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Design, synthesis, and evaluation of 2-aryl-3-heteroaryl-1,3-thiazolidin-4-ones as anti-HIV agents

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Abstract—Compounds having isothiourea or thiourea functional group have shown high anti-HIV-1 activity. Therefore, a series of 2-aryl-3-heteroaryl-1,3-thiazolidin-4-ones were designed, synthesized, and evaluated for anti-HIV-1 RT activity. The results of in vitro tests showed that the compound **9** exhibited EC_{50} at 0.26 μM with minimal toxicity in MT-4 cells as compared to 0.35 μM for thiazobenzimidazole (TBZ). It may be inferred from the present data that majority of compounds in this series exhibit higher selectivity index than TBZ.

1. Introduction

The pathogenesis of HIV-1 is due to uncontrolled viral replication in CD4+ T cells.[1] Several efforts have been made in the last two decades to understand and control virus replication. In this direction HIV-1 RT has been identified as a prime target for designing inhibitors for treatment of HIV/AIDS.[2–4] The introduction of anti-HIV-1 RT drugs has significantly reduced morbidity and mortality of HIV/AIDS patients.

Based on function of HIV-1RT there are two types of inhibitors that can inhibit RT enzyme activity. First, nucleoside reverse transcriptase inhibitors (NRTIs), analogs of nucleosides, which bind to the active site of enzyme and inhibit DNA template elongation. Second, non-nucleoside reverse transcriptase inhibitors (NNRTIs), which bind to the allosteric site of RT and inhibit enzyme activity. The FDA has approved several NNRTIs.[5] As it is often observed in anti-viral therapy, the continuous or discontinuous usage of NNRTIs in HIV/AIDS patients eventually leads to the development of resistant virus strains against the drug. Therefore, it is imperative to look for new chemical entities having broad–spectrum activity against a variety of clinically developed mutant RT enzymes.

In this direction, Barreca et al.[6] reported 2,3-diaryl-substituted-4-thiozolidone scaffold derived from retro-synthetic opening of the imidazole nucleus of thiazolo-benzimidazole (TBZ), which selectively inhibits HIV-1 RT. Since then, Barreca and Katti et al. are continuously trying to achieve enhanced anti-HIV-1 RT activity in 4-thiozolidinones by substituting functional groups at C-2 and N-3 positions. Detailed QSAR and docking studies have revealed that the biophoric space around N-3 can accommodate a variety of heterocyclic moieties namely, pyridine, pyrimidine, and furfuryl. This provides compelling rationale for further optimization at N-3 of thiazolidinone.[7–9]

The rationale behind the present study is a simple assumption made upon comparison of other NNRTIs with different skeleton having anti-HIV-RT activity that there is common thiourea or isothiourea functional group (Fig. 1). In the present study, the isothiourea or thiourea was substituted at N-3 position of 4-thiozolidinone skeleton. The isothiourea moiety with thiazolidin-4-one scaffold constitutes the body and remaining part of the thiazole or benzthiazole moiety and 2,6-dihalophenyl constitute the wings of the butterfly

Keywords: 4-Thiazolidinones; Anti-HIV activity; HIV-1RT; NNRTIs.
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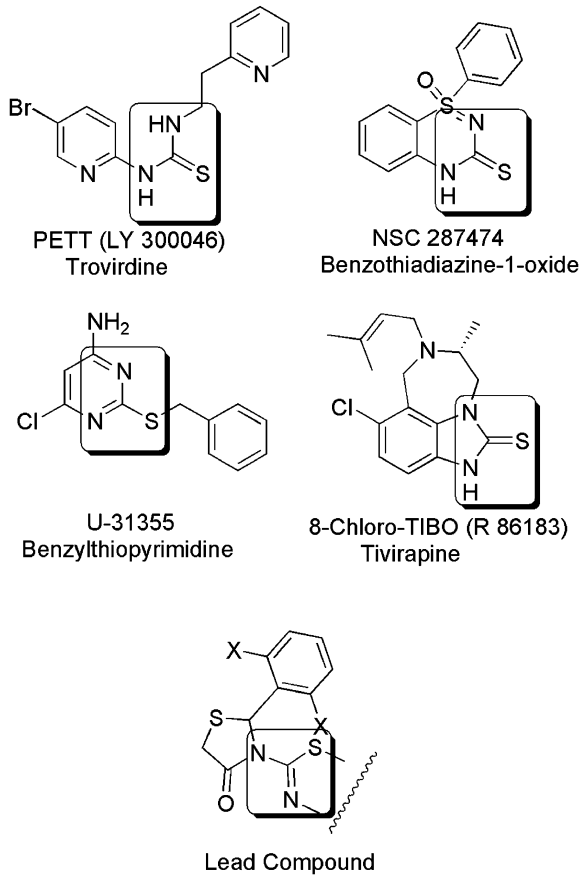


