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ORIGINAL ARTICLE

Design, synthesis, and anticancer activity of novel 4-thiazolidinone-phenylaminopyrimidine hybrids

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Abstract

4-Thiazolidinones and phenylaminopyrimidines are known as anticancer agents. Imatinib is the pioneer phenylaminopyrimidine derivative kinase inhibitor, which is used for the treatment of chronic myeloid leukemia. With a hybrid approach, a novel series of 5-benzylidene-2-arylimino-4-thiazolidinone derivatives containing phenylaminopyrimidine core were designed, synthesized, and tested for their anticancer activity on K562 (chronic myeloid leukemia), PC3 (prostat cancer), and SHSY-5Y (neuroblastoma) cells. Since superior anticancer activity was observed on K562 cells, further biological studies of selected compounds (**8**, **15**, and **34**) were performed on K562 cells. For the synthesis of designed compounds, thiourea compounds were converted to 2-imino-1,3-thiazolidin-4-ones with α-chloroacetic acid in the presence of sodium acetate. 5-Benzylidene-2-imino-1,3-thiazolidin-4-one derivatives were obtained by *Knoevenagel* condensation of 2-imino-1,3-thiazolidin-4-ones with related aldehydes. Compounds **8**, **15**, and **34** were evaluated for cell viability, apoptosis studies, cell cycle experiments, and DNA damage assays. IC_{50} values of compounds **8**, **15, and 34** were found as 5.26 ± 1.03, 3.52 ± 0.91, and 8.16 ± 1.27 µM, respectively, in K562 cells. Preferably, these compounds showed less toxicity towards L929 cells compared to imatinib. Furthermore, compounds **8** and **15** significantly induced early and late apoptosis in a time-dependent manner. Compounds **15** and **34** induced cell cycle arrest at G0/G1 phase and compound **8** caused cell cycle arrest at G2/M phase. Based on DNA damage assay, compounds **8** and **15** were found to be more genotoxic than imatinib towards K562 cells. To put more molecular insight, possible Abl inhibition mechanisms of most active compounds were predicted by molecular docking studies. In conclusion, a novel series of 5-benzylidene-2-arylimino-4-thiazolidinone derivatives and their promising anticancer activities were reported herein.

This work was presented as oral and poster presentation in 5th Bahçeşehir University Drug Design Congress on November 13–15, 2017, in İstanbul and selected as best poster presentation in mentioned congress.

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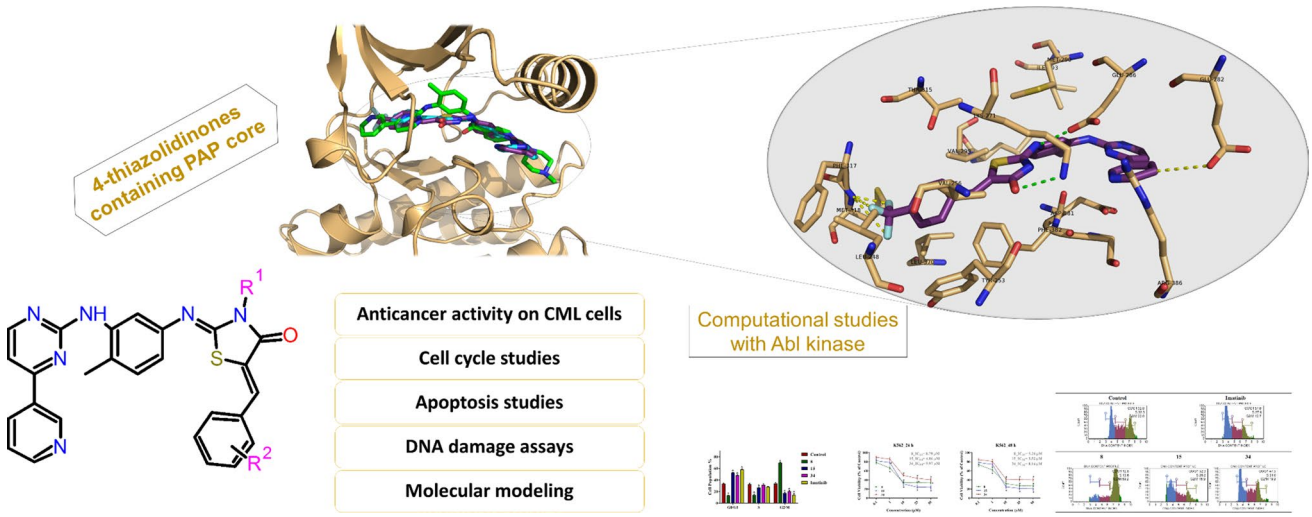
Graphic abstract



