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Design and Synthesis of 2-(2,6-Dibromo-phenyl)-3-heteroaryl-1,3-thiazolidin-4-ones as Anti-HIV agents

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Abstract— A series of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-ones were designed, synthesized and evaluated as selective HIV-1 RT enzyme inhibitors. The results of the HIV-1 RT kit based assay and MT-4 cell tests showed that eight compounds effectively inhibited human immunodeficiency virus type-1 (HIV-1) replication at 20-320 nM concentrations with minimal cytotoxicity in MT-4 as well as in CEM cells.

Key Words-4-thiazolidinones, Anti-HIV activity, HIV-1RT, NNRTIs

1. Introduction

4-Thiazolidinone scaffold has been gaining prominence due to the fact that its derivatives have been known to possess wide spectrum of activities like antibacterial,[1] antifungal,[2] anticonvulsant,[3] COX-1 inhibitor,[4] antituberculosis,[5] antihistaminic[6] and anticancer.[7] Furthermore, Berreca et al. and our group have reported 2,3-diaryl-1,3-thiazolidin-4-one scaffold as human immunodeficiency virus-1 (HIV-1) nonnucleoside reverse transcriptase inhibitor (NNRTI).[8-14] Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are selective inhibitors of reverse transcriptase enzyme (RT) of HIV-1, which do not inhibit HIV-2 or human cellular DNA polymerases.[15,17] NNRTIs are indicated for the treatment of HIV/AIDS in combination with other antiretroviral agents.

It is inferred from the previous literature[8-14] that the anti-HIV activity of 2,3-diaryl-1,3-thiazolidin-4-one derivatives is strongly dependent on the nature of the substituent at C-2 and N-3 of the thiazolidin-4-one ring. In particular, a high activity level was observed for compounds possessing a 2,6-dihalophenyl group at C-2 and heteroaryl with an appropriate substitution at N-3. In majority of the compounds, the 2,6-dichloro-phenyl substituted derivatives at C-2 are more active than the corresponding 2,6-difluoro-phenyl substituted ones, and the favourable effect of 2,6-dichloro derivative is confirmed by the finding that 2-chloro-6-fluoro-phenyl derivatives possessed intermediate activity between 2,6-dichloro and 2,6-difluoro analogues.[18-21] Based on this data it was surmised that there is a positive correlation between size of the halogen substituent and HIV-RT inhibitory activity and by extension of this logic it seemed appropriate to explore 2,6-dibromo-phenyl substituted 4-thiazolidinone for HIV-RT inhibitory activity. In the light of the above mentioned findings and as continuation of our effort to identify new candidate that may be of value in designing potent, selective and less toxic anti-HIV agents, we report

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in the present work the synthesis and anti-HIV activity of some new 2-(2,6-dibromophenyl)-3-aryl-1,3-thiazolidin-4-one derivatives (Fig. 1).

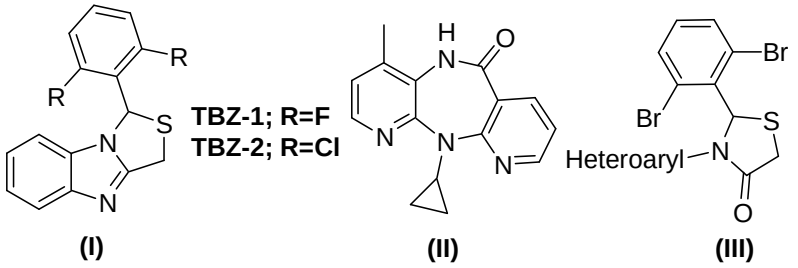


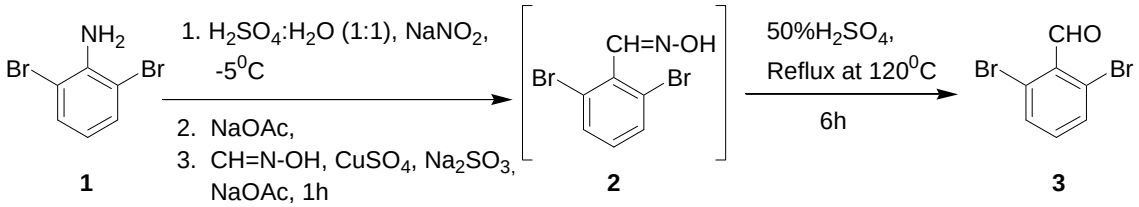
Fig. 1. Structure of TBZ's (I), Nevirapine (II) and 2-(2,6-Dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-one.