Figure 1. NNRTI lead compounds.

conformation of NNRTIs as depicted in Figure 1. The design, synthesis, and biological activities of these compounds are presented in this paper.

2. Results and discussion

2.1. Chemistry

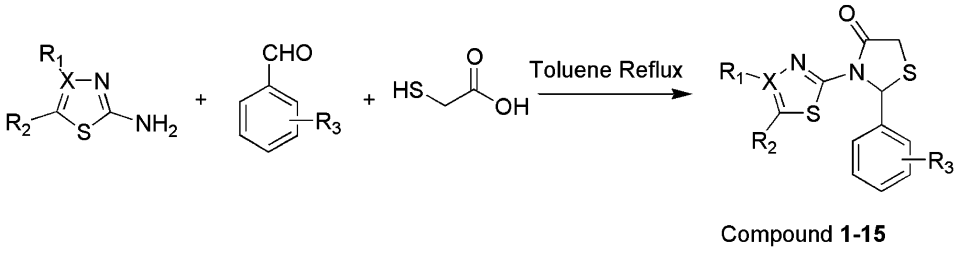
The synthesis of the new 2,3-diaryl-1,3-thiazolidin-4-ones (**1**–**15**) was carried out according to reported procedure,[10] by reacting a suitable (hetero)aromatic amine (**1**) with an equimolar amount of 2,6-dihalo-substituted benzaldehyde (**2**) in the presence of an excess of mercaptoacetic acid (**3**) in toluene reflux (Scheme 1). After completion of reaction, which takes usually 24 h, the desired products were obtained in moderate to excellent yields and purity. The spectral data including the elemental analysis of the compounds reported in this study correlate with the expected structure.

2.2. Biological evaluation

2.2.1. In vitro HIV-RT kit assay.[11] The HIV-RT inhibition assay was performed by using an RT assay kit (Roche), and the procedure for assaying RT inhibition was performed as described in the kit protocol (Roche Kit). Briefly, the reaction mixture consists of template/primer complex, dNTPs and reverse transcriptase (RT) enzyme in the lysis buffer with or without inhibitors. After 1-h incubation at 37 °C, the reaction mix was transferred to streptavidine-coated microtitre plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidine. The unbound dNTPs were washed using wash buffer and anti-DIG-POD was added to the MTP. The DIG-labeled dNTPs incorporated in the template were bound to anti-DIG-POD Antibody. The unbound anti-DIG-POD was washed and the peroxide substrate (ABST) was added to the MTP. A colored reaction product was produced during the cleavage of the substrate catalyzed by a peroxide enzyme. The absorbance of the sample was determined at O.D. 405 nm using microtiter plate ELISA reader. The resulting color intensity is directly proportional to the actual RT activity. The percentage inhibitory activity of RT inhibitors was calculated by comparing to a sample that does not contain an inhibitor. The percentage inhibition was calculated by formula as given below

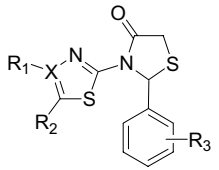
$$\% \text{ Inhibition} = 100 - \left[\frac{\text{O.D. 405 nm with inhibitor}}{\text{O.D. 405 nm without inhibitor}} \times 100\right].$$