Keywords Phenylaminopyrimidines · 2-Arylimino-4-thiazolidinone derivatives · Anticancer agents · Apoptosis · Molecular docking

Abbreviations

PAP Phenylaminopyrimidine
CML Chronic myeloid leukemia

Introduction

According to the report published by the World Health Organization, being the second leading cause of death worldwide after cardiovascular diseases, cancer is responsible for 9.6 million deaths in the year of 2018. The hole between the number of people suffering from cardiovascular diseases and cancer is getting narrow year after year. Cancer represents to be a global health problem that needs continuing development of novel treatment modalities [1, 2].

The disrupted equilibrium, aberrant signalization, abnormal cellular activity, increased proliferation, and decreased apoptosis levels are well-known drivers of tumorigenesis. This biological equilibrium is largely controlled by protein kinase enzymes in cells. Kinase proteins are established as cellular switches that are regulators of cells. For the regulation of biological reactions, 518 kinase enzymes have been identified in the human genome thus far [3]. Activation of any receptor kinase or cytosolic kinase commences different cellular signalization pathways [4]. However, unusual activity or overexpression of a kinase protein may role in cancer [5–7].

Kinase proteins are featured as outstanding molecular targets for cancer treatment. Imatinib, which has phenylaminopyrimidine (PAP) core in its molecular structure, is the pioneer kinase inhibitor, approved for chronic myeloid leukemia (CML) and gastrointestinal stromal tumors [8]. In the related literature, PAP derivatives are recorded as straight nominees for anticancer drug development [9–12]. Heterocycles are one of the important classes of organic molecules. In particular, nitrogenous heterocyles have proven to be prominent drug candidates. In this context, the synthesis of nitrogenous heterocycles has attracted the attention of many researchers in the community of synthetic chemistry and medicinal chemistry [13–18]. As of mentioned heterocyclic compounds, 2-imino-4-thiazolidinones has attracted great attention due to the diversity of their biological effects [19]. 2-Imino-4-thiazolidinones were reported as potential anticancer agents [20–22] as well as kinase inhibitors [23, 24]. Based on a hybrid approach of these mentioned ideas, novel PAP derivatives containing 5-substituted benzylidene-2-imino-4-thiazolidinone were designed as anticancer agents in this study. The design approach of the molecules is presented in Fig. 1.

Anticancer activity of the compounds was evaluated on K562, PC3, and SHSY-5Y cells, initially. As the compounds demonstrated better anticancer activity towards K562 cells compared to PC3 and SHSY-5Y cells; IC_{50} calculations, apoptotic, cell cycle, and DNA damage profiles of the most active compounds were evaluated on K562 cells as further studies. Moreover, because of the link between CML and Abl kinase [25], molecular docking studies were performed with Abl kinase domain and compounds **8**, **15** and **34**.

