Bioorganic & Medicinal Chemistry Letters 24 (2014) 1089–1093

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Antimycobacterial evaluation of novel hybrid arylidene thiazolidine-2,4-diones

Singanan Ponnuchamy [a], Selvaraj Kanchithalaivan [b], Raju Ranjith Kumar [b,*], Mohamed Ashraf Ali [c,d,e], Tan Soo Choon [c]

[a] Orchid Chemicals & Pharmaceuticals Ltd, R&D Center, Chennai 600119, India
[b] Department of Organic Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India
[c] Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia
[d] New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India
[e] New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan 301030, India

ARTICLE INFO

Article history:
Received 2 October 2013
Revised 31 December 2013
Accepted 6 January 2014
Available online 11 January 2014

Keywords:
Thiazolidine-2,4-dione
Knoevenagel condensation
Antimycobacterial activity
Mycobacterium tuberculosis
High Throughput Screen

ABSTRACT

A series of novel hybrid heterocycles comprising arylidene thiazolidine-2,4-dione and 1-cyclopropyl-2-(2-fluorophenyl)ethanone were synthesized. These compounds were evaluated for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ in High Throughput Screen. Most of the hybrid arylidene thiazolidine-2,4-diones displayed moderate to good activity with MIC of less than 50 μM. Compound **1m** exhibited maximum potency being 5.87 fold more active at EC_{50} and 6.26 fold more active at EC_{90} than the standard drug pyrimethamine.

Tuberculosis (TB) is a chronic bacterial infection caused by *Mycobacterium Tuberculosis* bacteria (MTB), which spreads through air and usually infects the lungs. TB can be treated by taking several drugs for a period of 6–9 months. Of the approved drugs, the first-line anti-TB agents that form the core of treatment regimen include isoniazid (INH), rifampin (RIF), ethambutol (EMB) and pyrazinamide (PZA). Regimens for treating TB disease have an initial phase of 2 months, followed by a choice of several options for the continuation phase of either 4 or 7 months. The initial empiric treatment of TB starts with a 4-drug regimen: isoniazid, rifampin, pyrazinamide and either ethambutol or streptomycin. Once the TB isolate is known to be fully susceptible, ethambutol (or streptomycin, if it is used as a fourth drug) can be discontinued.[1] After 2 months of therapy (for a fully susceptible isolate), pyrazinamide can be stopped. Isoniazid and rifampin are continued as daily or intermittent therapy for 4 more months. If isolated isoniazid resistance is documented, isoniazid is discontinued and treatment with rifampin, pyrazinamide and ethambutol is continued for the entire 6 months. The Directly Observed Therapy (DOT) is the most recommended for all patients. With DOT, patients on the above regimens can be switched to 2–3 times per week dosing after an initial 2 weeks of daily dosing.[2]

One of the most significant methods for the development of highly active compounds is the combination of active pharmacophores into a single unit.[3] The biological screening of such compounds may result in new lead compounds with better activity than the standard drugs. In this context, we envisage to investigate the antimycobacterial activity of novel hybrid thiazolidine-2,4-diones **1** (Fig. 1) that comprise 5-arylidene-thiazolidine-2,4-dione and 1-cyclopropyl-2-(2-fluorophenyl)ethanone unit. The latter is the key component of the platelet inhibitor prasugrel **2**.[4] Incidentally, thiazolidine-2,4-dione derivatives have been identified as the privileged class of organic heterocycles with profound biological activities. The well known drugs used for the treatment of type II diabetes mellitus troglitazone **3**[5] and pioglitazone **4**[6] comprise thiazolidine-2,4-dione moiety (Fig. 1). Troglitazone **3**[7] has been shown to exhibit anticancer activities through the inhibition of the Raf/MEK/ERK signaling pathway. Further, the 5-arylidene thiazolidine-2,4-dione derivatives have been identified as potent and highly selective inhibitors of the PIM kinase.[8] However, the antimycobacterial activity of these heterocyclic derivatives are still unknown. The above importance of 5-arylidene thiazolidine-2,4-diones and in view of our continued interest in the synthesis and biological evaluation of novel heterocycles,[9] we herein report the

* Corresponding author. Tel.: +91 9655591445.
E-mail address: raju.ranjithkumar@gmail.com (R. Ranjith Kumar).