2.2.2. In vitro anti-HIV assay. The methodology of the anti-HIV assays has been previously described.[12] Briefly, MT-4 or CEM cells were infected with HIV-1_{IIIB} and HIV-2_{ROD} at 100 times the $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Then, 100 μl of the infected cell suspension was then transferred to microtiter plate wells, mixed with 100 μl of the appropriate dilutions of test compounds, and further incubated at 37 °C. In CEM cells, after 4 days of incubation, HIV-1-induced syncytium formation was recorded. The 50% effective concentration EC_{50} was defined as the compound concentration required to inhibit virus-induced syncytium formation by 50%.[13] After 5 days of incubation of MT-4 cells, the number of viable cells



Scheme 1. General synthetic scheme of 4-thiazolidinones.

Table 1. Anti-HIV-1 activity, cytotoxicity, and selectivity index in MT-4 cells and in CEM cells for selected compounds with HIV-1 RT kit assay for compounds, **1**–**15**



Compound	R_1	R_2	X	R_3		Anti-HIV-1 activity						% HIV-RT inhibition at 100 μg/ml
						EC_{50}[a](μM)		CC_{50}[b](μM)		SI[c]		
				2′	6′	$HIV\text{-}1_{IIIB}$MT-4	$HIV\text{-}1_{IIIB}$CEM	MT-4 cells	CEM cells	MT-4 cells	CEM cells	
1	H	H	C	Cl	Cl	1.51 ± 1.51	0.41 ± 0.06	>377.37	>377.37	>251	>893	78.56
2	H	H	C	Cl	F	0.76 ± 0.35	0.60 ± 0.35	44.16 ± 2.00	44.16 ± 2.00	58	72	89.80
3	H	H	C	F	F	1.34 ± 0.20	1.17 ± 0.30	>419	>419	>313	>358	94.50
4	Me	H	C	Cl	Cl	1.51 ± 0.23	1.85 ± 0.64	249.31 ± 32.99	249.31 ± 32.99	167	135	53.30
5	Me	H	C	Cl	F	1.12 ± 0.46	1.16 ± 0.34	162.56 ± 144.67	162.56 ± 144.67	143	140	69.60
6	Me	H	C	F	F	3.20 ± 1.44	2.72 ± 1.31	>400.18	>400.18	>125	>147	81.00
7	Ph	H	C	Cl	Cl	>125.20	NT[d]	126.26	NT[d]	<1	NT[d]	60.08
8	Me	Me	C	Cl	Cl	2.67 ± 2.37	2.23 ± 1.84	>347.90	>347.90	131	>157	39.10
9	Me	Me	C	Cl	F	0.26 ± 0.03	0.23 ± 0.06	31.01 ± 15.11	32.73 ± 13.65	113	138	81.59
10	Me	Me	C	F	F	0.46 ± 0.15	0.37 ± 0.18	56.37	>382.98	123	>1001	87.88
11	—	Et	N	Cl	Cl	>53.57	NT[d]	60.59 ± 6.94	NT[d]	<1	NT[d]	26.30
12	—	Et	N	Cl	F	>18.88	NT[d]	18.88 ± 11.84	NT[d]	<1	NT[d]	18.50
13	—	Et	N	F	F	>51.01	NT[d]	156.49 ± 78.23	NT[d]	<1	NT[d]	16.70
14	Benzthiazol-2-yl			Cl	Cl	>327.83	NT[d]	>327.83	NT[d]	<1	NT[d]	19.90
15	Benzthiazol-2-yl			Cl	F	25.73 ± 19.13	NT[d]	213.51 ± 86.75	NT[d]	12	NT[d]	45.06
16	Compound **16**					0.030 ± 0.013	0.030 ± 0.002	32.0 ± 0.54	⩾269.4	1066	⩾8980	NT[d]
	TBZ					0.35 ± 0.14	1.10 ± 0.32	19.20 ± 2.80	50.0 ± 3.2	54.5	45	NT[d]

