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Original article

Synthesis and in vivo antidiabetic activity of novel dispiropyrrolidines through [3 + 2] cycloaddition reactions with thiazolidinedione and rhodanine derivatives

Ramalingam Murugan [a], S. Anbazhagan [b], S. Sriman Narayanan [c,*]

[a] Department of Medicinal Chemistry, Orchid Research Laboratories Limited, Sholinganallur, Chennai 600119, India
[b] Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Chennai 600 097, India
[c] Department of Analytical Chemistry, School of Chemical Science, University of Madras, Guindy Campus, Chennai 600 025, India

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ABSTRACT

The synthesis of a series of novel dispiropyrrolidines has been accomplished by 1,3-dipolar cycloaddition reaction with 5-arylidene-1,3-thiazolidine-2,4-dione and 5-arylidene-4-thioxo-1,3-thiazolidine-2-one derivatives as dipolarophiles. The structure and stereochemistry of the cycloadduct have been established by single crystal X-ray structure and spectroscopic techniques. Molecular docking studies were performed on 1FM9 protein. The synthesized compounds were screened for their antidiabetic activity on male Wistar rats.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a long-term disease, characterized by a state of fasting hyperglycemia [1]. Type 2 or adult-onset diabetes is a chronic metabolic disorder defined by high levels of glucose in blood due to non-secretion of insulin. According to recent estimates, the world diabetic population could rise to 300 million by the year 2025 due to contemporary lifestyle and obesity [2,3]. The hyperglycemia that characterizes T2DM and that promotes the development of complications is a consequence of at least three metabolic abnormalities: resistance of skeletal muscle, adipose tissue and liver to the action of insulin, inadequate insulin secretion such that glucose is taken up from the bloodstream into the tissues efficiently, and excessive hepatic glucose output [4]. Chronic hyperglycemia leads to a number of disorders including cardiovascular, renal, and neurological as well as ophthalmic infections [5–7].

The treatment of Type 2 diabetes has been revolutionized with the advent of thiazolidinediones (TZDs) class of molecules that normalize elevated blood glucose level. Unlike sulfonylureas (e.g., glipizide, glyburide), which enhance insulin resistance, and metformin, which reduces hepatic glucose output, TZDs (e.g., troglitazone, rosiglitazone, pioglitazone) improve insulin sensitivity in liver, muscle and fat tissues and thus counteract insulin resistance [8] (Fig. 1). Frances C. Brown reported the structural relationship among the various 4-thiazolidinones [9]. TZDs are effective in reducing glycosylated hemoglobin (HbA1c). It is generally recommended that thiazolidinediones should be used in combination with metformin only in obese patients [10].

Like TZDs, rhodanine based molecules have been popular as small molecule inhibitors of numerous targets such as HCV NS3 protease [11], antidiabetic agents [12], aldose reductase [13], β-lactamase [14], histidine decarboxylase [15] and inhibitors of JSP-1 [16].

The peroxisome proliferator activated receptors (PPARs) are a group of nuclear receptor isoforms that play a key role in the regulation of dietary fat storage and are a target for the development of treatments for Type 2 diabetes, obesity and cardiovascular disease. TZDs at these receptors act as insulin sensitizers, and PPARα and PPARγ receptor subtypes show different tissue and ligand specificities, PPARγ agonists improve glycemic control and dyslipidemia in type 2 diabetic patients by down regulating cytokines in adipose tissue, while agonists of the PPARα subtype improve the atherogenic lipoprotein profile of insulin resistance

* Corresponding author. Tel.: +91 44 22351269x226; fax: +91 44 22352494.
E-mail address: sriman55@yahoo.com (S. Sriman Narayanan).

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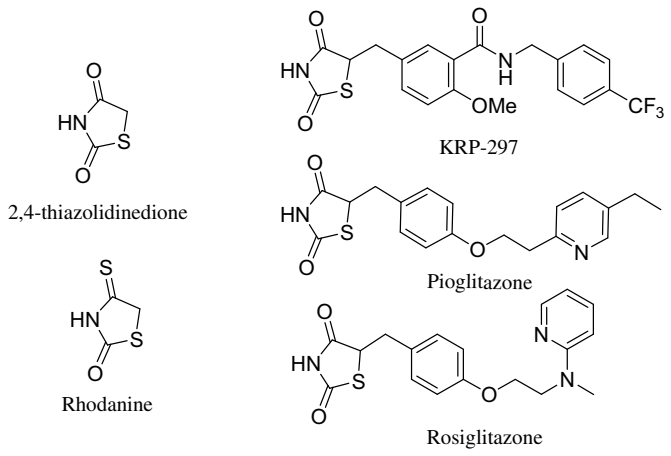
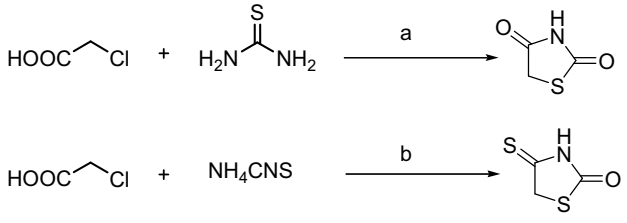


Fig. 1. Structure of known compounds contains thiazolidinedione.

[17–20]. Apart from rosiglitazone and pioglitazone, the recent drug KRP-297 is a potent agonist of PPARγ [21]. Consequently, there continues to be interest in new compounds for clinical development. This provides an opportunity to bring diverse class of dispiropyrrolidine ligands through 1,3-dipolar cycloaddition that could normalize both insulin and glucose levels.

2. Chemistry

Multicomponent 1,3-dipolar cycloaddition reactions are considered to be one of the most useful processes for the construction of five membered heterocyclic ring systems [22–24]. These five membered heterocycles are most important because of their high regioselectivity and stereoselectivity. Spiro compounds represent an important class of naturally occurring substances characterized by highly pronounced biological properties [25–32]. Azomethine ylides generated by the decarboxylative route offer a convenient method for the synthesis of nitrogen containing heterocyclic compounds [33,34]. We herein report for the first time the cycloaddition reaction of azomethine ylides with the olefinic bond of 5-arylidene-1,3-thiazolidine-2,4-dione derivatives **3a**–**h** and 5-arylidene-4-thioxo-1,3-thiazolidine-2-one derivatives **3i**–**p**. Thiazolidinedione [35,36] was synthesized according to the procedure from monochloroacetic acid and thiourea, rhodanine [37] was synthesized from monochloroacetic acid and ammonium thiocyanate (Scheme 1). The different substituted derivatives of 5-arylidene-1,3-thiazolidine-2,4-dione and 5-arylidene-4-thioxo-1,3-thiazolidine-2-one derivatives **3a**–**p** were synthesized in accordance with the literature procedure [38]. The azomethine ylides generated by the reaction of sarcosine **5** with isatin **4** in boiling methanol for 20–25 h reacted with double bond of **3a**–**h** and 15–20 h reacted with double bond of **3i**–**p** to the corresponding cycloadducts **6a**–**p** as single regioisomers with overall yields of