0960-894X/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2014.01.007

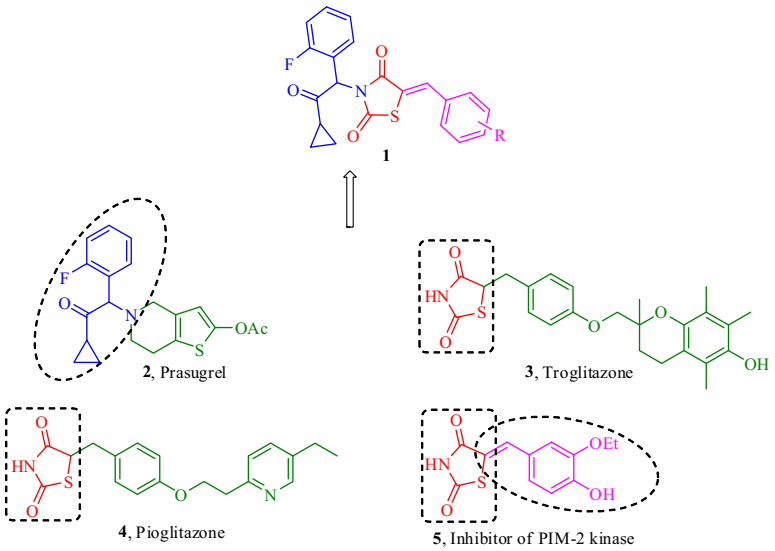


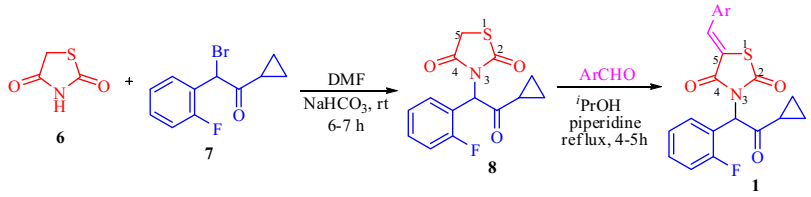
Figure 1. Design of the target molecule.

preliminary results of the antimycobacterial activity of novel 3-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5-[(*Z*)-(aryl)methylidene]-1,3-thiazolane-2,4-diones **1**.

In the present investigation, the starting material 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone **7** was synthesized from the reaction of 2-bromo-2-(2-fluorophenyl)acetonitrile and cyclopropyl magnesium bromide solution, following a literature report.[10] Further, the reaction of **7** with thiazolidine-2,4-dione **6** in DMF in the presence of $NaHCO_3$ at ambient temperature afforded novel 3-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)thiazolidine-2,4-dione **8** in 93% yield (Scheme 1).[11] The structure of **8** is in complete agreement with its elemental analysis, mass, IR and NMR spectroscopy. The mass spectrum of **8** has a characteristic molecular ion peak at 292.0 (M^+). The IR spectrum of **8** shows strong absorptions at 1765, 1703 and 1687 cm^{-1} which are due to the three carbonyl groups. Further, in the ^1H NMR spectrum of **8** the diastereotopic 5-CH_2 protons appeared as doublets at 3.94 and 4.01 ppm with *J* value 17.7 Hz. The N–CH appeared as a singlet at 6.40 ppm whereas the cyclopropyl ring protons were observed as multiplets between 0.96 and 1.92 ppm. In the ^{13}C NMR of **8**, the carbonyls of the thiazolidine ring appeared at 170.4 and 170.5 ppm whilst the signal at 200.8 ppm is due to the carbonyl of the 1-cyclopropyl-2-(2-fluorophenyl)ethanone moiety.