2. Results and Discussion

2.1. Methods

2.1.1. Chemistry:

2,6-dibromobenzaldehyde (**3**) was synthesized following the literature procedure[22] as shown in scheme 1. In the first step 2,6-dibromoaniline (**1**) was diazotized by using H_2SO_4 and $NaNO_2$ at -5°C then the cold solution of diazonium salt was neutralized by NaOAc and treated with formaldoxime solution followed by reflux in 50% H_2SO_4 in water to obtain the aldehyde **(3)**. The heteroaromatic amines not commercially available namely, 4-methy-6-trifluoromethylpyrimidin-2-ylamine (**4l**), 4-methyl-6-phenylpyrimidin-2-ylamine (**4m**) and 4,5,6-trimethylpyrimidin-2-ylamine (**4n**) were synthesized in good yields by refluxing free base guanidine solution in ethanol with corresponding 1,3-dicarbonyl compounds according to the reported procedure.[23] Whereas the synthesis of 4-amino-2,6-dimethyl pyrimidine was carried out by heating the mixture of acetonitrile and NaOMe at 150 °C in a glass sealed tube.[24] The synthesis of new series of 2-(2,6-dibromophenyl)-3-(heteroaryl-2-yl)-1,3-thiazolidin-4-ones (**6a-o**) was carried out according to the reported procedure,[9] by reacting a suitably substituted heteroaromatic amine (**4a-o**) with 2,6-dibromobenzaldehyde (**3**) in the presence of an excess of mercaptoacetic acid (**5**) in reflux toluene for 24 h (Scheme 2). After completion of the reaction, desired final products were obtained by conventional work-up procedure followed by flash column chromatography purification on slica-gel 230-400 mesh size in moderate to good yields(**6a-o**). All the synthesized compounds were well characterized by spectroscopic methods and elemental analysis.



Scheme 1. Preparation of 2,6-dibromobenzaldehyde.

Scheme 2. Synthesis of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-ones **6a-o**.

2.1.2. Anti-HIV activity Evaluation

2.1.2.1. *In vitro* HIV-RT kit assay[25]

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, 2'-deoxy-nucleotide-5'-triphosphates (dNTP's) and reverse transcriptase (RT) enzyme in the lysis buffer with or without inhibitors. After 1 hr 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-digoxigenin-peroxidase (DIG - POD) was added in MTP. The DIG-labeled dNTPs incorporated in the template was 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 catalyses 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

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

2.1.2.2. *In vitro* Anti-HIV assay

The methodology of the anti-HIV assays has been previously described.[26] 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 were 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%.[27] After 5 days of incubation of MT-4 cells, the number of viable cells was determined. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}) in MT-4 cells. The cytotoxic concentration CC_{50} were determined as the concentrations of compound to inhibit by 50% the number of viable cells in mock-infected MT-4 and CEM cell cultures.

2.2. Biological Activity

All Compounds **4a-o** were evaluated for HIV-1 RT inhibitory activity by determining their percentage inhibition of HIV-1 RT activity in HIV-1 RT kit.[25] Further,

the compounds **4a-o,** TBZ's and nevirapine were evaluated for anti-HIV activity by determining their ability to inhibit the replication of HIV-1_{IIIB} and HIV-2_{ROD} reverse transcriptase enzyme in acutely infected MT-4 and CEM cell lines and the data are shown in Tables 1 and 2 respectively. The in-vitro anti-HIV activities in MT-4 and CEM cell lines were also in agreement to the HIV-1 RT inhibition assay which was performed by using RT assay kit. Those compounds showing inhibitory activity against RT in HIV-1 RT kit also show anti-HIV-1 activity in both cell lines (Table 1 & 2). Compound induced cytotoxicity was also measured in MT-4 and CEM cells in parallel with the HIV-RT inhibitory activity[26-27] (Table 1 & 2).

Table 1. Anti-HIV-1 activity, cytotoxicity and selectivity index in MT-4 cells and HIV-1 RT kit assay for compounds **6a-o**.