[a] EC_{50} is the 50% effective concentration required to reduce HIV-1 induced cytopathic effect of $HIV\text{-}1_{IIIB}$ in MT-4 cells and $HIV\text{-}1_{RF}$ in CEM cells.
[b] The CC_{50} is the 50% cytotoxic concentration for MT-4 cell and mock-infected CEM cells.
[c] Selectivity index ratio CC_{50}/EC_{50}.
[d] Not tested. All data represent mean values for at least two separate experiments.

was determined. The 50% effective concentration EC_{50} was defined as the concentrations of compound required for reducing the number of cell viability in MT-4 cells. The cytotoxic concentration CC_{50} was determined as the concentrations of compound required to inhibit by 50% the number of viable cells in mock-infected MT-4 and CEM cell cultures.

2.3. Biological activity

All compounds (**1**–**15**) were initially analyzed for anti-RT activity by HIV-RT kit (Roche). The compounds showed varied inhibition activity against RT in vitro. Further, the compounds (**1**–**15**) and TBZ were evaluated for anti-HIV-1 activity by determining their ability to inhibit the HIV-1_{IIIB} or HIV-2_{ROD} reverse transcriptase enzyme in MT-4 and CEM cells (Table 1). Those compounds showing inhibitory activity against RT in the kit also showed anti-HIV-1 activity in MT-4 and CEM cells with few exceptions (Table 1). The results obtained show that our approach has led to develop a highly potent anti-HIV-1 compound (**9**), which is active at a concentration of 0.26 μM compared to 0.35 μM of TBZ in MT-4 cells. The increase in activity of compound **9** than TBZ could be because it can adopt more flexible conformation, which enables an appropriate binding with the non-nucleoside reverse transcriptase inhibitory binding pocket. Second, compound **9** is more potent within the series because of additional lipophilic character at the thiazol-2-yl moiety in terms of 4,5-dimethyl substitution. Interestingly, other compounds of the series **1**–**6** and **8**–**10** in MT-4 cells were less active compared to TBZ but were having a high selective index (Table 1). Similarly, compounds **1**, **2**, **9**, and **10** were active at 0.41, 0.60, 0.23, and 0.37 μM concentration which were 2.68, 1.80, 4.78, and 2.97 times, respectively, more active than TBZ in CEM cells. Other compounds **3**–**8** were less active compared to TBZ but have demonstrated high selective index (Table 1). The compounds that were less toxic and having high selective index compared to TBZ clearly suggest that these compounds could be used at high concentration for conferring high anti-HIV activity.

From the biological activity data reported in Table 1, it may be inferred that the anti-HIV activity is strongly dependent on the nature of the substituent at C-2 and N-3 of the thiazolidinone ring. In particular, a high activity level was observed for compounds possessing a 2,6-dihalophenyl group at C-2 and a pyridine-2-yl or pyrimidine-2-yl ring at N-3. As suggested by molecular modeling studies, the introduction of a lipophilic substituent in the hetero (aromatic) ring led to a substantial increase in the antiviral activity. In the present study, the anti-HIV activity was enhanced by introducing a substituted and unsubstituted 2-thiazolyl moieties at the N-3 position by keeping an intact 2,6-dihalo-phenyl substituent at C-2 of the 4-thiazolidinone ring. In fact, the compounds with the best combination of high potency and low toxicity were unsubstituted or 4,5-dimethyl substituted thiazol-2-yl derivatives, such as **1**, **2**, **9**, and **10**. The effect of halogen substituent on the phenyl ring at C-2, was apparent in compounds **2**, **5**, and **9**. These compounds were more active than the corresponding 2,6-dichloro substituted (**1**, **4**, and **8**) and 2,6-difluoro substituted (**3**,**6**, and **10**) compounds. Further the favorable effect of 2-chloro-6-fluoro was confirmed by the finding that 2,6-difluoro derivative (**3**, **6**, and **10**) possessed intermediate activity between 2,6-dichloro and 2-chloro-6-fluoro analogues. On the other hand, the introduction of the 5-ethyl-[1,3,4]-thiadiazol-2-yl (**11**–**13**) at N-3 led to a substantial decrease in the activity than the thiazol-2yl and benzthiazol-2-yl moiety-containing compounds. Although present series of compounds are less active than 3-(6-bromopyridin-2-yl)-2-(2,6-difluorophenyl)-1,3-thiazolidin-4-one (compound **16** in Table 1)[6] it may be appropriate to mention that thiazolidinone scaffold can accommodate heteroaryl moiety other than pyridine moiety and with further optimization it may be possible to obtain compounds with improved activity.