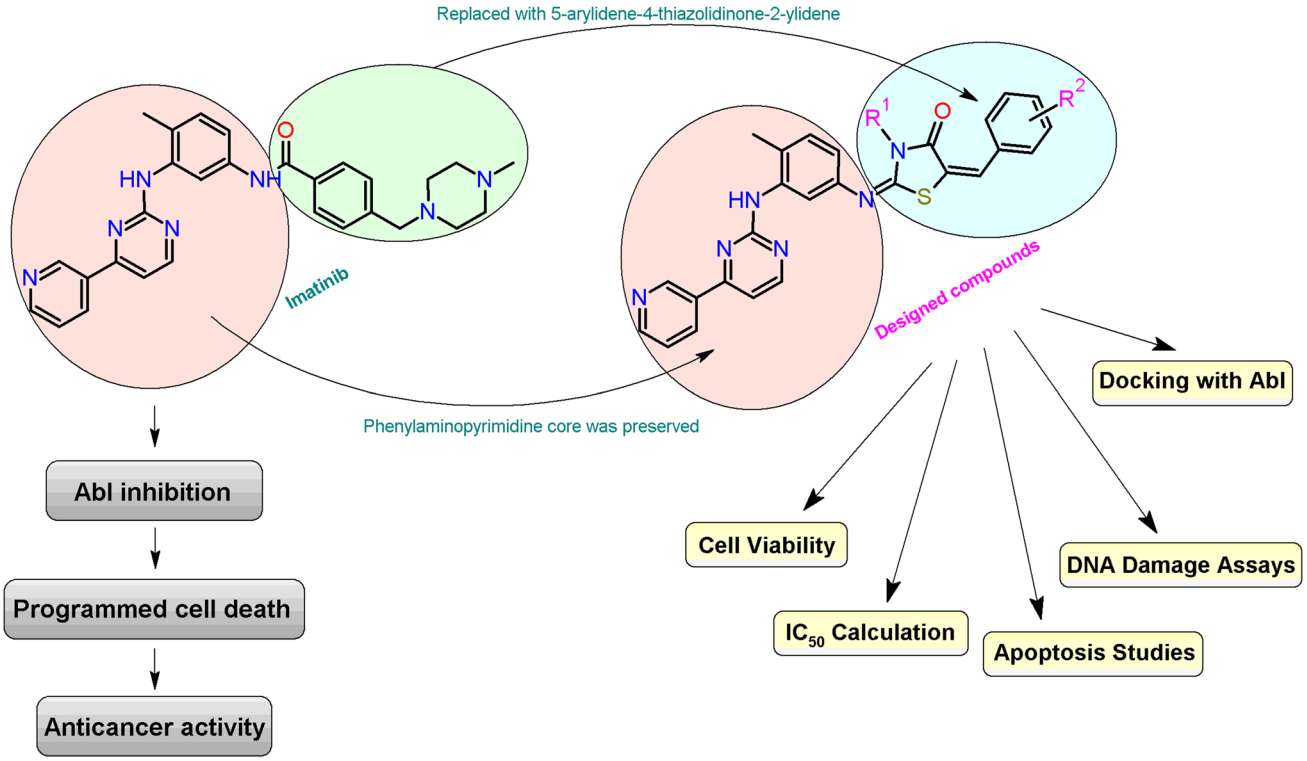


Fig. 1 Design of some novel 5-substituted benzylidene-2-arylimino-4-thiazolidinone derivatives containing pharmacophoric PAP core