Comp.	%Yield	Anti-HIV-1 activity			*In vitro* % inhibition (HIV-RT kit assay) 100 µg/ml
		EC_{50}[a] (µM)	CC_{50}[b] (µM)	SI[c]	
6a	62	0.50±0.14	108.56±120.20	218	69.50
6b	64	0.58±0.21	34.63±0.37	59	84.30
6c	58	0.21±0.06	36.39±1.65	171	99.00
6d	56	0.84±0.02	173.61±10.42	209	97.60
6e	50	0.22±0.02	13.06±8.11	60	85.20
6f	48	0.09±0.06	233.22±19.53	2663	79.50
6g	49	4.56±0.14	29.97±4.00	7	23.87
6h	37	1.03±0.41	97.98±25.22	96	61.36
6i	68	0.24±0.02	172.13±28.36	687	100
6j	61	0.04±0.00	33.86±8.18	757	100
6k	42	0.02±0.01	184.76±42.22	7123	100
6l	36	0.32±0.02	32.59±1.53	99	85.37
6m	28	>8.65	183.78±13.16	21	17.75
6n	25	0.06±0.00	227.48	3721	100
6o	59	5.21±1.35	33.35±1.80	6	66.36
TBZ-1		0.35±0.14	19.20±2.80	54.50	-
TBZ-2		0.60±0.09	9.40±6.50	16	-
Nevirapine		0.05	>50.00	>1000	100

[a], Concentration required to reduce HIV-1 induced cytopathic effect by 50% in MT-4 cells.
[b], Concentration required to reduce MT-4 cell viability by 50%.
[c], Selectivity index ratio CC_{50}/EC_{50}.

Table 2. Anti-HIV-1 activity, cytotoxicity and selectivity index in CEM cells for selected compounds.

C.No.	Anti-HIV-1 Activity		
	EC_{50}[a] (µM)	CC_{50}[b] (µM)	SI[c]
6a	0.56±0.14	108.56±120.20	194
6b	0.60±0.14	35.60±2.19	59
6c	0.18±0.07	36.63±1.53	200
6d	0.63±0.35	117.46±12.50	283
6e	0.27±0.07	17.07±11.37	66
6f	0.08±0.04	233.22±19.53	2954
6g	3.96±1.06	29.79±3.48	8
6h	1.83±1.44	97.98±25.22	53
6i	0.23±0.04	178.70±28.62	769
6j	0.04±0.01	36.26±8.90	930
6k	0.03±0.01	186.12±36.69	6726
6l	0.36±0.08	59.30±59.74	161
6n	0.06±0.01	>273.42	>5020
6o	4.67±1.31	33.53±1.62	7
TBZ-1	1.10±0.32	50.00±3.20	45
Nevirapine	0.01±0.01	>4.00	>370

[a], Concentration required to reduce HIV-1 induced cytopathic effect by 50% in CEM cells.
[b], Concentration required to reduce CEM cell viability by 50%.
[c], Selectivity index ratio CC_{50}/EC_{50}.

The results of the present study show that our approach has led to the development of potent anti-HIV agents, up to 14 folds more active than the corresponding TBZ lead compound. This is in conformity with the earlier observation namely, nature of the substituent at C-2 and N-3 of the thiazolidin-4-one ring and butterfly conformation collectively facilitate better interaction with the allosteric binding pocket residues of HIV-1 RT enzyme. This has a direct bearing on the anti-HIV activity.

Structure activity relationship studies (SARs) suggest that the anti-HIV activity of this series was strongly enhanced by introducing different heteroaryl moiety with /or without an appropriate lipophilic substitution at the N-3 atom of the thiazolidinone ring and in particular by 2,6-dibromophenyl ring at C-2. Compounds **6f, 6j, 6k & 6n** prevented the cytopathic effect of HIV-1 IIIB with an EC_{50} value ranging from 20 to 90nM and were minimally toxic to MT-4 cells resulting in remarkably high selectivity indices up to 7123. It was worth noting that eight compounds (**6c, 6e, 6f, 6i-6l & 6n**) of this series were found most active from 1.1 to 17.5 fold as compared to the lead compound TBZ-1 in MT-4 cells. Similarly, these eight compounds exhibited the most promising activity at nanomolar concentrations in CEM cells. Whereas compounds (**6a-6c, 6e-6f, 6i-6l & 6n**) were found most active from 1.04 to 30 fold as compared to the lead compound TBZ-2 in

MT-4 cells. Compound **6j** and **6k** were found 1.25 to 2.5 fold more active than nevirapine in MT-4 cells. While none of the compounds were more effective than nevirapine but the compounds **6f**, **6i-6k** and **6n** were having more selectivity indicies as compared to nevirapine in CEM cell line. As observed for other classes of NNRTIs, none of the compounds inhibited the replication of HIV-2 (ROD) in MT-4 as well as in CEM cells at subtoxic concentrations.