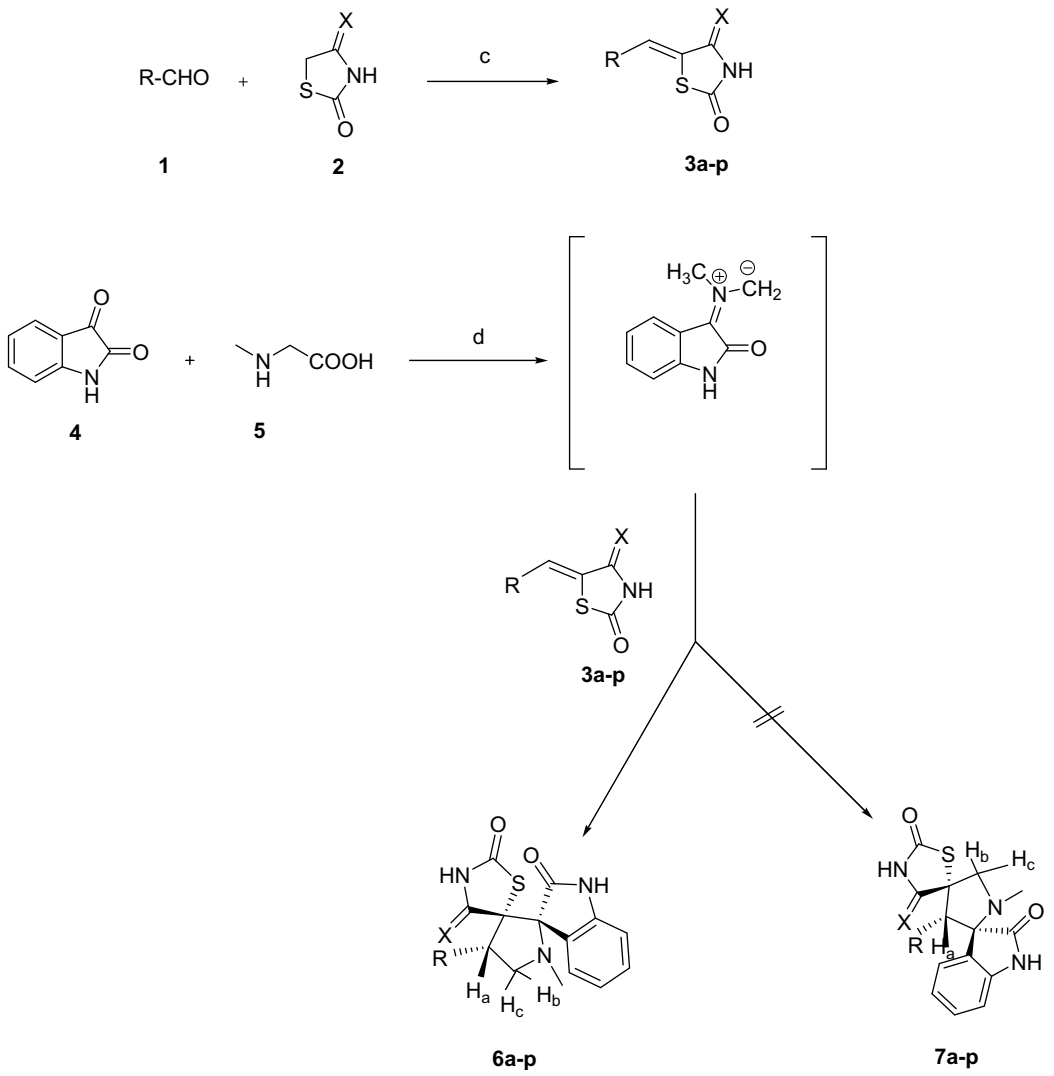
Scheme 1. Synthesis of 2,4-thiazolidinedione and rhodanine: (a) conc. HCl, water, reflux 10 h. (b) Et_3N, dichloromethane, rt 12 h.

54–65% (Scheme 2, Table 1). There is no trace of other regioisomers **7a**–**p** [39].

IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies confirmed the dispiroheterocyclic ring structures of products **6a**–**p**. The IR spectrum of **6l** revealed the presence of a thiocarbonyl stretching vibration band at 1750 cm^{-1}, showing an increase of 40 cm^{-1} from the normal value of 1710 cm^{-1} for 3,4-dihydroxybenzylidene-4-thioxo-1,3-thiazolidine-2-one **6l** indicating the loss of conjugation. The ^{1}H NMR spectrum of **6l** revealed one sharp singlet at δ 2.20 due to the *N*-methyl protons. The benzylic proton H_a exhibited a doublet of doublets at δ 4.44 ($J = 10.5$, 8.2 Hz). H_c of the *N*-CH_2 proton appeared as a doublet of doublets at δ 3.82 ($J = 11.5$, 8.4 Hz). A doublet of doublets was also observed at δ 3.45 ($J = 11.5$, 8.4 Hz) for the H_b proton. The aromatic protons appeared as a multiplet in the region δ 6.80–7.80 and there were two broad singlets at δ 9.22 and δ 10.81 for the NH proton of oxindole and rhodanine. Similarly, the benzylic proton H_a exhibited a doublet of doublets at δ 4.45 ($J = 9.6$, 8.2 Hz). H_c of the *N*-CH_2 proton appeared as a doublet of doublets at δ 3.71 ($J = 12.3$, 8.4 Hz) in the ^{1}H NMR spectrum of **6a**. A doublet of doublets was also observed at δ 3.42 ($J = 12.3$, 8.4 Hz) for the H_b proton. The regiochemistry of the products **6l** and **6a** were confirmed by its ^{1}H NMR spectrum. If the other isomers had been formed one would expect a singlet instead of doublet of doublets for the benzylic proton. The regiochemistry of the product **6l** was also confirmed by its ^{13}C NMR spectrum. The off resonance decoupled ^{13}C NMR of **6l** exhibited signals at δ 72.6 ppm due to the spiro carbon C3 of the pyrrolidine ring and at δ 81.7 ppm due to the C2 spiro carbon of the pyrrolidine ring. In the same way, ^{13}C NMR of **6a** and **6g** exhibited signals at δ 69.2 ppm and 68.7 ppm due to the spiro carbon C3 of the pyrrolidine ring and at δ 80.1 ppm and 80.2 ppm respectively due to the C2 spiro carbon of the pyrrolidine ring. The resonance at δ 170.2 ppm and δ 202.1 ppm of the product **6l** is due to the keto carbonyl and thiocarbonyl carbons of rhodanine. The resonance at δ 172.1 ppm is due to the oxindole carbonyl carbon. Signals at δ 37.5 ppm due to *N*-CH_3 and at δ 54.5 ppm due to *N*-CH_2 carbons were also observed. The mass spectrum of **6l** showed a molecular ion peak at *m*/*z* 426.5 (M^-), which further confirmed the formation of the cycloadduct by the single crystal X-ray structural analysis (Fig. 2). Likewise, the resonance at δ 172.2 ppm and δ 180.1 ppm of the product **6a** is due to the keto carbonyl carbons of thiazolidinedione. The mass spectrum of **6a**–**d** showed the molecular ion peak at *m*/*z* 378.4 (M^-), 414.4 (M^-), 384.5 (M^-), 410.4 (M^-), which further confirmed the formation of the cycloadduct. Identical results were obtained with other derivatives of thiazolidinedione and rhodanine **6a**–**p** [40].