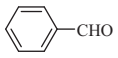
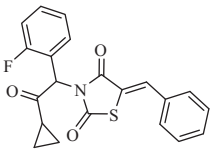
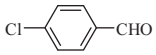
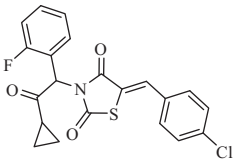
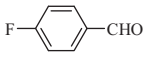
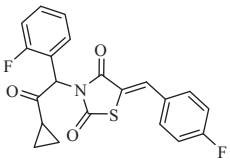
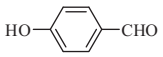
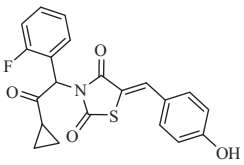
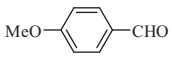
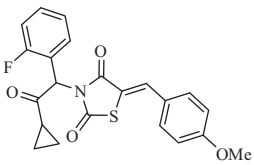
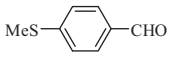
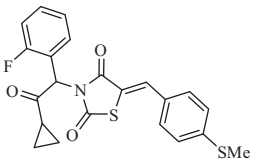
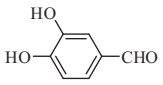
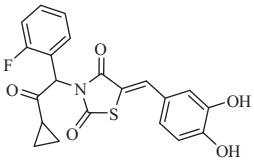
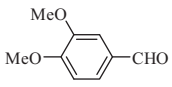
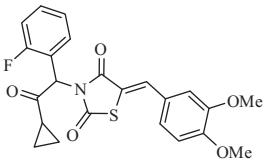
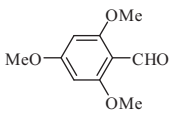
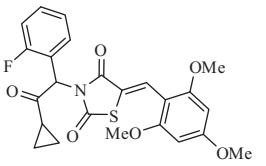
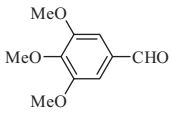
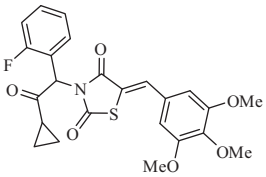
In the next step, the synthesis of novel (*Z*)-5-benzylidene-3-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)thiazolidine-2,4-diones **1** via the Knoevenagel condensation of **8** with various aromatic aldehydes was performed (Scheme 1).[12] The reaction proceeded in isopropyl alcohol in the presence of piperidine as base under reflux condition affording **1a–q** in excellent yields (73–88%). A total of seventeen novel arylidene thiazolidine-2,4-dione hybrid heterocycles were synthesized (Table 1). The reaction occurred smoothly with aromatic aldehydes bearing electron-withdrawing or electron-donating group affording **1** in excellent yields. Further, the presence of sterically hindered groups in the aldehyde and bulky indoline, quinoxaline and benzo[*d*][1,3]dioxazole too had no adverse effects in the yield of the product. However, the reaction failed to occur with aliphatic aldehydes. The structure of all the arylidene thiazolidine-2,4-dione hybrid heterocycles **1a–q** were elucidated with the help of elemental analysis, mass, IR and NMR spectroscopic techniques. Moreover, the NMR spectra of **1** and thiazolidine-2,4-dione **8** were similar except for the presence of new signals in the ^1H and ^{13}C NMR spectra of **1** due to the aromatic ring and benzylidene protons and carbons. Further, the DEPT-135 spectrum of **1** reveals the absence of one 'CH_2' carbon and appearance of new signals due to aromatic 'C' and 'CH' carbons. The structure of **1** assigned from NMR spectroscopy was further solved from single crystal X-ray studies. The ORTEP diagram of **1f** reveals (*Z*)-configuration for the exocyclic alkene (Fig. 2).[13]

All the hybrid thiazolidine-2,4-diones **1a–q** and **8** were screened for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_V$ (MTB-$H_{37}Rv$) in a High Throughput Screen (HTS) using an assay adapted from the microdilution alamar Blue (AB) broth assay reported by Collins and Franzblau[14] and additionally an alternative method for end-point detection using the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG). Five standard drugs were used as references together with the synthesized compound for the assay. Data were analyzed using the IDBS Activity Base software and the dose response result was analyzed using a four parameter logistic fit to the data (Excel Fit equation 205) with the maximum and minimum locked at 100 and 0. From these curves, EC_{50} and EC_{90} values were calculated. The MIC is the minimum concentration of the compound required to inhibit 90% of bacterial growth and MIC's of the compounds are given in Table 2 along with standard drugs for comparison.



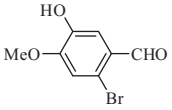
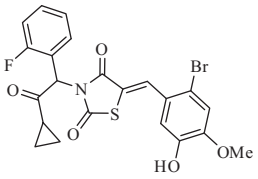
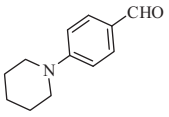
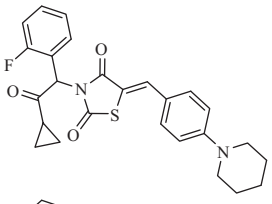
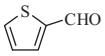
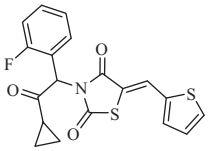
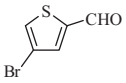
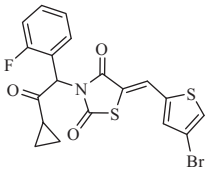
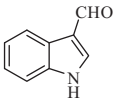
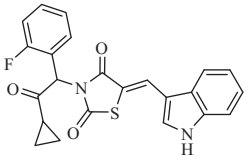
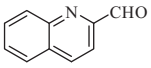
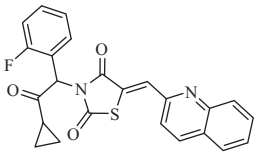
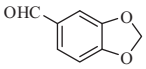
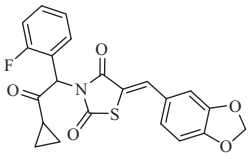
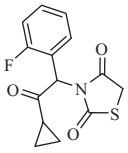
Scheme 1. Synthesis of 1,3-thiazolane-2,4-diones **1**.