3. Conclusion

In conclusion, novel 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-one series were synthesized and characterized. Eight compounds were found to be the most promising of this series and proved to be potent anti-HIV-1 agents, inhibiting virus replication at 20-320 nM concentrations with minimal cytotoxicity and high selectivity index up to 7123 in MT-4 and 6726 in CEM cells respectively. Furthermore these compounds were found to be 1-17.5 folds more active in MT-4 cells and 1.7 to 36.7 fold more effective in CEM cells than TBZ-1 respectively. This study suggest that only possible position for modulating anti-HIV activity in the case of 4-thiazolidinone skeleton is N-3 position of this privileged scaffold. It may be inferred that 4-thiazolidinone holds promise for further activity optimization studies.

4. Experimental

Melting points (mp) were determined on a Complab melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on an FT-IR Perkin-Elmer 621 spectrometer. The ^{1}H-NMR spectra were recorded on a DPX-200 and DPX-300 Bruker FT-NMR spectrometer using $CDCl_3$ as a solvent. 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 using glycerol or *m*-nitrobenzyl alcohol as matrix either by fast atom bombardment (FAB positive) or by electro spray ionization (ESI-MS) technique. Elemental analyses were carried out on CARLO-ERBA EA1108 C, H, N elemental analyzer and values were in the acceptable limits of the calculated values. Column chromatography separations were performed on silica gel (230-400 mesh).

4.1. General synthetic procedure for 2,6-dibromo-benzaldehyde (3)

2,6-dibromoaniline **1** (1 mmol) was diazotized by using H_2SO_4 and $NaNO_2$ (1.2 mmol) at -5°C then the cold solution of diazonium salt was neutralized by NaOAc and treated with catalytic amount of $CuSO_4$, $NaSO_3$ and formaldoxime (1.5 mmol) solution. The reaction mixture was stirred at room temperature for 1 hour and then stirred at 80°C for 4 hour. After 4 hours 50 ml of 50% H_2SO_4 was added and refluxed for 8 hours at 150°C. After completion of the reaction, the reaction mixture was neutralized with $NaHCO_3$ and extracted in ethylacetate. The organic layer was washed with brine and concentrated under vaccum. The crude product was purified by column chromatography. The structures of 2,6-dibromobenzaldehyde **3** was characterized by means of TLC, IR, FAB-MS, ^{1}H-NMR and ^{13}C-NMR.

4.1.1. 2,6-dibromobenzaldehyde (3)

This compound was obtained as solid in 40 % yield, mp 90-91°C; IR (KBr): ν_{max} C=O 1696 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.41 (m, 2H, H_3 & H_5-Ph), 7.49 (m, 1H, H_4-

Ph), 10.48 (s, 1H, -CHO); ^{13}C-NMR (50 MHz, $CDCl_3$): δ 189.15, 137.23, 133.99 (2C), 130.16 (2C); FAB-MS: m/z 265 $[M+1]^+$. Anal. Calcd for $C_7H_4Br_2O$: C, 31.86; H, 1.53. Found: C, 32.08; H, 1.35.

4.2. General synthetic procedure for compounds (6a-o)

The synthesis of compounds **6a-o** was performed according to the previously reported procedure.[9] The appropriate (hetero)aromatic amine (1.0 mmol) and 2,6-dibromo-benzaldehyde **3** (1.2 mmol) were stirred in dry toluene under reflux condition followed by addition of mercaptoacetic acid (2.0 mmol). The reaction mixture was refluxed under stirring for an additional 24-48 h till the complete consumption of the amine component. 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 sulphate 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 final compounds were characterized by means of TLC, IR, FAB-MS, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

4.2.1. 2'-(2,6-dibromo-phenyl)-[2,3]bithiazolyl-4'-one (6a)