3. Conclusions

The introduction of a thiazol-2-yl moiety (with or without substitution) at the N-3 position of 4-thiazolidinone scaffold has led to an increase in the anti-HIV-1 RT activity and, in some compounds, activity is higher than for the lead molecule TBZ. In this series, compound **9** was found to be the most promising with an EC_{50} of 0.26 and 0.23 μM, and selectivity index of 113 and 138 in MT-4 cells and CEM cells, respectively. Other compounds in this series namely, **1**, **2**, and **10** were more active than the lead compound TBZ in CEM cells with higher selectivity indices. Taken together these results indicate that changes at N-3 position of 4-thiazolidinone scaffolds with different heterocyclic moiety, with an appropriate lipophilic character, may provide compounds with improved activity. It may be noted that substitution at N-3 position of 4-thiazolidinone skeletons deserves further consideration toward optimizing anti-HIV1 RT activity.

4. Experimental

Melting points (mp) were determined on a Complab melting point apparatus and are uncorrected. The C, H, and N analyses were carried out on CARLO-ERBA EA1108 elemental analyser. Infrared (IR) spectra were recorded on an FT-IR Perkin-Elmer (model) spectrometer. The ^1H spectra were recorded on a DPX-200 and DPX-300 Bruker FT-NMR spectrometer. The chemical shifts are reported as parts per million (δ ppm) from $(CH_3)_4Si$ (TMS) as an internal standard. The ^{13}C NMR spectra were recorded on a DPX-300 Bruker FT-NMR (75 MHz) spectrometer. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive) technique. Column chromatography separations were obtained on silica gel (230–400 mesh).

4.1. General synthetic procedure for compounds 1–15

The synthesis of compounds **1**–**15** was performed according to the previously reported procedure.[10,14] The appropriate (hetero)aromatic amine (1.0 mmol)

and 2,6-dihalo-substituted benzaldehyde (1.2 mmol) were stirred in dry toluene under reflux condition followed by addition of mercapto acid (2.0 mmol). The reaction mixture was refluxed under stirring for an additional 24–48 h untill the complete consumption of (hetero) aromatic amine. The reaction mixture was concentrated to dryness under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was successively washed with 5% aq citric acid, water, 5% aq sodium hydrogen carbonate, and then finally with brine. The organic layer was dried over sodium sulfate and solvent was removed under reduced pressure to get a crude product that was purified by column chromatography on silica gel using hexane–ethyl acetate as eluent. The structures of all synthesized compounds were characterized by TLC, IR, FAB-MS, ^{1}H NMR, and ^{13}C NMR.

4.2. 2′-(2,6-Dichloro-phenyl)-[2,3′]bithiazolyl-4′-one (1)

This compound was obtained as solid in 70% yield, mp 183–185 °C IR (KBr): ν_{max} C=O 1693 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.93 (d, J = 16.02 Hz, 1H, 5-H_A), 4.16 (dd, J = 1.56 and 16.02 Hz, 1H, 5-H_B), 6.97 (d, J = 3.54 Hz, 1H, H-2), 7.10–7.20 (m, 2H, H_3 and H_5-Ph), 7.32–7.41 (m, 3H, H_4-Ph and H'_4 and H'_5-thiazole); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.35, 155.21, 136.50, 134.19, 132.82, 131.38, 129.12, 128.15, 127.52, 112.82, 58.34, 33.32; FAB-MS: m/z 331 [M]$^+$ and 333 [M+2]. Anal. Calcd for $C_{12}H_8Cl_2N_2OS_2$: C, 43.51; H, 2.43; N, 8.46. Found: C, 43.21; H, 2.11; N, 8.08.