3. Results and discussion

In the in vivo antidiabetic study in male Wistar rats, the selective six compounds showed better reduction in glucose levels than rosiglitazone. Among the six compounds, particularly compounds **6b**, **6h**, **6j** and **6p** showed the best antidiabetic activity (Table 2). Molecular docking studies were carried out on the synthesized compounds to get insight about their binding preferences at the active site of the receptor. The docking studies were performed on 1FM9 protein, viz. PPARγ employing Cerius2 4.8.1 program installed on silicon graphics Octane2 workstation equipped with a 400 MHz MIPS RI2000 (512 MB RAM) running in Irix 6.5 operating system. All the PPARγ inhibitors were modeled in cerius2 using standard bond length and bond angle parameters. Ligands and the standards Pioglitazone, Rosiglitazone, Farglitazar and Rivoglitazone were docked in the active site of the PPARγ using ligand fit module [41]. The active sites were assigned at a radius of 5 Å around the reference ligand. The docking scores of all molecules are listed (Table 3). In the subsequent synthesis and biological



Scheme 2. Synthesis of dispiropyrrolidine derivatives of thiazolidinedione and 4-thioxothiazolidine-2-one: Reagents and conditions: (c) piperidine, Toluene, reflux 3 h (d) methanol, reflux 15–20 h.

evaluation of hit molecules; we found compounds **6b** and **6h** as high affinity ligands for PPARγ. Fig. 3(a) and (b) shows the interaction of the ligands with few of the critical residues (His 449, Tyr473, Ser289 and His323) [42,43] in the active site of the PPARγ. Fig. 4(a) shows the superimposition of active compounds with the crystallized ligand GI262570 and Fig. 4(b) and (c) shows the connolly surfaces of compounds **6h** and **6b**. The most active compound **6h** fitted the best in the active site of PPARγ and attained the best score of 71.18 amongst all molecules. It showed the important additional H bonding interactions with the residue Tyr^{473} at the active site of PPARγ. This is due to the methoxy group, which is present in fourth position of phenyl ring in compound **6h**.

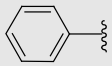
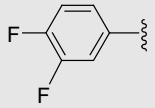
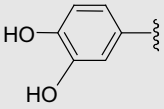
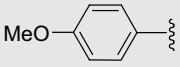
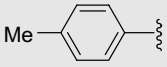
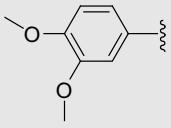
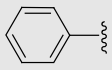
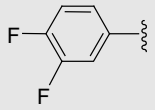
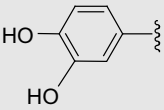
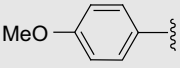
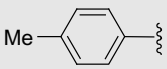
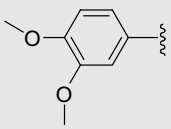
The synthesized molecules were showed moderate binding score when compared to standard molecule, though they have good antidiabetic activity in vivo. Some of the molecules like netoglitazone, NC-2100 containing thiazolidinedione moiety undergoing phase-II clinical trials and (Δ^5 – unsaturated) thiazolidinediones were reported to have low or no activity at PPARγ in vitro than rosiglitazone, at the same time have high antidiabetic activity in vivo [44–46]. These reports corroborate our surveillance presented herein, which raise the possibility that some dispiropyrrolidines with thiazolidinediones and rhodanine derivatives arbitrate their antidiabetic activity through a mechanism other than PPARγ.

In general, thiazolidinediones have shown hemotoxicity and hepatoxicity in both animal and clinical trials [47]. Serum GPT, blood urea nitrogen and creatinine were elevated in alloxan-induced diabetes [48] indicating that alloxan administration produced hepatic and renal damage (Table 4). The data obviously specify that the elevation of liver GPT and ALP values in alloxan-induced diabetes was reduced when treated with synthesized compounds.

4. Conclusion

This communication illustrates simple and efficient synthesis of regio- and stereocontrolled dispiropyrrolidine derivatives of thiazolidinedione and rhodanine which are found to exhibit attractive anti-diabetic properties to male Wistar rats. The synthesized compounds are more effective than rosiglitazone in ameliorating stress condition. Albeit more discovery work is needed to assess the mechanism of such dispiropyrrolidine derivatives of thiazolidinedione and rhodanine, present results substantiate some of its

Table 1
Compounds (**3a**–**p**) used for 1,3-dipolar cycloaddition reaction.

S. No	Compound	R	X	Yield (%)	Time (h)
1	**6a**		O	61	21
2	**6b**		O	59	25
3	**6c**		O	54	22
4	**6d**		O	55	24
5	**6e**		O	59	23
6	**6f**		O	62	20
7	**6g**		O	61	21
8	**6h**		O	65	20
9	**6i**		S	62	16
10	**6j**		S	60	20
11	**6k**		S	55	15
12	**6l**		S	56	16
13	**6m**		S	59	17
14	**6n**		S	62	16
15	**6o**		S	61	16
16	**6p**		S	65	15

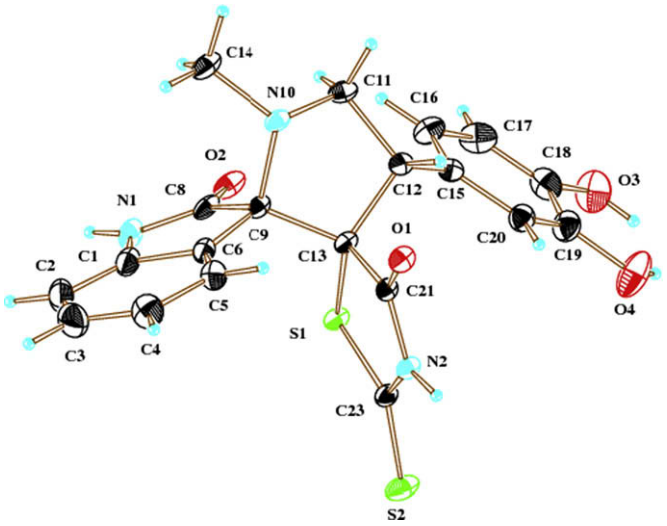


Fig. 2. ORTEP diagram of **6l** (CCDC 653170).

antidiabetic properties. Further advanced drug design and pharmacological studies on these compounds are underway.