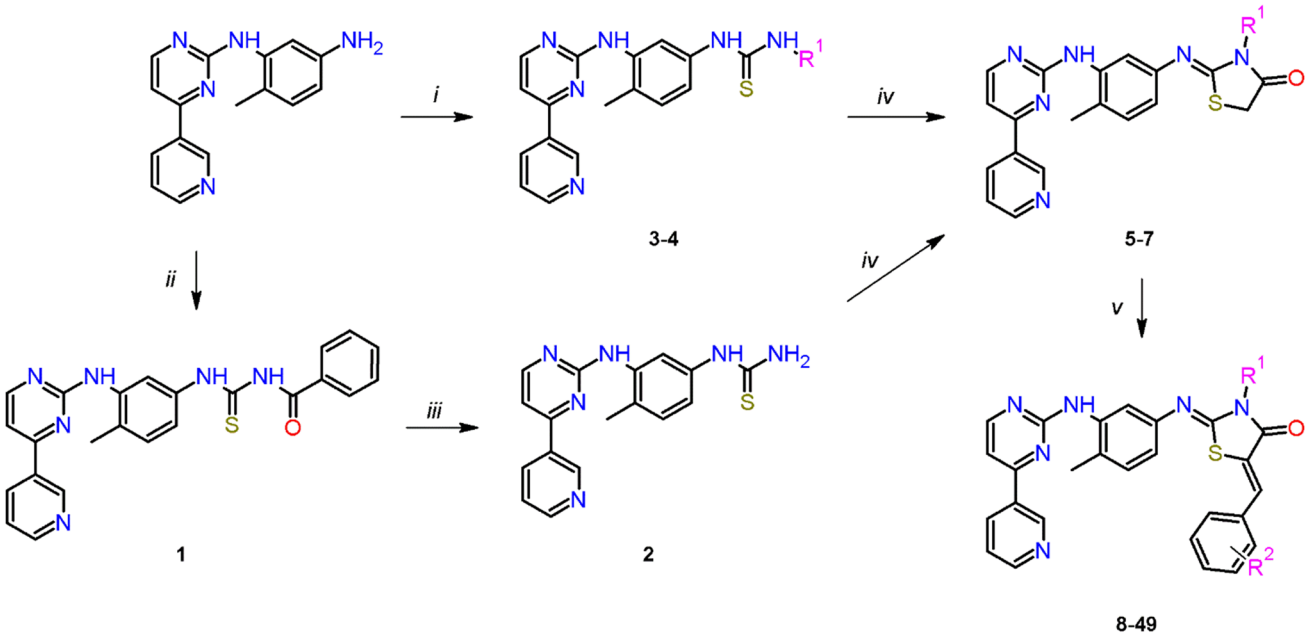
Results and discussion

Chemistry

It is known that 2-imino-1,3-thiazolidin-4-one ring system can be synthesized via different methods. One of them includes cyclization of chloroacetamide compounds in the presence of ammonium thiocyanate [26–33] or potassium thiocyanate [34]. In other respects, the treatment of α-halo carboxylic acid derivatives with thiourea compounds affords 2-imino-1,3-thiazolidin-4-ones [35]. Reaction of chloroacetyl chloride [36], ethyl chloroacetate [20], ethyl bromoacetate [37–39], or ethyl chloroacetate [40] reagents with thiourea compounds were reported to give 2-imino-1,3-thiazolidin-4-one derivatives. In this study, we followed the latter method to obtain 2-imino-1,3-thiazolidin-4-one compounds. We carried out cyclization reaction with α-chloroacetic acid owing to the preferable yield after the purification procedure. Furthermore, whether hydrogen substitution of third position nitrogen is necessary for structure–activity relationship or not, we obtained 3-substituted-2-imino-1,3-thiazolidin-4-ones as well by starting the reaction with di-substituted thioureas.

In previous communications reported for the synthesis of designed compounds, thioureas were produced by the reaction of amines with isothiocyanates [41, 42]. For the in situ preparation of benzoyl isothiocyanate, ammonium thiocyanate was reacted with benzoyl chloride in dry acetone. To this medium that contains benzoyl isothiocyanate, 4-methyl-N^3-[4-(pyridin-3-yl)pyrimidin-2-yl]benzene-1,3-diamine was then added. The reaction was monitored with TLC. The precipitate was obtained by filtration after the TLC spot of the above-mentioned diamine was disappeared. The resultant crude was washed with hot methanol (**1**). For the synthesis of the targeted mono-substituted thiourea **2**, compound **1** was partially hydrolyzed using methanolic solution of 1 N NaOH (aq.). For the synthesis of the di-substituted thiourea compounds **3**–**4**, 4-methyl-N^3-[4-(pyridin-3-yl) pyrimidin-2-yl]benzene-1,3-diamine was refluxed with substituted ethyl and methyl isothiocyanates in dry acetone [12]. Thioureas **2**–**4** were converted to 2-imino-1,3-thiazolidin-4-ones **5**–**7** with α-chloroacetic acid in the presence of sodium acetate. Excess amounts of α-chloroacetic acid and sodium acetate were used for the cyclization. And lastly, synthesis of 5-benzylidene-2-imino-1,3-thiazolidin-4-one derivatives was performed by *Knoevenagel* condensation of 2-imino-1,3-thiazolidin-4-ones with related benzaldehydes [28, 29]. Scheme 1 represents the route to synthesize compounds **1**–**49**.