This compound was obtained as solid in 62 % yield, mp 158-162°C; IR (KBr): ν_{max} C=O 1693 cm^{-1}; ^{1}H-NMR (300 Mz, $CDCl_3$): δ 3.93 (dd, *J* = 1.98 & 16.02 Hz, 1H, 5-H_A), 4.17 (dd, *J* = 2.19 & 15.96 Hz, 1H, 5-H_B), 6.98 (d, *J* = 3.42 Hz, 1H, H_5-thiazole), 7.03-7.09 (m, 1H, H_4-Ph), 7.14-7.28 (m, 1H, H_3–Ph), 7.33 (d, *J* = 3.33 Hz, 1H, H_4-thiazole), 7.39-7.63 (m, 2H, H-2 & H_5-Ph); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.39, 155.21, 136.50, 133.39, 131.54, 130.93, 129.86, 128.49, 128.14, 112.81, 61.74, 33.58; ESI-MS: m/z 421 $[M+1]^+$. Anal. Calcd for $C_{12}H_8Br_2N_2OS_2$: C, 34.30; H, 1.92; N, 6.67. Found: C, 34.28; H, 2.02; N, 6.61.

4.2.2. 2-(2,6-dibromo-phenyl)-3-thiophen-2-ylmethyl-thiazolidin-4-one (6b)

This compound was obtained as semisolid in 64 % yield, IR (Neat): ν_{max} C=O 1682 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.65 (d, *J* = 15.32 Hz, 1H, C*H*$_2$ thiophene-2yl-methyl), 3.75 (d, *J* = 14.94 Hz, 1H, H_A), 3.90 (d, *J* = 14.94 Hz, 1H, H_B), 5.13 (d, *J* = 15.32 Hz, 1H, C*H*$_2$ thiophene-2yl-methyl), 5.84 (s, 1H, H-2), 6.87-7.00 (m, 3H, H_3 & H_5-Ph and H_4-thiophene), 7.09 (d, *J* = 3.00 Hz, 1H, H_3-thiophene), 7.40 (d, *J* = 4.70 Hz, 1H, H_5-thiophene), 7.61 (t, 1H, H_4-Ph); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.87, 142.27, 134.07, 132.15, 129.79, 126.58, 126.28, 125.61, 125.42, 125.06, 124.77, 56.75, 39.78, 31.18 ;ESI-MS m/z 434 $[M+1]^+$. Anal. Calcd for $C_{14}H_{11}Br_2NOS_2$: C, 38.82; H, 2.56; N, 3.23. Found: C, 38.78; H, 2.44; N, 3.20.

4.2.3. 2-(2,6-dibromo-phenyl)-3-furan-2-ylmethyl-thiazolidin-4-one (6c)

This compound was obtained as semisolid in 58 % yield, IR (Neat): ν_{max} C=O 1684 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.57 (d, *J* = 15.64 Hz, 1H, C*H*$_2$-Furfuryl), 3.73 (d, *J* = 15.80 Hz, 1H, H_A), 3.82 (d, *J* = 15.80 Hz, 1H, H_B), 4.92 (d, *J* = 15.56 Hz, 1H, C*H*$_2$-Furfuryl), 6.10 (s, 1H, H-2), 6.26 (d, *J* = 3.08 Hz, 1H, H_3-Fu), 6.51 (m, 1H, H_4-Fu), 7.10 (m, 1H, H_4-Ph), 7.36 (d, *J* = 7.30 Hz, 1H, H_5-Fu), 7.51 (m, 2H, H_3 & H_5-Ph); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.10, 146.90, 141.63, 131.66, 130.98, 129.59, 129.28, 128.90,

127.56, 109.20, 108.41, 60.59, 38.08, 33.17; FAB-MS m/z 418 $[M+1]^+$. Anal. Calcd for $C_{14}H_{11}Br_2NO_2S$: C, 40.31; H, 2.66; N, 3.36; S, 7.69. Found: C,40.55; H,2.64; N, 3.21; S, 7.79.

4.2.4. 2-(2,6-dibromo-phenyl)-3-pyridin-2-ylmethyl-thiazolidin-4-one (6d)

This compound was obtained as white solid in 56 % yield, mp 184-187°C; IR (KBr): ν_{max} C=O 1694 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.70 (d, *J* = 15.16 Hz, 1H, CH_2-Py), 3.80 (d, *J* = 16.92 Hz, 1H, H_A), 3.90 (dd, *J* = 2.2 & 16.92 Hz, 1H, H_B), 5.11 (d, *J* = 15.16 Hz, 1H, CH_2-Py), 6.62 (d, *J* = 2.03 Hz, 1H, H-2), 7.11-7.35 (m, 5H, H_3, H_4 & H_5-Ph and H_3 & H_5-Py), 7.60 (t, 1H, H_4-Py), 8.51 (d, 1H, *J* = 3.78 Hz, H_6-Py); ESI-MS m/z 429 $[M+1]^+$. Anal. Calcd for $C_{15}H_{12}Br_2N_2OS$: C, 42.08; H, 2.83; N, 6.54; Found: C, 42.04; H, 2.74; N, 6.43.