5. Experimental protocols

5.1. Pharmacology

Male Wistar rats 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 given standard chow (National Institute of Nutrition, India) and water ad libitum. The experiments were designed and conducted in accordance with the guidelines of institutional animal's ethics committee. The acclimatized animals were kept fasting for 24 h with water ad libitum and alloxan monohydrate (120 mg/kg i.p.) in normal saline was then administered [49]. Serum glucose level was checked after 72 h. Animals with serum glucose levels > 250 mg/dl were considered diabetic and were used for the study [50]. The diabetic rats were divided into four groups of six rats each. Control animals received distilled water only (Group I), diabetic animals received alloxan injection (Group II), diabetic animals orally fed with rosiglitazone (Rg) [as 0.25% carboxymethyl cellulose suspension] at dose of 36 mg/kg (Group III) and the diabetic animals orally fed with synthesized dispiropyrrolidine derivatives (**6b**, **6g**, **6h**, **6j**, **6l** and **6p**) [as 0.25%

Table 2
In vivo antidiabetic activity screening of dispiropyrrolidine derivatives.

Compound	Blood glucose in mg/dl			
	0 h	1 h	3 h	6 h
Group I	185.0 ± 10.5	164.0 ± 6.3	157.0 ± 4.5	151.0 ± 3.8
Group II	322.2 ± 8.7	315.2 ± 7.2	310.4 ± 6.5	297.4 ± 4.5
Group III	317.0 ± 7.4	155.0 ± 5.8	145.5 ± 4.3	131.0 ± 4.8
6b	310.0 ± 10.5[a]	134.5 ± 3.7	123.5 ± 4.4	115.3 ± 2.2[a]
6g	320.5 ± 12.5[a]	140.5 ± 2.7	136.5 ± 5.4	125.5 ± 4.9[b]
6h	315.0 ± 10.2	133.5 ± 4.8	123.8 ± 3.7	115.8 ± 1.8[a]
6j	230.5 ± 8.6	137.5 ± 11.3	112.8 ± 6.7	106.5 ± 5.7[a]
6l	275.5 ± 14.5[b]	175.5 ± 10.7	158.5 ± 5.5	120.5 ± 3.5[b]
6p	213.5 ± 17.5	140.5 ± 14.5	106.5 ± 5.8	95.8 ± 5.3[a]

The mean ± S.E. of the blood glucose level was calculated for each group and the results were analyzed by ANOVA and student *t*-test by comparing the results of 1, 3 and 6 h with 0 h. This test is applied to assess the statistical significance of difference between two independently drawn sample means.
[a] $p < 0.01$ indicates statistically more significant when compared with Group I.
[b] $p < 0.05$ indicates statistically significant when compared with Group I.

Table 3
Compounds with their Cerius2 docking scores in 1FM9 (PPARγ).

S. No	Compound	Dock score
1	**6a**	35.62
2	**6b**	68.80
3	**6c**	35.72
4	**6d**	47.72
5	**6e**	38.56
6	**6f**	42.35
7	**6g**	34.56
8	**6h**	71.18
9	**6i**	28.32
10	**6j**	57.65
11	**6k**	29.44
12	**6l**	58.34
13	**6m**	49.25
14	**6n**	45.28
15	**6o**	30.25
16	**6p**	56.87
17	Farglitazar	130.38
18	Pioglitazone	95.27
19	Rivoglitazone	105.78
20	Rosiglitazone	96.48

carboxymethyl cellulose suspension] at dose of 36 mg/kg (Group IV). The quantity of spiropyrrolidine derivatives equivalent to average human intake of 200 mg/kg at a time was calculated for a single dose of 36 mg/kg (for acute study). Fasting blood samples were collected from the tail vein on third day of alloxan treatment prior to the administration of the synthesized compounds and at 0, 1, 3, 6 h intervals. For biochemical study of the synthesized compounds, the same animals were continued with the same dose of the synthesized compounds once daily for 15 days. Serum glucose levels in the blood collected at random were measured on 6, 9, 12, 15 and 18th day after alloxan treatment.

5.2. Synthesis

Melting points were determined on Veego melting point apparatus and are uncorrected. Infra red spectra were recorded on Perkin–Elmer FT-IR 1600 spectrometer. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Bruker Avance 400 MHz spectrometer with TMS as the internal standard. Mass spectra were recorded on Agilent MSD VL mass spectrometer. Elemental analysis was performed with Perkin–Elmer, 2400 series II CHN–O analyzer. The preparative chromatography was performed by Waters LC 4000 system includes a Waters 2487 dual wavelength absorbance detector, Millennium software and a YMC C18 (ODS) column (250 × 20 mm, 10 μ). Purification of synthesized compounds was carried out using water/acetonitrile (60:40 v/v) as mobile phase at a flow rate of 25 ml/min. Eluate from the column was monitored by UV detector at 254 nm.

5.2.1. General procedure for the synthesis of dispiropyrrolidine derivatives of thiazolidinedione ***6a–h***

A mixture of isatin **4** (1.0 mmol), sarcosine **5** (1 mmol), and 5-arylidene-1,3-thiazolidine-2,4-dione (1 mmol) was refluxed in methanol. Completion of the reaction was evidenced by TLC analysis. The solvent was then removed in vacuo and the crude compound was subjected to preparative chromatography to afford the cycloadduct, which was lyophilized and then crystallized from ethanol. In the same manner, the following compounds were obtained.

*5.2.1.1. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] thiazolidine-2^{11}, 4^{11}-dione-4(-phenyl)-pyrrolidine (**6a**).* Yield = 61%; $[\alpha]_D = -35.2$ (c 1.0, CH_2Cl_2); mp 152 °C IR (KBr) 1750, 1697, 1685 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 12.1 (s, 1H), 10.41 (s, 1H), 6.8–7.7 (m, 9H), 4.45 (dd, J = 9.6, 8.2 Hz, 1H$_a$), 3.71 (dd, J = 12.3, 8.4 Hz, 1Hc), 3.42 (dd, J = 12.3, 8.4 Hz, 1H$_b$), 2.11 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 180.1, 172.2, 169.4, 153.2, 140.2, 139.7, 128.9, 128.5, 128.2, 126.1, 124.4, 121.5, 80.1, 69.2, 54.5, 36.5, 32.2 ppm; m/z 378.4 (M$^-$). Analysis calcd for $C_{20}H_{17}N_3O_3S$: C, 63.31; H, 4.52; N, 11.07; S, 8.45. Found: C, 63.28; H, 4.62; N, 11.02; S, 8.43%.