Table 1
Yield and melting point of hybrid arylidene thiazolidine-2,4-diones **1** and **8**

Entry	Compd	Aldehyde	Product	Yield (%)	Mp (°C)
1	**1a**			82	134–135
2	**1b**			79	181–183
3	**1c**			82	154–156
4	**1d**			79	165–168
5	**1e**			84	127–128
6	**1f**			84	142–143
7	**1g**			74	199–202
8	**1h**			87	75–79
9	**1i**			87	142–143
10	**1j**			88	121–123

(*continued on next page*)

Table 1 (*continued*)

Entry	Compd	Aldehyde	Product	Yield (%)	Mp (°C)
11	**1k**			82	76–79
12	**1l**			80	159–163
13	**1m**			81	94–98
14	**1n**			73	120–121
15	**1o**			81	161–164
16	**1p**			78	194–196
17	**1q**			86	156–158
18	**8**	NA		93	94–95

All the hybrid thiazolidine-2,4-diones **1a–q** and **8** showed EC_{50} <25 and were more potent than the standard drug pyrimethamine (EC_{50} = 37.35). Seven compounds **1c–e**, **1h**, **1m**, **1q** and **8** with EC_{50} value 11, 10, 11, 8, 6, 9 and 6 μM, respectively, were found to be more potent than the standard drug cycloserine (EC_{50} = 12.47 μM). Two compounds **1m** and **8** were nearly equipotent of which **1m** comprising a thiophene ring was the most active with EC_{50} of 6 μM and was 1.95 and 5.85 times more potent than the standard drugs cycloserine and pyrimethamine, respectively. Further, three compounds **1d**, **1h** and **1m** with EC_{90} value 19, 16 and 11 μM, respectively, were found to be more active than pyrimethamine. These observations clearly showed that the presence of unsubstituted thiophene ring in the hybrid thiazolidine-2,4-diones makes remarkable improvement in antimycobacterial activity when compared to the other substituents.

From the data in Table 2 it is evident that twelve compounds **1c–h**, **1l**, **1m**, **1o–q** and **8** with MIC of 25 μM were found to be equipotent to cycloserine and 4 times more potent than pyrimethamine. However, none of these compounds were found to be active than other standard drugs. The hybrid thiazolidine-2,4-diones **1a–q** and **8** were also tested for cytotoxicity in VERO cells at concentrations of 62.5 μg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay. All the compounds were found to be non-toxic up to 62.5 μg/mL. The compounds were bactericidal and the cytotoxic profile was within the acceptable range.

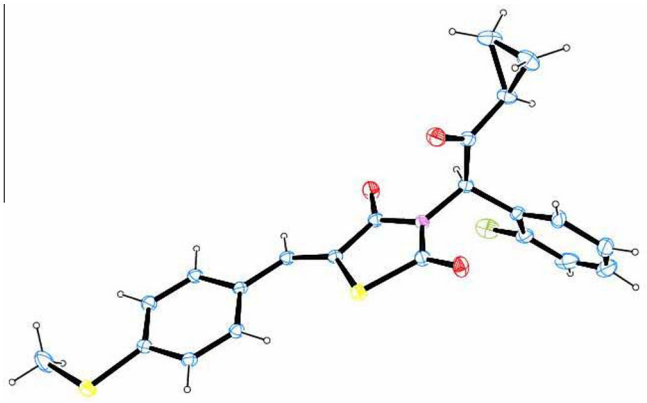


Figure 2. ORTEP diagram of **1f**.