The purity of all the synthesized compounds was checked with thin layer chromatography (TLC) and reverse phase-high performance liquid chromatography (RP-HPLC).



Scheme 1 Synthetic route to compounds **1**–**49**. *i* Acetone, methyl or ethyl isothiocyanate, reflux. *ii* Acetone, NH_4SCN benzoyl chloride, reflux. *iii* MeOH, 1 N NaOH, reflux. *iv* EtOH, $ClCH_2COOH$, NaOAc, reflux. *v* NaOMe, ArCHO, reflux

Structures of the compounds were confirmed by FTIR, ^{1}H-NMR, ^{13}C-NMR, HMBC, and mass spectral data besides elemental analysis.

We have previously published the chemistry of thioureas [12]. In FTIR spectra, C=S stretching bends of thioureas **1**–**4** were observed at 1232–1288 cm^{-1}. Discriminately, C=O stretching bend of compound **1** was identified at 1666 cm^{-1}. C=O stretching bends of 1,3-thiazolidin-4-ones **5**–**7** were detected at 1708–1712 cm^{-1}. C=O stretching bends of 5-benzylidene-1,3-thiazolidin-4-ones **8**–**49** were identified at 1697–1720 cm^{-1}.

In ^{1}H-NMR spectra of compounds 1,3-thiazolidin-4-one rings **5**–**7**, $–SCH_2–$ protons were observed 3.92–4.01 ppm. In ^{13}C-NMR spectra of compounds **5**–**6**, $–SCH_2–$ signals were observed at 33–35 ppm and C=O carbon signals of the compounds were identified at ppm 171–188 ppm [35, 42]. Benzylidene substitution to 1,3-thiazolidin-4-one rings was confirmed with the ^{1}H- and ^{13}C-NMR results of compounds **8**–**49**. Absence of $-SCH_2-$ signals at 32–36 ppm and merge of signals at 127–130 ppm demonstrated the structures of final compounds **8**–**49** [43, 44]. Similarly, in ^{1}H-NMR spectra, while $-SCH_2-$ protons were disappeared, methylidene proton signals of compounds **8**–**49** were observed between 7.58 and 7.99 ppm which confirms Z isomer of methylidene structure [29, 31, 45, 46]. Methylidene carbon signals of =CH–Ar structures were identified at 134–135 ppm [47].

In ^{1}H-NMR studies, secondary NH proton signals of the compounds that belong to PAP core were detected in the range of 8.90 and 9.10 ppm. These NH signals disappeared in the ^{1}H-NMR spectra of the compounds when $CDCl_3$ was used as a solvent. D_2O exchange method was also used to identify exchangeable protons. Moreover, it was observed that 2-amino(/imino)-4-thiazol(idin)one NH protons in addition to secondary NH protons exchange with deuterium of D_2O as well as $CDCl_3$ [48, 49]. Unless 2-amino(/imino)-4-thiazol(idin)one compounds **8**–**21** NH protons are changed with deuterium, they were observed between 11.40 and 12.90 ppm in DMSO-d_6 as broad singlets that confirm imino structures of the compounds. 2-Amino-4-thiazolone protons would appear at much higher field around 9.00 ppm [31]. Amino-imino tautomeric equilibrium for compounds **5** and **8**–**21** was monitored in their both ^{1}H- and ^{13}C-NMR data. Detailed spectral data are presented in supporting information.

In the literature, keto/enol and amino/imino tautomeric forms of 2-amino(/imino)-4-thiazol(idin)one rings were investigated elaborately [50–53]. As can be seen in Fig. 2, 2-phenylimino-1,3-thiazolidin-4-one structure can be found in eight different forms depending on keto/enol tautomerism, amino/imino tautomerism in addition to E/Z geometrical isomers. In light of this information, modeling studies of compounds **5** and **8**–**21**, possessing amino/imino tautomerism, were held with both forms of the compounds.