*5.2.1.2. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] thiazolidine-2^{11}, 4^{11}-dione-4-(3,4-difluorophenyl)-pyrrolidine (**6b**).* Yield = 59%; $[\alpha]_D = -44.1$ (c 1.0, CH_2Cl_2); mp 158 °C IR (KBr) 1747, 1687, 1677 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.8 (s, 1H), 10.32 (s, 1H), 6.8–7.7 (m, 7H), 4.32 (dd, J = 9.7, 8.3 Hz, 1H$_a$), 3.65 (dd, J = 12.4, 8.2 Hz, 1H$_c$), 3.32 (dd, J = 12.4, 8.2 Hz, 1H$_b$), 2.08 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 179.6, 172.0, 167.3, 153.1, 149.4, 147.1, 140.2, 135.9, 128.4, 126.2, 125.1, 122.4, 121.3, 118.1, 80.2, 69.1, 53.5, 37.4, 33.4 ppm; m/z 414.4 (M$^-$). Analysis calcd for $C_{20}H_{15}F_2N_3O_3S$: C, 57.83; H, 3.64; N, 10.12; S, 7.72. Found: C, 57.78; H, 3.61; N, 10.10; S, 7.82%.

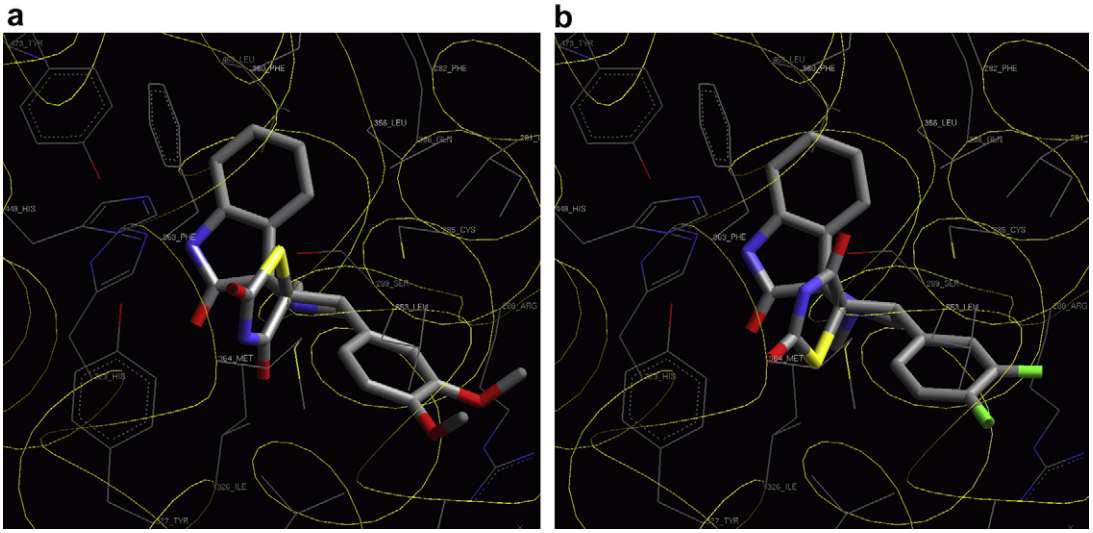


Fig. 3. (a) Compound **6h** with PPARγ (1FM9). (b) Compound **6b** with PPARγ (1FM9). It shows the binding mode of ligands in the active site. The side chains of the residues are shown in stick model. Yellow color ribbon represents the secondary structure of the protein.

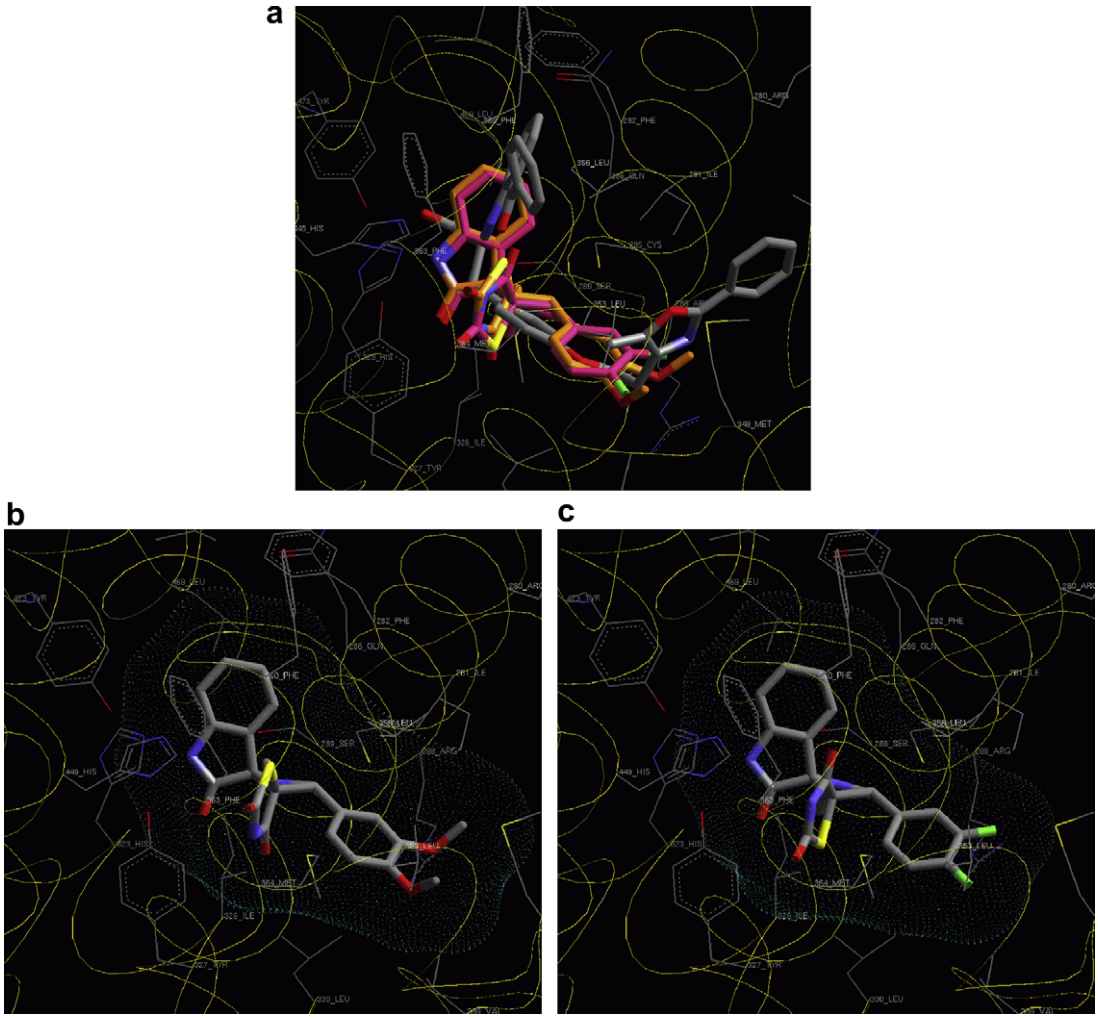


Fig. 4. (a) The superimposition of active compounds **6h** and **6b** with GI262570 (crystallized ligand). (b) Connolly surface of compounds **6h** and **6b**.

