° axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 A° and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, that is, all non-ring torsions were allowed. This approach seems to be consistent with the structural similarity observed between rosiglitazone and compounds under investigation. The results of molecular docking studies are given in Table 4.

In order to gain insight into the putative binding mode of TZDs (**2a**–**x**) with PPARγ, these compounds were docked with a crystallographic structure of human PPARγ. The crystallographic structure was obtained from the Protein Data Bank (PDB), accession code 3CS8.[69] This is the unique study describing the molecular docking of these TZDs (**2a**–**x**) with PPARγ. Before docking TZDs, the docking protocol of Molegro Virtual Docker (MVD) was validated by predicting the binding mode of the crystallographic ligand (rosiglitazone), a full agonist of PPARγ. The Figure 1 shows a comparison between binding mode of the crystallographic ligand and the binding mode predicted by MVD. Figure 1 clearly shows that MVD successfully predicted the binding mode of crystallographic mode with a root-mean square (RMS) deviation of 1.78 A°.

Table 4
Synthesized TZDs (**2a-x**) with their Moldock scores and corresponding H-bond interacting residues with 3CS8 (PPARγ).

Compound	R	Moldock score (kcal/mol)	No of H-bonds/interacting residues
2a	C_6H_5	−133.14	3/Glu 259, Arg 280, Cys 285
2b	4-MeC_6H_4	−140.547	5/Glu 259, Arg 280, Gly 284, Cys 285
2c	3-$OMeC_6H_4$	−152.395	2/Cys 285, Gly 344
2d	4-$OMeC_6H_4$	−135.297	4/Glu 259, Glu 272, Arg 280
2e	2-OHC_6H_4	−139.881	1/Phe 282
2f	4-OHC_6H_4	−149.367	4/Pro 227, Leu 228, Leu 340, Gly 344
2g	2,4-diOHC_6H_3	−142.347	6/Glu 259, Cys 285, Arg 288
2h	2,5-diOHC_6H_3	−145.75	5/Glu 259, Cys 285, Arg 288
2i	2-OH,5-MeC_6H_3	−144.919	4/Glu 259, Cys 285
2j	6-OH,5-MeC_6H_3	−135.386	2/Ile 281, Gln 345
2k	3-$NH_2C_6H_4$	−145.035	5/Glu 259, Glu 272, Arg 280, Glu 291
2l	4-$NH_2C_6H_4$	−144.691	5/Glu 259, Glu 272, Arg 280, Glu 291
2m	3-$NO_2C_6H_4$	−120.87	4/Lys 261, Arg 288, Ser 342
2n	4-$NO_2C_6H_4$	−119.159	1/Ile 281
2o	3-ClC_6H_4	−143.839	1/Tyr 473
2p	4-ClC_6H_4	−140.932	4/Arg 280, Cys 285
2q	3-FC_6H_4	−140.733	2/Glu 259
2r	4-FC_6H_4	−142.35	5/Glu 259, Glu 272, Arg 280, Glu 291
2s	3,5-di$C_7H_7OC_6H_3$	−204.925	6/Leu 228, Lys 261, Gly 284, Arg 288, Glu 291
2t	Thiophen-2-yl	−137.203	2/Ile 281, Arg 288
2u	Pyridin-2-yl	−130.577	2/Ile 281, Arg 288
2v	Pyridin-3-yl	−130.181	2/Ile 281, Arg 288
2w	Naphthalen-2-yl	−157.389	5/Glu 259, Glu 272, Arg 280, Glu 291
2x	Fluoren-2-yl	−170.113	5/ Glu 259, Glu 272, Arg 280, Cys 285
Rosiglitazone	—	−131.877	4/His 449, Tyr 473, Cys 285