Table 2
HTS Antimycobacterial activity of **1** and **8**

Compd	EC_{50} (μM)	EC_{90} (μM)	MIC (μM)
1a	17	36	50
1b	25	55	50
1c	11	25	25
1d	10	19	25
1e	11	27	25
1f	16	25	25
1g	21	55	25
1h	8	16	25
1i	13	27	50
1j	19	53	50
1k	15	54	50
1l	24	36	25
1m	6	11	25
1n	18	27	50
1o	18	27	25
1p	14	68	25
1q	9	21	25
8	6	26	25
Amikacin	0.07	0.08	0.16
Cycloserine	12.47	13.49	25.00
Ethambutol	<1.56	32.79	12.50
Isoniazid	0.18	0.29	0.31
Pyrimethamine	37.35	74.96	100.00

In conclusion, the present work reports a simple synthesis of novel hybrid heterocycles comprising biologically active components viz. arylidene thiazolidine-2,4-dione and 1-cyclopropyl-2-(2-fluorophenyl)ethanone. The structural features of these compounds were established with the help of NMR and single crystal X-ray studies. In addition, all these hybrid thiazolidine-2,4-diones were screened for their antimycobacterial activity against MTB $H_{37}Rv$ in High Throughput Screen wherein compound **1m** was found to be the most active.

Acknowledgments

R.R.K., S.P. and S.K. thank the University Grants Commission, New Delhi for funds through Major Research Project F. No. 42-242/2013 (S.R.) and Department of Science and Technology, New Delhi for funds under (i) DST-SERC Fast Track Scheme No. SR/FT/CS-073/2009 and (ii) IRHPA program for the high resolution NMR facility in the Department. S.P. thanks the management, Orchid Chemicals and Pharmaceuticals Ltd, Chennai, for providing laboratory facilities. R.R.K. and S.P. thank the Sophisticated Analytical Instrument Facility, Indian Institute of Technology Madras for single crystal X-ray diffraction studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.007.