*5.2.1.3. 1-N-methyl-spiro [2.3[1]] oxindole-spiro [3.5[11]] thiazolidine-2[11], 4[11]-dione-4-(thiophene-2-yl)-pyrrolidine (**6c**).* Yield = 54%; $[\alpha]_D = -37.4$ (c 1.0, CH_2Cl_2); mp 153 °C IR (KBr) 1755, 1688, 1679 cm^{-1}; ^1H NMR (δ in $CDCl_3$, 400 MHz) 11.2 (s, 1H), 9.61 (s, 1H), 6.9–7.5 (m, 7H), 4.12 (dd, $J = 8.9$, 7.8 Hz, $1H_a$), 3.45 (dd, $J = 11.8$, 8.2 Hz, $1H_c$), 3.32 (dd, $J = 11.8$, 8.2 Hz, $1H_b$), 2.20 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 179.7, 172.2, 167.2, 152.3, 144.8, 140.1, 128.2, 126.3, 126.2, 124.1, 123.2, 121.4, 79.8, 69.6, 54.4, 37.5, 32.4 ppm; *m/z* 384.5 (M^-). Analysis calcd for $C_{18}H_{15}N_3O_3S_2$: C, 56.09; H, 3.92; N, 10.90; S, 10.64. Found: C, 56.11; H, 3.87; N, 10.79; S, 16.78%.

Table 4
Biochemical parameters in alloxan-induced diabetic rats at 18th day.

Serum[a]		Blood[a]	Liver[a]		Treatment (dose/kg b.wt)
GPT (U/ml)	Creatinine (mg/dl)	Urea nitrogen (mg/dl)	GPT (U/mg protein)	ALP (KA/dl)	
82.5 ± 15.5	0.8 ± 0.25	17.5 ± 2.0	525.1 ± 55.3	34.0 ± 3.5	Group I
312.5 ± 15.5	3.0 ± 0.50	62.0 ± 3.5	870.5 ± 59.0	55.5 ± 6.5	Group II
150.2 ± 15.5	2.2 ± 0.10	55.0 ± 2.5	725.0 ± 32.0	55.5 ± 6.5	Group III
132.0 ± 26.2[b]	2.1 ± 0.15	35.0 ± 2.5[b]	582.5 ± 48.5	41.3 ± 5.5	**6b**
129.8 ± 28.3[b]	1.8 ± 0.15	37.5 ± 1.8[b]	557.5 ± 46.5	38.3 ± 3.8	**6g**
140.5 ± 15.8	1.8 ± 0.15	41.8 ± 3.2	698.0 ± 54.6	48.5 ± 4.5	**6h**
137.5 ± 19.5[b]	1.5 ± 0.10	38.5 ± 1.5[b]	625.6 ± 69.6	37.0 ± 5.5	**6j**
142.8 ± 17.2	2.0 ± 0.10	39.0 ± 3.8	645.5 ± 42.5	43.8 ± 5.5	**6l**
121.0 ± 12.2[b]	2.2 ± 0.15	35.5 ± 2.5[b]	575.6 ± 35.5	42.3 ± 4.0	**6p**

[a] All values are mean ± SD, n = 6.
[b] $p < 0.001$ when compared with Group I.

*5.2.1.4. 1-N-methyl-spiro [2.3[1]] oxindole-spiro [3.5[11]] thiazolidine-2[11], 4[11]-dione-4-(3,4-dihydroxyphenyl)-pyrrolidine (**6d**).* Yield = 55%; $[\alpha]_D = -37.6$ (c 1.0, CH_2Cl_2); mp 155 °C IR (KBr) 1765, 1689, 1665 cm^{-1}; ^1H NMR (δ in $CDCl_3$, 400 MHz) 10.53 (s, 1H), 8.95 (s, 1H), 6.8–7.8 (m, 7H), 4.25 (dd, $J = 9.6$, 8.2 Hz, $1H_a$), 3.41 (dd, $J = 12.3$, 8.4 Hz, $1H_c$), 3.22 (dd, $J = 12.3$, 8.4 Hz, $1H_b$), 2.11 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 179.5, 172.8, 167.4, 152.4, 147.6, 144.4, 139.8, 132.8, 128.4, 126.6, 124.3, 122.7, 121.2, 117.3, 79.5, 68.7, 53.6, 37.5, 33.2 ppm; *m/z* 410.4 (M^-). Analysis calcd for $C_{20}H_{17}N_3O_5S$: C, 58.38; H, 4.16; N, 10.21; S, 7.79. Found: C, 58.34; H, 4.24; N, 10.22; S, 7.74%.

*5.2.1.5. 1-N-methyl-spiro [2.3[1]] oxindole-spiro [3.5[11]] thiazolidine-2[11], 4[11]-dione-4-(4-flurophenyl)-pyrrolidine (**6e**).* Yield = 59%; $[\alpha]_D = -45.3$ (c 1.0, CH_2Cl_2); mp 150 °C IR (KBr) 1755, 1692, 1668 cm^{-1}; ^1H NMR (δ in $CDCl_3$, 400 MHz) 11.51 (s, 1H), 9.31 (s, 1H), 6.8–7.7 (m, 8H), 4.25 (dd, $J = 9.4$, 8.2 Hz, $1H_a$), 3.42 (dd, $J = 11.8$, 8.2 Hz, $1H_c$), 3.24 (dd, $J = 11.8$, 8.2 Hz, $1H_b$), 2.08 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 180.1, 172.5, 167.4, 160.3, 152.3, 139.5, 134.7, 129.8, 128.4, 126.2, 124.2, 121.4, 115.6, 79.7, 68.9, 53.6, 37.4, 33.2 ppm; *m/z* 396.4 (M^-). Analysis calcd for $C_{20}H_{16}FN_3O_3S$: C, 60.44; H, 4.06; N, 10.57; S, 8.07: Found: C, 60.38; H, 4.13; N, 10.48; S, 8.15%.