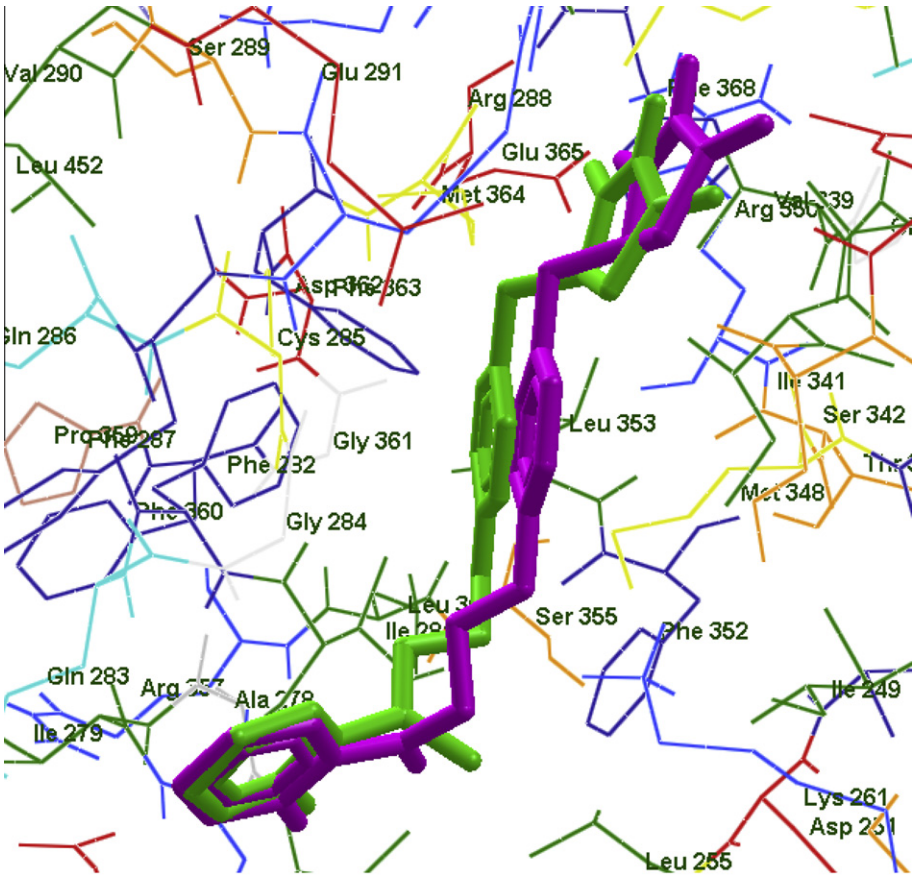


Figure 1. Superimposed binding orientation of the crystallographic ligand (pink) and docked conformer (green) predicted by MVD in the 3CS8 active binding site. The amino acid residues are shown in stick model. The binding site residues involved are labeled (dark green).

The predicted binding energies and the corresponding hydrogen bond forming residues for the TZDs (**2a–x**) are summarized in Table 4. According to the crystallographic structure of rosiglitazone in the Ligand Binding Domain (LBD) of PPARγ, the thiazolidinedione ring revealed some specific interactions with neighboring amino acid residues of the LBD. These interactions include hydrogen bonding with amino acids Cys 285, His 449 and Tyr 473 as reported in the literature.[70–72] The binding profile of TZDs (**2a–x**) was compared with the profile of the rosiglitazone molecule. The Figure 2 (a–h) summarizes the binding modes and hydrogen bond interactions of the pharmacologically active TZDs (**2x**, **2j**, **2d** and **2h**) predicted by MVD. According to the docking models, all TZDs (**2a–x**) were predicted to bind into the LBD, thus sharing a very similar binding mode. Interestingly, we found the docking scores of TZDs (**2a–x**) except **2m**, **2n**, **2u** and **2v** are favorable for the most stable conformations (most negative docking energies ranging from −204.925 to −133.14 kcal/mol) than that of the crystallographic ligand (rosiglitazone) and it could be the remarkable in silico confirmation to evaluate these compounds for their in vivo antihyperglycemic activity.

However, the docking analysis of the TZDs (**2a–x**) in the present study revealed the PPARγ activating process, termed "dock and lock" as seen in case of compounds **2a**, **2b**, **2c**, **2g**, **2h**, **2i**, **2p** and **2x** in silico binding covalently to Cys 285 within the PPARγ LBD.[73] Thus these compounds suggested that the formation of a covalent bond with Cys 285 causes a conformational transmission to the receptor surface. We consequently found α,β-unsaturated ketone group is the fundamental moiety of naturally occurring ligands to exhibit PPARγ agonistic activity as reported earlier[74] and also the same observation is valid in the TZDs (**2a–x**) synthesized in the present investigation, as they also consist of similar α,β-unsaturated ketone moiety in the general structure. Finally, we evaluated all the synthesized compounds for their in vivo antihyperglycemic activity. The synthesized compounds were showed moderate antihyperglycemic activity when compared to standard molecule, though they have significant binding scores in silico. Therefore, this correlation is less consistent, because the docking results are related exclusively to the ligand-receptor interaction, while the experimental results take into account the pharmacokinetic parameters also.[75] However, we did not analyze whether the antihyperglycemic effects of our compounds were derived from a PPAR agonist effect, since we did not evaluate their direct effects or binding affinities to the PPARγ.

In summary, we could synthesis and characterize a series of some novel 2,4-thiazolidinediones (**2a–x**). These compounds were screened for cytotoxicity, antimicrobial and antihyperglycemic activities and the results revealed the positive contribution of α,β-unsaturated ketone and 2,4-thiazolidinedione moieties towards the observed cytotoxicity, antimicrobial and antihyperglycemic properties. The structure-activity relationship studies indicated that the introduction of an aromatic or heteroaromatic ring system at position 3 of the α,β-unsaturated ketone bridge plays a key role in determining the potency of observed pharmacological activities. Computational molecular docking study rationalizes the selectivity and provides a binding model for the further refinement of this chemo type. Therefore, this series of TZDs (**2a–x**) have considerable promise for development as potential cytotoxic, antimicrobial and antihyperglycemic agents.