4.2.5. 2-(2,6-dibromo-phenyl)-3-pyridin-2-yl-thiazolidin-4-one (6e)

This compound was obtained as solid in 37 % yield, mp 145-149°C; IR (KBr): ν_{max} C=O 1692 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.89 (dd, *J* = 4.90 & 15.76 Hz, 1H, 5-H_A), 4.13 (dd, *J* = 1.74 & 15.76 Hz, 1H, 5-H_B), 6.87 (d, *J* = 8.06 Hz, 1H, H_5-Py), 6.96-7.02 (m, 2H, H_3 & H_5-Ph), 7.38 (m, 1H, H_4-Ph), 7.54 (dd, *J* = 7.76 & 8.98 Hz, 1H, H_4-Py), 8.09 (d, *J* = 8.50 Hz, 1H, H_3-Py), 8.18 (d, *J* = 3.04 Hz, 1H, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.17, 149.27, 146.09, 136.31, 133.46, 131.34, 129.89, 128.63, 128.25, 119.43, 115.59, 115.28, 62.11, 34.53; ESI-MS: m/z 415 $[M+1]^+$. Anal. Calcd for $C_{14}H_{10}Br_2N_2OS$: C, 40.60; H, 2.43; N, 6.76; Found: C, 40.54; H, 2.45; N, 6.74.

4.2.6. 2-(2,6-dibromo-phenyl)-3-(6-methyl-pyridin-2-yl)-thiazolidin-4-one (6f)

This compound was obtained as solid in 48 % yield, mp 173-175°C; IR (KBr): ν_{max} C=O 1692 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3 at C_6-Py), 3.94 (d, *J* = 15.75 Hz, 1H, 5-H_A), 4.14 (dd, *J* = 2.19 & 15.75 Hz, 1H, 5-H_B), 6.83 (m, 2H, H_4-Py & H_4-Ph), 7.40 (d, 1H, *J* = 7.92 Hz, H_5-Py), 7.52 (m, 2H, H_3 & H_5-Ph), 7.64 (d, 1H, *J* = 2.79 Hz, H-2), 7.94 (dd, 1H, *J* = 3.24 & 8.28 Hz, H_3-Py); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.02, 155.21, 148.46, 136.57 (2C), 133.44, 131.14, 128.54, 125.15, 118.75 (2C), 112.09, 62.21, 34.59, 22.27; ESI-MS: m/z 429 $[M+1]^+$. Anal. Calcd for $C_{15}H_{12}Br_2N_2OS$: C, 42.08; H, 2.83; N, 6.54; Found: C, 42.01; H, 2.65; N, 6.34.

4.2.7. 2-(2,6-dibromo-phenyl)-3-(6-trifluoromethyl-pyridin-2-yl)-thiazolidin-4-one (6g)

This compound was obtained as solid in 49 % yield, mp 150-152°C; IR (KBr): ν_{max} C=O 1691 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.96 (dd, *J* = 2.70 & 16.05 Hz, 1H, 5-H_A), 4.17 (dd, *J* = 1.83 & 16.05 Hz, 1H, 5-H_B), 6.88 (m, 1H, H_5-Py), 7.14 (m, 1H, H_4-Ph), 7.34-7.69 (m, 3H, H-2 and H_3 & H_5-Ph), 7.87 (t, 1H, H_4-Py), 8.48 (t, 1H, H_3-Py); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.08, 158.00, 149.32, 137.86 (2C), 133.22, 131.44, 130.82, 129.66, 128.75, 128.02, 117.70, 115.52, 62.06, 34.26; ESI-MS: m/z 483 $[M+1]^+$.