*5.2.1.6. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] thiazolidine-2^{11}, 4^{11}-dione-4-(4-methoxyphenyl)-pyrrolidine (**6f**).* Yield = 62%; $[\alpha]_D = -42.3$ (c 1.0, CH_2Cl_2); mp 154 °C IR (KBr) 1762, 1689, 1665 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.2 (s, 1H), 9.87 (s, 1H), 6.8–7.7 (m, 8H), 4.32 (dd, J = 9.4, 8.3 Hz, 1H$_a$), 3.42 (dd, J = 12.3, 8.4 Hz, 1H$_c$), 3.35 (dd, J = 12.3, 8.4 Hz, 1H$_b$), 3.75 (s, 3H) 2.12 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 180.2, 173.4, 168.3, 158.0, 152.2, 139.7, 131.8, 129.4, 128.5, 126.2, 124.2, 121.4, 114.5, 80.2, 69.4, 58.5, 54.5, 38.1, 33.7 ppm; *m*/*z* 408.4 (M$^-$). Analysis calcd for $C_{20}H_{19}N_3O_4S$: C, 61.60; H, 4.68; N, 10.26; S, 7.83. Found: C, 61.57; H, 4.68; N, 10.33; S, 7.79%.

*5.2.1.7. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] thiazolidine-2^{11}, 4^{11}-dione-4-(4-methylphenyl)-pyrrolidine (**6g**).* Yield = 61%; $[\alpha]_D = -41.3$ (c 1.0, CH_2Cl_2); mp 157 °C IR (KBr) 1755, 1693, 1658 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.8 (s, 1H), 10.34 (s, 1H), 6.8–7.7 (m, 8H), 4.32 (dd, J = 9.6, 8.2 Hz, 1H$_a$), 3.52 (dd, J = 12.3, 8.4 Hz, 1H$_c$), 3.25 (dd, J = 12.3, 8.4 Hz, 1H$_b$), 2.62 (s, 3H) 2.11 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 179.2, 172.9, 168.5, 152.6, 140.2, 135.8, 134.9, 129.2, 128.4, 128.1, 126.2, 124.2, 121.4, 80.2, 68.7, 54.3, 37.5, 33.8 ppm; *m*/*z* 392.5 (M$^-$). Analysis calcd for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68; S, 8.15. Found: C, 64.21; H, 4.77; N, 10.72; S, 8.10%.

*5.2.1.8. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] thiazolidine-2^{11}, 4^{11}-dione-4-(3,4-dimethoxyphenyl)-pyrrolidine (**6h**).* Yield 65%; $[\alpha]_D = -46.2$ (c 1.0, CH_2Cl_2); mp = 150 °C; IR (KBr) 1752, 1697, 1666 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.10 (s, 1H), 9.82 (s, 1H), 6.8–7.7 (m, 7H), 4.42 (dd, J = 9.4, 8.2 Hz, 1H$_a$), 3.71 (dd, J = 12.3, 8.4 Hz, 1H$_c$), 3.42 (dd, J = 12.3, 8.4 Hz, 1H$_b$), 3.75 (s, 6H) 2.11 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 180.2, 173.2, 168.4, 153.1, 150.2, 147.4, 140.2, 132.8, 128.6, 126.4, 124.2, 121.8, 116.4, 113.5, 70.2, 69.3, 56.8, 56.7, 55.3, 38.4, 32.5 ppm; *m*/*z* 422.5 (M$^-$). Analysis calcd for $C_{22}H_{21}N_3O_4S$: C, 62.40; H, 5.00; N, 9.92; S, 7.57. Found: C, 62.38; H, 5.12; N, 9.87; S, 7.52%.

*5.2.2. General procedure for the synthesis of dispiropyrrolidine derivatives of rhodanine **6i–p***

A mixture of isatin **4** (1.0 mmol), sarcosine **5** (1 mmol), and 5-arylidene-4-thioxo-1,3-thiazolidine-2-one (1 mmol) was refluxed in methanol. Completion of the reaction was evidenced by TLC analysis. The solvent was then removed in vacuo and the crude compound was subjected to preparative chromatography to afford the cycloadduct, which was lyophilized and then crystallized from ethanol. In the same manner, the following compounds were obtained.

*5.2.2.1. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(phenyl)-pyrrolidine (**6i**).* Yield = 62%; $[\alpha]_D = -33.1$ (c 1.0, CH_2Cl_2); mp 156 °C IR (KBr) 1714, 1645 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.0 (s, 1H), 8.82 (s, 1H), 6.8–7.8 (m, 9H), 4.48 (dd, J = 10.1, 8.5 Hz, 1H$_a$), 3.85 (dd, J = 11.8, 8.4 Hz, 1Hc), 3.44 (dd, J = 11.8, 8.4 Hz, 1H$_b$), 2.21 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 202.4, 172.5, 170.6, 153.1, 140.4, 138.9, 128.9, 128.4, 128.2, 126.2, 126.1, 124.2, 121.4, 85.2, 81.4, 55.3, 39.4, 37.6 ppm; *m*/*z* 394.5 (M$^-$). Analysis calcd for $C_{20}H_{17}N_3O_2S_2$: C, 60.74; H, 4.33; N, 10.62; S, 16.22: Found: C, 60.66; H, 4.41; N, 10.52; S, 16.32%.

*5.2.2.2. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(3,4-difluro phenyl)-pyrrolidine (**6j**).* Yield = 60%; $[\alpha]_D = -45.6$ (c 1.0, CH_2Cl_2); mp 150 °C IR (KBr) 1757, 1638 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.10 (s, 1H), 9.85 (s, 1H), 6.8–7.8 (m, 7H), 4.51 (dd, J = 10.2, 8.4 Hz, 1H$_a$), 3.82 (dd, J = 11.8, 8.4 Hz, 1Hc), 3.42 (dd, J = 11.8, 8.4 Hz, 1H$_b$), 2.17 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 203.8, 173.4, 170.4, 153.3, 150.2, 147.4, 140.2, 136.5, 128.4, 126.2, 125.2, 124.2, 121.4, 117.8, 115.6, 85.6, 82.4, 55.6, 39.2, 37.9 ppm; *m*/*z* 430.5 (M$^-$). Analysis calcd for $C_{20}H_{15}F_2N_3O_2S_2$: C, 55.67; H, 3.50; N, 9.74; S, 14.86. Found: C, 55.64; H, 3.54; N, 9.68; S, 14.91%.