4.3. 2′-(2-Chloro-6-fluoro-phenyl)-[2,3′]bithiazolyl-4′-one (2)

This compound was obtained as solid in 65% yield, mp 155–157 °C IR (KBr): ν_{max} C=O 1694 cm$_{-1}$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.81 (dd, J = 3.11 and 16.01 Hz, 1H, 5-H_A), 4.15 (d, J = 15.99 Hz, 1H, 5-H_B), 6.97 (d, J = 3.52 Hz, 1H, H-2),7.14–7.37 (m, 5H, Ar-H); FAB-MS: m/z 315 [M+1]$^+$.

4.4. 2′-(2,6-Difluoro-phenyl)-[2,3′]bithiazolyl-4′-one (3)

This compound was obtained as solid in 58% yield, mp 145–148 °C IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.83 (d, J = 16.14 Hz, 1H, 5-H_A), 4.21 (d, J = 16.05 Hz, 1H, 5-H_B), 6.82 (s, 1H, H-2), 7.86–7.39 (m, 5H, Ar-H); FAB-MS: m/z 299 [M+1]$^+$.

4.5. 2′-(2,6-Dichloro-phenyl)-4-methyl-[2,3′]bithiazolyl-4′-one (4)

This compound was obtained as solid in 68% yield, mp 168–170 °C IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 1H, CH_3), 3.93 (d, J = 16.02 Hz, 1H, 5-H_A), 4.14 (dd, J = 2.04 and 16.02 Hz, 1H, 5-H_B), 6.51 (s, 1H, H'_5-thiazole), 7.09–7.20 (m, 2H, H-2 and H_4-Ph), 7.33–7.40 (m, 2H, H_3 and H_5-Ph); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.14, 154.06, 146.25, 134.61, 132.84, 131.73, 129.05, 128.07, 127.27, 107.28, 58.22, 33.45, 15.70; FAB-MS: m/z 345 [M]$^+$ and 347 [M+2]. Anal. Calcd for $C_{12}H_8ClFN_2OS_2$: C, 45.22; H, 2.92; N, 8.11. Found: C, 45.11; H, 2.76; N, 8.01.

4.6. 2′-(2-Chloro-6-fluoro-phenyl)-4-methyl-[2,3′]bithiazolyl-4′-one (5)

This compound was obtained as solid in 62% yield, mp 130–132 °C IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 1H, CH_3), 3.83 (dd, J = 3.33 and 15.96 Hz, 1H, 5-H_A), 4.18 (dd, J = 1.38 and 15.96 Hz, 1H, 5-H_B), 6.52 (s, 1H, H'_5-thiazole), 6.89 (m, 1H, H4-Ph), 7.15 (s, 1H, H-2), 7.18–7.24 (m, 2H, H_3 and H_5-Ph); FAB-MS: m/z 329 [M]$^+$.

4.7. 2′-(2,6-Difluoro-phenyl)-4-methyl-[2,3′]bithiazolyl-4′-one (6)

This compound was obtained as solid in 54% yield, mp 145–148 °C IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.20 (s, 3H, CH_3), 3.80 (dd, J = 2.00 and 15.98 Hz, 1H, 5-H_A), 4.18 (d, J = 16.02 Hz, 1H, 5-H_B), 6.51 (s, 1H, H_5-thiazole), 6.81 (t, 1H, H_4-Ph), 6.97 (s, 1H, H-2), 7.17–7.22 (m, 2H, H_3-Ph and H_5-Ph); FAB-MS: m/z 313 [M+1]$^+$.

4.8. 2′-(2,6-Dichloro-phenyl)-4-phenyl-[2,3′]bithiazolyl-4′-one (7)

This compound was obtained as solid in 40% yield, IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.80 (d, 16.08, 1H, 5-H_A), 4.18 (d, J = 16.02 Hz, 1H, 5-H_B), 7.16 (s, 1H, H_5-thiazole), 7.18–7.67 (m, 9H, H-2 and Ar-H);FAB-MS: m/z 313 [M+1]$^+$.