4.2.8. 3-(5-Bromo-6-methyl-pyridin-2-yl)-2-(2,6-dibromo-phenyl)-thiazolidin-4-one (6h)

This compound was obtained as solid in 37 % yield, mp 148-152°C; IR (KBr): ν_{max} C=O 1692 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3 at C_6-Py), 3.88 (dd, *J* = 4.65 & 15.88 Hz, 1H, 5-H_A), 4.11 (dd, *J* = 1.91 & 15.88 Hz, 1H, 5-H_B), 6.87 (m, 2H, H_3 & H_5-Ph), 7.18 (m, 1H, H_4-Ph), 7.52 (d, *J* = 8.89 Hz, 1H, H_3-Py), 7.71 (d, *J* = 8.75 Hz, 1H, H_4-Py), 7.90 (s, 1H, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.07, 153.82, 147.22, 139.99,

133.56, 131.23, 128.70, 128.31, 124.98, 120.19, 115.49, 113.59, 62.04, 34.47, 22.59; ESI-MS: m/z 508 $[M+1]^+$. Anal. Calcd for $C_{15}H_{11}Br_3N_2OS$: C, 35.53; H, 2.19; N, 5.52. Found: C, 35.48; H, 2.10; N, 5.39.

4.2.9. 2-(2,6-dibromo-phenyl)-3-pyrimidin-2-yl-thiazolidin-4-one (6i)

This compound was obtained as solid in 68 % yield, mp 180-184°C; IR (KBr): ν_{max} C=O 1703 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.94 (dd, *J* = 1.56 & 15.87 Hz, 1H, 5-H_A), 4.17 (dd, *J* = 1.86 & 15.90 Hz, 1H, 5-H_B), 6.89 (t, 1H, H_4-Ph), 7.00 (m, 1H, H_5-Py), 7.01 (s, 1H, H-2), 7.44 (m, 2H, H_3 & H_5-Ph), 8.60 (d, *J* = 4.82 Hz, 2H, H_4 & H_6-Py); ESI-MS: m/z 416 $[M+1]^+$. Anal. Calcd for $C_{13}H_9Br_2N_3OS$: C, 37.61; H, 2.19; N, 10.12. Found: C, 37.44; H, 2.29; N, 9.98.

4.2.10. 2-(2,6-dibromo-phenyl)-3-(4-methyl-pyrimidin-2-yl)-thiazolidin-4-one (6j)

This compound was obtained as solid in 61 % yield, mp 170-174°C; IR (KBr): ν_{max} C=O 1718 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.93 (d, *J* = 15.83 Hz, 1H, 5-H_A), 4.15 (dd, *J* = 1.90 & 15.87 Hz, 1H, 5-H_B), 6.85-7.04 (m, 3H, H_3, H_4 & H_5-Ph), 7.43 (d, *J* = 4.82 Hz, 1H, H_5-Py), 7.50 (d, *J* = 1.78 Hz, 1H, H-2), 8.50 (d, *J* = 5.04 Hz, 1H, H_6-Py) ; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.25, 167.04, 156.43, 134.70, 133.65, 131.14, 128.74, 128.36, 125.05, 120.73, 116.08, 61.97, 34.31, 22.43; ESI-MS: m/z 430 $[M+1]^+$. Anal. Calcd for $C_{14}H_{11}Br_2N_3OS$: C, 39.18; H, 2.58; N, 9.79; S, 7.47. Found: C, 39.24; H, 2.59; N, 9.91; S, 7.62.

4.2.11. 2-(2,6-dibromo-phenyl)-3-(4,6-dimethyl-pyrimidin-2-yl)-thiazolidin-4-one (6k)

This compound was obtained as solid in 42 % yield, mp >220°C; IR (KBr): ν_{max} C=O 1717 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 6H, $2CH_3$ at C_4 & C_6-Pym), 3.91 (d, *J* = 15.73 Hz, 1H, 5-H_A), 4.14 (d, *J* = 15.73 Hz, 1H, 5-H_B), 6.72 (s, 1H, H_5-Pym), 6.91 (t, 1H, H_4-Ph), 7.42 (m, 2H, H_3 & H_5-Ph), 7.50 (s, 1H, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.15, 166.67 (2C), 154.95, 134.67, 133.62, 131.06, 128.72, 125.13, 121.05, 115.79, 62.03, 34.31, 22.46 (2C); ESI-MS: m/z 444 $[M+1]^+$. Anal. Calcd for $C_{15}H_{13}Br_2N_3OS$: C, 40.65; H, 2.96; N, 9.48; S, 7.24; Found: C, 40.85; H, 3.01; N, 9.58; S, 7.09.