*5.2.2.3. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(thiophene-2-yl)-pyrrolidine (**6k**).* Yield = 55%; $[\alpha]_D = -35.2$ (c 1.0, CH_2Cl_2); mp 141 °C IR (KBr) 1758, 1645 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 9.90 (s, 1H), 8.85 (s, 1H), 6.8–7.8 (m, 7H), 4.42 (dd, J = 10.5, 8.2 Hz, 1H$_a$), 3.85 (dd, J = 11.5, 8.4 Hz, 1H$_c$), 3.42 (dd, J = 11.8, 8.4 Hz, 1H$_b$), 2.18 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 203.8, 174.5, 170.9, 155.4, 145.5, 140.7, 128.4, 126.3, 126.2, 123.2, 122.4, 121.4, 85.6, 82.7, 55.6, 38.9, 38.2 ppm; *m*/*z* 400.5 (M$^-$). Analysis calcd for $C_{18}H_{15}N_3O_2S_3$: C, 53.84; H, 3.77; N, 10.47; S, 23.96. Found: C, 53.79; H, 3.85; N, 10.37; S, 24.03%.

*5.2.2.4. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(3,4-dihydroxyphenyl)-pyrrolidine (**6l**).* Yield = 56%; $[\alpha]_D = -36.1$ (c 1.0, CH_2Cl_2); mp 138 °C IR (KBr) 1725, 1654 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 10.81 (s, 1H), 9.22 (s, 1H), 6.8–7.8 (m, 7H), 4.88 (s, 2H), 4.44 (dd, J = 10.5, 8.2 Hz, 1H$_a$), 3.82 (dd, J = 11.5, 8.4 Hz, 1H$_c$), 3.45 (dd, J = 11.8, 8.4 Hz, 1H$_b$), 2.20 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 202.1, 172.1, 170.2, 154.5, 147.6, 145.7, 139.2, 128.6, 126.5, 124.6, 122.3, 121.4, 117.8, 115.5, 81.7, 72.6, 54.5, 38.1, 37.5 ppm; *m*/*z* 426.5 (M$^-$). Analysis calcd for $C_{20}H_{17}N_3O_4S_2$: C, 56.19; H, 4.01; N, 9.83; S, 15.00. Found: C, 56.10; H, 3.98; N, 9.88; S, 15.07%.

*5.2.2.5. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(4-flurophenyl)-pyrrolidine (**6m**).* Yield = 59%; $[\alpha]_D = -43.8$ (c 1.0, CH_2Cl_2); mp 139 °C IR (KBr) 1748, 1658 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.2 (s, 1H), 10.32 (s, 1H), 6.8–7.8 (m, 8H), 4.41 (dd, J = 9.5, 7.9 Hz, 1H$_a$), 3.82 (dd, J = 11.8, 8.5 Hz, 1H$_c$), 3.44 (dd, J = 11.8, 8.5 Hz, 1H$_b$), 2.18 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 204.3, 173.2, 170.4, 161.4, 153.7, 139.8, 134.5, 129.8, 129.2, 126.4, 122.2, 121.4, 115.4, 85.7, 82.6, 54.5, 39.7, 38.2 ppm; *m*/*z* 412.5 (M$^-$). Analysis calcd for $C_{20}H_{16}FN_3O_2S_2$: C, 58.09; H, 3.90; N, 10.16; S, 15.51. Found: C, 58.11; H, 4.03; N, 10.08; S, 15.44%.

*5.2.2.6. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(4-methoxyphenyl)-pyrrolidine (**6n**).* Yield = 62%; $[\alpha]_D = -41.4$ (c 1.0, CH_2Cl_2); mp 138 °C IR (KBr) 1767, 1644 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.50 (s, 1H), 9.85 (s, 1H), 6.8–7.8 (m, 8H), 4.42 (dd, J = 9.5, 7.9 Hz, 1H$_a$), 3.75 (dd, J = 11.4, 8.5 Hz, 1Hc), 3.39 (dd, J = 11.4, 8.5 Hz, 1H$_b$), 3.82 (s, 3H); 2.15 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 202.6, 172.8, 170.4, 159.2, 153.4, 140.7, 132.6, 129.2, 128.4, 126.2, 124.3, 121.6, 114.4, 84.7, 82.4, 56.0, 54.1, 38.6, 37.4 ppm; *m*/*z* 424.5 (M$^-$). Analysis calcd for $C_{21}H_{19}N_3O_3S_2$: C, 59.27; H, 4.50; N, 9.87; S, 15.07. Found: C, 59.31; H, 4.49; N, 9.81; S, 15.10%.

*5.2.2.7. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(4-methyl phenyl)-pyrrolidine (**6o**).* Yield = 61%; $[\alpha]_D = -39.7$ (c 1.0, CH_2Cl_2); mp 142 °C IR (KBr) 1754, 1632 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 10.88 (s, 1H), 9.86 (s, 1H), 6.8–7.8 (m, 8H), 4.28 (dd, J = 9.6, 8.1 Hz, 1H$_a$), 3.45 (dd, J = 11.4, 8.5 Hz, 1Hc), 3.24 (dd, J = 11.4, 8.5 Hz, 1H$_b$), 2.56 (s, 3H) 2.21 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 201.3, 172.8, 170.2, 152.6, 139.7, 135.7, 135.6, 129.2, 128.4, 128.1, 126.2, 124.2, 121.4, 85.3, 82.4, 58.4, 38.6, 36.5, 25.4 ppm; *m*/*z* 408.5 (M$^-$). Analysis calcd for $C_{21}H_{19}N_3O_2S_2$: C, 61.59; H, 4.68; N, 10.26; S, 15.66. Found: C, 61.55; H, 4.71; N, 10.19; S, 15.74%.

*5.2.2.8. 1-*N*-methyl-spiro [2.3^1] oxindole-spiro [3.5^{11}] 4^{11}-thioxo-thiazolidine-2^{11}-one-4-(3,4-dimethoxyphenyl)-pyrrolidine (**6p**).* Yield = 65%; $[\alpha]_D = -45.4$ (c 1.0, CH_2Cl_2); mp 140 °C IR (KBr) 1736, 1647 cm^{-1}; ^{1}H NMR (δ in $CDCl_3$, 400 MHz) 11.5 (s, 1H), 10.20 (s, 1H), 6.8–7.8 (m, 7H),

4.44 (dd, $J = 9.5$, 7.9 Hz, $1H_a$), 3.82 (dd, $J = 11.4$, 8.5 Hz, H_c), 3.42 (dd, $J = 11.4$, 8.5 Hz, $1H_b$), 3.88 (s, 6H) 2.21 (s, 3H); ^{13}C NMR (δ in $CDCl_3$, 400 MHz) 201.4, 171.6, 170.2, 153.0, 148.4, 145.6, 128.4, 126.2, 124.2, 122.2, 121.4, 84.6, 80.3, 53.4, 38.7, 36.8 ppm; m/z 454.5 (M^-). Analysis calcd for $C_{20}H_{17}N_3O_4S_2$: C, 56.19; H, 4.01; N, 9.83; S, 15.00. Found: C, 56.11; H, 4.08; N, 9.76; S, 15.08%.

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