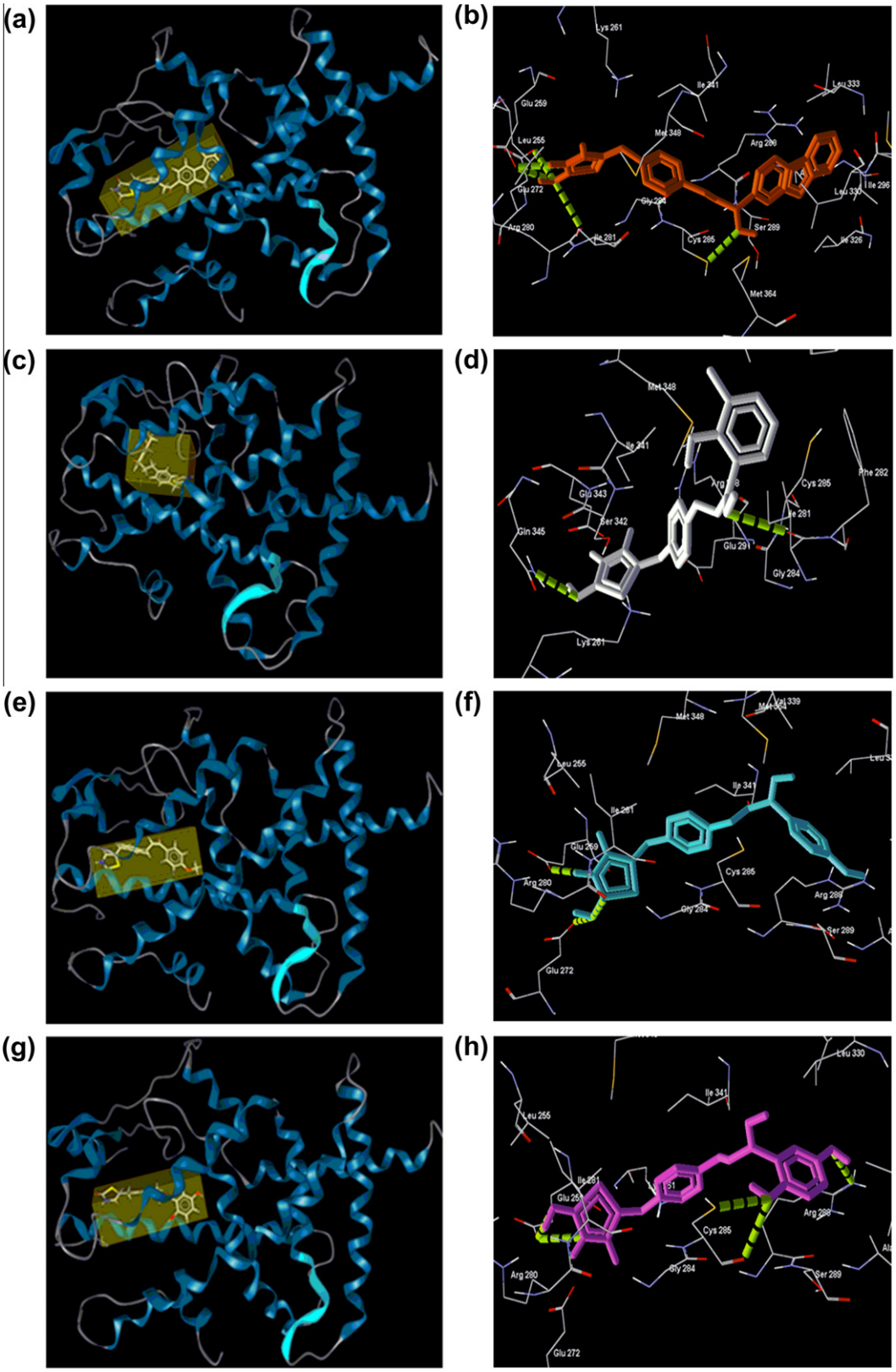


Figure 2. (a) Compound **2x** with PPARγ (3CS8). (b) Hydrogen bond interactions (green dashes) of predicted binding mode of **2x** (orange). (c) Compound **2j** with PPARγ (3CS8). (d) Hydrogen bond interactions (green dashes) of predicted binding mode of **2j** (white). (e) Compound **2d** with PPARγ (3CS8). (f) Hydrogen bond interactions (green dashes) of predicted binding mode of **2d** (blue). (g) Compound **2h** with PPARγ (3CS8). (h) Hydrogen bond interactions (green dashes) of predicted binding mode of **2h** (pink). It shows the binding mode and hydrogen bond interactions of active ligands in the ligand binding site. The side chains of the residues are shown in stick model. Blue ribbon represents the secondary structure of the protein PPARγ (3CS8).

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Supplementary data

Supplementary data (Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.052.

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