References and notes

1. Kohn, W. G.; Collins, A. S.; Cleveland, J. L.; Harte, J. A.; Eklund, K. J.; Malvitz, D. M. *MMWR Recomm. Rep.* **2003**, *52*, 1.
2. Swaminathan, S.; Narendran, G.; Venkatesan, P.; Iliayas, S.; Santhanakrishnan, R.; Menon, P. A.; Padmapriyadarsini, C.; Ramachandran, R.; Chinnaiyan, P.; Suhadev, M.; Sakthivel, R.; Narayanan, P. R. *Am. J. Respir. Crit. Care Med.* **2010**, *181*, 743.
3. (a) Liu, A.; Huang, L.; Wang, Z.; Luo, Z.; Mao, F.; Shan, W.; Xie, J.; Lai, K.; Li, X. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1548; (b) Schlager, T.; Schepmann, D.; Lehmkuhl, K.; Holenz, J.; Vela, J. M.; Buschmann, H.; Wunsch, B. *J. Med. Chem.* **2011**, *54*, 6704; (c) Solomon, V. R.; Hu, C.; Lee, H. *Bioorg. Med. Chem.* **2009**, *17*, 7585; (d) Yogeeswari, P.; Sriram, D.; Sahitya, P.; Ragavendran, J. V.; Ranganadh, V. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3712.
4. (a) Wiviott, S. D.; Braunwald, E.; McCabe, C. H.; Montalescot, G.; Ruzyllo, W.; Gottlieb, S.; Neumann, F.-J.; Ardissino, D.; De Servi, S.; Murphy, A. A.; Riesmeyer, J.; Weerakkody, G.; Gibson, C. M.; Antman, E. M. *N. Eng. J. Med.* **2007**, *357*, 2001; (b) Baker, W. L.; White, C. M. *Am. J. Cardiovasc. Drugs* **2009**, *9*, 213.
5. Minamikawa, J.; Tanaka, S.; Yamauchi, M.; Inoue, D.; Koshiyama, H. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 1818.
6. Momose, Y.; Mequro, K.; Ikeda, H.; Hatanaka, C.; Oi, S.; Sohda, T. *Chem. Pharm. Bull.* **1991**, *39*, 1440.
7. (a) Yao, C.-J.; Lai, G.-M.; Chan, C.-F.; Cheng, A.-L.; Yang, Y.-Y.; Chuang, S.-E. *Int. J. Cancer* **2006**, *118*, 773; (b) Yan, K.-H.; Yao, C.-J.; Chang, H.-Y.; Lai, G.-M.; Cheng, A.-L.; Chuang, S.-E. *Mol. Carcinog.* **2010**, *49*, 235.
8. Dakin, L. A.; Block, M. H.; Chen, H.; Code, E.; Dowling, J. E.; Feng, X.; Ferguson, A. D.; Green, I.; Hird, A. W.; Howard, T.; Keeton, E. K.; Lamb, M. L.; Lyne, P. D.; Pollard, H.; Read, J.; Wua, A. J.; Zhang, T.; Zheng, X. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4599.
9. (a) Jeyachandran, V.; Ranjith Kumar, R.; Ali, M. A.; Choon, T. S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2101; (b) Sivakumar, S.; Ranjith Kumar, R.; Ali, M. A.; Choon, T. S. *Eur. J. Med. Chem.* **2013**, *65*, 240.
10. Pan, X.; Huang, R.; Zhang, J.; Ding, L.; Li, W.; Zhang, Q.; Liu, F. *Tetrahedron Lett.* **2012**, *53*, 5364.
11. *Procedure for the synthesis of **8**:* A mixture of 1,3-thiazolidine-2,4-dione (**6**, 1 mmol), 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (**7**, 1 mmol) and sodium bicarbonate (2 equiv) were dissolved in DMF and stirred at ambient temperature (27–28 °C) for 7 h. After completion of the reaction as evidenced by TLC (eluent 7:3/hexane–ethyl acetate) the reaction mixture was poured into water (25 mL), extracted with ethyl aceate and the solvent distilled under vacuum to obtain pure **8**. *3-(2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)thiazolidine-2,4-dione **8**:* Isolated as white crystalline powder. Yield: 93%; mp 94–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.96–1.03 (m, 2H), 1.16–1.30 (m, 2H), 1.85–1.9 (m, 1H), 3.98–4.00 (s, 2H), 6.39 (s, 1H), 7.10–7.61 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 12.4, 13.0, 18.9, 33.3, 59.3, 115.6 ($^2J_{C,F}$ = 22 Hz), 119.7 ($^2J_{C,F}$ = 13 Hz), 123.9 ($^4J_{C,F}$ = 4 Hz), 131.1 ($^3J_{C,F}$ = 8 Hz), 132.7, 161.3 ($^1J_{C,F}$ = 248 Hz), 170.4, 170.8, 200.8; IR (KBr) ν cm^{-1} 1687, 1703, 1764; MS (*m*/*z*) [M−1] 292. Anal. Calcd for $C_{14}H_{12}FNO_3S$: C, 57.33; H, 4.12; N, 4.78. Found: C, 57.38; H, 4.15; N, 4.77.
12. *Synthesis of (Z)-5-benzylidene-3-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-thiazolidine-2,4-diones **1**. General procedure:* A mixture of **8** (1 mmol), aromatic aldehyde (1 mmol) and piperdine (2 equiv.) in isopropyl alcohol was refluxed for 4–5 h. After completion of the reaction as seen from the TLC, (eluent 7:3/hexane–ethyl acetate) the mixture was allowed to cool to room temperature. Then the reaction mixture was poured onto ice-water and neutralized with dilute HCl. The precipitated solid was filtered, washed with water and dried under vacuum. *(Z)-3-(2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-5-(4-(methylthio)benzylidene)thiazolidine-2,4-dione (**1f**):* Isolated as yellow powder. Yield: 84%; mp 142–143 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.95–0.99 (m, 2H), 1.00–1.03 (m, 2H), 2.17–2.23 (m, 1H), 2.50 (s, 3H), 6.48 (s, 1H), 7.23–7.30 (m, 2H), 7.39–7.42 (m, 2H), 7.44–7.49 (m, 2H), 7.57 (d, *J* = 6.3 Hz, 2H), 7.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 12.3, 12.4, 14.0, 18.6, 59.5, 115.5 ($^2J_{C,F}$ = 21 Hz), 118.4, 120.0 ($^2J_{C,F}$ = 14.0 Hz), 124.2 ($^4J_{C,F}$ = 2 Hz), 125.7, 128.8, 130.7, 131.2 ($^3J_{C,F}$ = 9 Hz), 131.7, 134.1, 160.6 ($^1J_{C,F}$ = 246 Hz), 164.8, 166.4, 201.0; IR (KBr) ν cm^{-1} 1688, 1710, 1737; MS (*m*/*z*) [M+1] 428. Anal. Calcd for $C_{22}H_{18}FNO_3S_2$: C, 61.81; H, 4.24; N, 3.28. Found: C, 61.84; H, 4.24; N, 3.32.
13. Suresh, J.; Venkateshan, M.; Ponnuchamy, S.; Ranjith Kumar, R.; Lakshman, P. L. N. *Acta Crystallogr.* **2013**, *E69*, 188.
14. Colins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.