4.9. 2′-(2,6-Dichloro-phenyl)-4,5-dimethyl-[2,3′]bithiazolyl-4′-one (8)

This compound was obtained as solid in 65% yield, mp above 210 °C IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, CH_3 at C_5-thiazole), 2.23 (s, 3H, CH_3 at C_4-thiazole), 3.91 (d, J = 15.99 Hz, 1H, 5-H_A), 4.10 (dd, J = 2.10 and 15.93 Hz, 1H, 5-H_B), 7.10 (m, 2H, H-2 and H_4-Ph), 7.33–7.38 (m, 2H, H_3-Ph and H_5-Ph); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.85, 150.41, 141.24, 134.59, 132.96, 131.76, 129.04, 127.99, 127.23, 119.50, 57.98, 33.46, 13.08, 9.46; FAB-MS: m/z 359 [M]$^+$ and 361 [M+2]. Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS_2$: C, 46.80; H, 3.37; N, 7.80. Found: C, 46.99; H, 3.20; N, 7.56.

4.10. 2′-(2-Chloro-6-fluoro-phenyl)-4,5-dimethyl-[2,3′]-bithiazolyl-4′-one (9)

This compound was obtained as solid in 60% yield, mp 159–161 °C IR (KBr): ν_{max} C=O 1692 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H, CH_3 at C_5-thiazole), 2.24 (s, 3H, CH_3 at C_4-thiazole), 3.81 (dd, J = 3.36 and 15.93 Hz, 1H, 5-H_A), 4.15 (dd, J = 1.50 and 15.93 Hz, 1H, 5-H_B), 6.88 (m, 1H, H_4-Ph), 7.11 (s, 1H, H-2), 7.14–7.22 (m, 1H, H_3 and H_5-Ph); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.62, 150.64, 141.29, 133.18, 128.35, 125.38, 124.30, 119.44, 114.15, 113.84, 56.44,

33.47, 13.12, 9.46; FAB-MS: *m/z* 343$[M]^+$. Anal. Calcd for $C_{14}H_{12}ClFN_2OS_2$: C, 49.05; H, 3.53; N, 8.17. Found: C, 49.09; H, 3.36; N, 7.94.

4.11. 2′-(2,6-Difluoro-phenyl)-4,5-dimethyl-[2,3′]bithiazolyl-4′-one (10)

This compound was obtained as solid in 52% yield, mp 158–160 °C IR (KBr): ν_{max} C=O 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.07 (s, 3H, CH_3 at C_5-thiazole), 2.22 (s, 3H, CH_3 at C_4-thiazole), 3.78 (dd, J = 2.02 and 15.92 Hz, 1H, 5-H_A), 4.16 (d, J = 15.90 Hz, 1H, 5-H_B), 6.80 (t, 1H, H_4-Ph), 6.93 (d, J = 1.2 Hz, 1H, H-2), 7.13–7.25 (m, 2H, J = 2.08 Hz, H_3-Ph and H_5-Ph); FAB-MS: *m/z* 327 $[M+1]^+$. Anal. Calcd for $C_{14}H_{12}F_2N_2OS_2$: C, 51.52; H, 3.71; N, 8.58. Found: C, 51.50; H, 3.59; N, 8.36.

4.12. 2-(2,6-Dichloro-phenyl)-3-(5-ethyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-4-one (11)

This compound was obtained as solid in 58% yield, mp 162–165 °C IR (KBr): ν_{max} C=O 1693 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, 3H, CH_3), 2.94 (q, 2H, CH_2), 3.91 (d, J = 16.29 Hz, 1H, 5-H_A), 4.14 (dd, J = 1.84 and 16.26 Hz, 1H, 5-H_B), 7.09 (t, 1H, H_4-Ph), 7.33 (d, J = 2.21 Hz, 1H, H-2), 7.37–7.43 (m, 2H, H_3-Ph and H_5-Ph); FAB-MS: *m/z* 360 $[M]^+$ and 362 [M+2]. Anal. Calcd for $C_{12}H_8ClFN_2OS_2$: C, 43.51; H, 2.43; N, 8.46. Found: C, 43.33; H, 2.40; N, 8.26.