4.2.12. 2-(2,6-dibromo-phenyl)-3-(4-methyl-6-trifluoromethyl-pyrimidin-2-yl)-thiazolidin-4-one (6l)

This compound was obtained as solid in 36 % yield, mp 133-137°C; IR (KBr): ν_{max} C=O 1724 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.60 (s, 3H, CH_3), 3.95 (dd, *J* = 2.40 & 15.99 Hz, 1H, 5-H_A), 4.17 (dd, *J* = 1.80 & 15.96 Hz, 1H, 5-H_B), 6.90-7.04 (m, 1H, H_3–Ph), 7.19 (s, 1H, H_5-Py), 7.37-7.49 (m, 1H, H_5-Ph), 7.50 (m, 1H, H_4-Ph), 7.57 (d, *J* = 1.08 Hz, 1H, H-2**);** ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.75, 169.00, 133.51, 131.28, 130.66, 129.92, 128.95, 128.56, 127.85, 125.27, 120.38, 111.56, 61.77, 34.00, 23.40; ESI-MS: m/z 498 $[M+1]^+$.

4.2.13. 2-(2,6-dibromo-phenyl)-3-(4-methyl-6-phenyl-pyrimidin-2-yl)-thiazolidin-4-one (6m)

This compound was obtained as solid in 28 % yield, mp 192-194°C; IR (KBr): ν_{max} C=O 1718 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.54 (s, 3H, CH_3 at C_6-Pym), 3.90 (d, *J* = 15.79 Hz, 1H, 5-H_A), 4.16 (d, *J* = 15.49 Hz, 1H, 5-H_B), 6.69 (s, 1H, H_5-Pym), 6.91 (t, 1H, H_4-Ph), 7.43 (m, 2H, H_3 & H_5-Ph), 7.87 (s, 1H, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.00, 164.47 (2C), 152.34, 134.34, 133.27, 133.10, 129.26, 127.94, 126.98, 122.79,

57.71, 34.21, 21.00 (2C), 12.34; ESI-MS: m/z 506 $[M+1]^+$. Anal. Calcd for $C_{20}H_{15}Br_2N_3OS$: C, 47.55; H, 2.99; N, 8.32; S, 6.35. Found: C, 47.77; H, 2.89; N, 8.44; S, 6.22.

4.2.14. 2-(2,6-dibromo-phenyl)-3-(4,5,6-trimethyl-pyrimidin-2-yl)-thiazolidin-4-one (6n)

This compound was obtained as solid in 25 % yield, mp >220°C; IR (KBr): ν_{max} C=O 1720 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 3H, CH_3 at C_5-pym), 2.37 (s, 6H, $2CH_3$ at C_4 & C_6-Pym), 3.90 (d, *J* = 15.69 Hz, 1H, 5-H_A), 4.14 (dd, *J* = 1.86 & 15.69 Hz, 1H, 5-H_B), 6.89 (t, 1H, H_4-Ph), 7.40-7.51 (m, 2H, H_3 & H_5-Ph), 7.54 (d, *J* = 1.17 Hz, 1H, H-2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.00, 164.44 (2C), 152.34, 134.62, 133.27, 133.10, 128.64, 127.94, 126.98, 122.79, 62.05, 34.29, 20.95 (2C), 12.31; ESI-MS: m/z 458 $[M+1]^+$. Anal. Calcd for $C_{16}H_{15}Br_2N_3OS$: C, 42.03; H, 3.31; N, 9.19; Found: C, 42.00; H, 3.16; N, 9.01.

4.2.15. 2-(2,6-dibromo-phenyl)-3-(2,6-dimethyl-pyrimidin-4-yl)-thiazolidin-4-one (6o)

This compound was obtained as solid in 59 % yield, mp 122-126°C; IR (KBr): ν_{max} C=O 1708 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3 at C_6-pym), 2.47 (s, 3H, CH_3 at C_2-pym), 3.91 (d, *J* = 16.14 Hz, 1H, 5-H_A), 4.13 (dd, *J* = 1.92 & 16.20 Hz, 1H, 5-H_B), 6.93 (m, 1H, H_4-Ph), 7.40 (s, 1H, H-2), 7.45-7.59 (m, 2H, H_3 & H_5-Ph), 7.95 (s, 1H, H_5-pym); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.05, 167.15, 165.20, 155.33, 134.31, 133.52, 131.79, 129.16, 127.97, 127.21, 105.77, 57.33, 34.46, 23.93, 23.26; ESI-MS: m/z 444 $[M+1]^+$. Anal. Calcd for $C_{15}H_{13}Br_2N_3OS$: C, 40.65; H, 2.96; N, 9.48; Found: C, 40.54; H, 2.74; N, 9.39.

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