4.13. 2-(2-Chloro-6-fluoro-phenyl)-3-(5-ethyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-4-one (12)

This compound was obtained as solid in 53% yield, IR (KBr): ν_{max} C=O 1693 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.34 (t, 3H, CH_3), 2.96 (q, 2H, CH_2), 3.84 (d, J = 16.14 Hz, 1H, 5-H_A), 4.15 (d, J = 16.01 Hz, 1H, 5-H_B), 6.85 (t, 1H, H_4-Ph), 7.10 (s, 1H, H-2), 7.17–7.21 (m, 2H, H_3-Ph and H_5-Ph); FAB-MS: *m/z* 344 $[M]^+$.

4.14. 2-(2,6-Difluoro-phenyl)-3-(5-ethyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-4-one (13)

This compound was obtained as solid in 44% yield, mp 110–114 °C IR (KBr): ν_{max} C=O 1705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, 3H, CH_3), 2.98 (q, 2H, CH_2), 3.86 (d, J = 16.23 Hz, 1H, 5-H_A), 4.21 (d, J = 16.26 Hz, 1H, 5-H_B), 6.85 (t, 2H, H_3-Ph and H_5-Ph), 7.01 (s, 1H, H-2), 7.19–7.27 (m, 1H, H_4-Ph); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.98, 166.42, 160.92, 157.60, 155.53, 128.88, 115.06, 110.93, 110.60, 52.37, 32.00, 22.20, 12.42; FAB-MS: *m/z* 328 $[M]^+$.

4.15. 3-Bezothiazol-2-yl-2-(2,6-dichloro-phenyl)-thiazolidin-4-one (14)

This compound was obtained as white solid in 49% yield, mp >220 °C; IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.96 (dd, J = 16.22 Hz, 1H, 5-H_A), 4.19 (dd, J = 1.92 and 16.24 Hz, 1H, 5-H_B), 7.12–7.42 (m, 5H, H_3, H_4 and H_5-Ph and H'_5 and H'_6-benzthiazole), 7.52 (d, J = 1.8 Hz, 1H, H-2), 7.67 (d, J = 8.37 Hz, 1H, H'_4-benzthiazole), 7.77 (d, J = 8.38 Hz, 1H, H'_5-benzthiazole); FAB-MS: *m/z* 381 $[M]^+$ and 383 [M+2]. Anal. Calcd for $C_{16}H_{10}Cl_2N_2OS_2$: C, 50.40; H, 2.64; N, 7.35; S, 16.82. Found: C, 50.03; H, 2.50; N, 7.06; S, 16.88.

4.16. 3-Bezothiazol-2-yl-2-(2-chloro-6-fluoro-phenyl)-thiazolidin-4-one (15)

This compound was obtained as white solid in 41% yield, mp 160–162 °C; IR (KBr): ν_{max} C=O 1709 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.93 (dd, J = 2.79 and 16.21 Hz, 1H, 5-H_A), 4.20 (d, J = 16.21 Hz H_Z, 1H, 5-H_B), 7.15–7.33 (m, 5H, H_3, H_4 and H_5-Ph and H'_5 and H'_6-benzthiazole), 7.36 (s, 1H, H-2), 7.67 (d, J = 8.37 Hz, 1H, H'_4-benzthiazole), 7.67 (d, J = 8.38 Hz, 1H, H'_5-benzthiazole); FAB-MS: *m/z* 365 $[M+H]^+$. Anal. Calcd for $C_{16}H_{10}ClFN_2OS_2$: C, 52.67; H, 2.76; N, 7.68; S, 17.58. Found: C, 52.84; H, 2.56; N, 7.58; S, 17.92.

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