Biological activity

Anticancer activity of compounds **5**–**49** was evaluated against K562, PC3 and SHSY-5Y cells. For preliminary

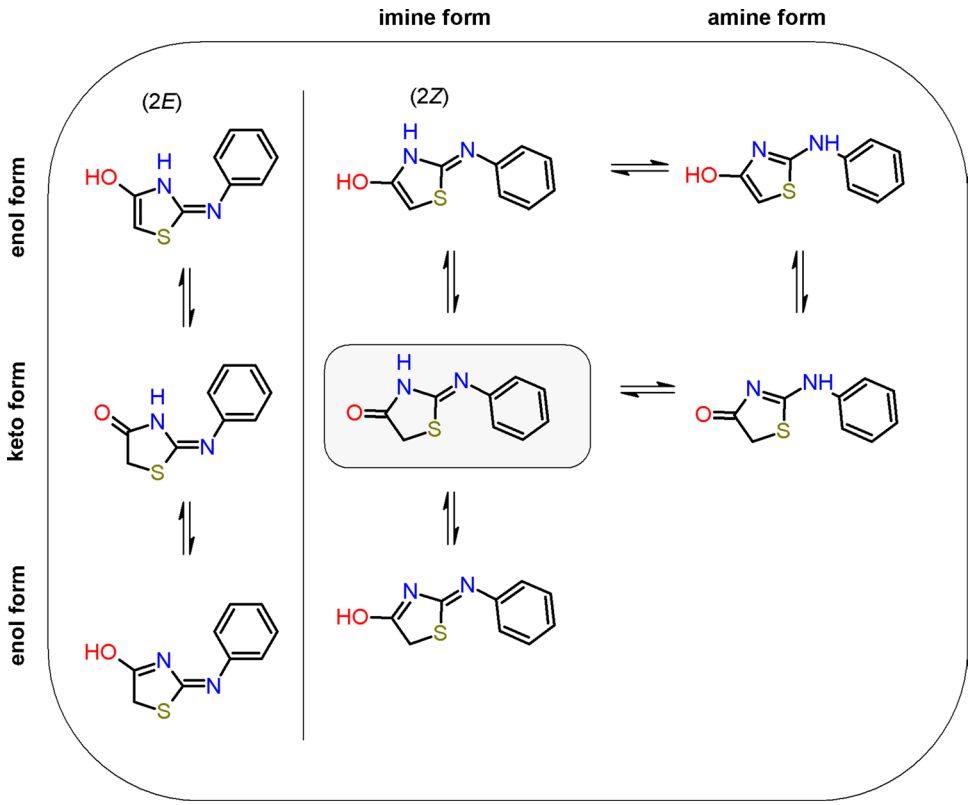


Fig. 2 Keto/enol and amino/imino tautomeric forms of 2-phenylimino-1,3-thiazolidin-4-one ring

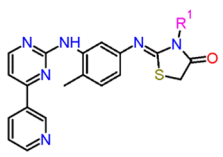
screening, cytotoxic bioactivity of synthesized compounds was evaluated in vitro against K562, PC3 and SHSY-5Y cells with the XTT assay. To evaluate the anticancer potency of target compounds, the cancer cells were treated with the compounds at 10 µM constant concentration. Cell viability percentages were calculated after the treatment of cells for 24 and 48 h. Imatinib was used as a positive control. Preliminary anticancer activity results of compounds **5**–**49** against K562, PC3, and SHSY-5Y are presented in Tables 1, 2 and 3 respectively. It was observed that while compounds **5**–**49** showed moderate antiproliferative activity against K562 cells, compounds **5**–**49** demonstrated weak antiproliferative activity against PC3 and SHSY-5Y cells. Since compounds **5**–**49** displayed better anticancer activity towards K562 cells compared to PC3 and SHSY-5Y cells, further biological studies were held with K562 cells. As of compounds, **8**, **15** and **34** exhibited better anticancer activity and further activity studies were carried on with compounds **8**, **15** and **34**. It was also observed that the introduction of substituted benzylidene substitution at the 5th position of 2-imino-4-thiazolidinone ring significantly enhances anticancer activity on K562 cells.

As seen in Table 1, the percentage of viability values of compounds **8**, **15** and **34** were determined as 34.1, 31.1 and 49.8 for 24 h and 31.8, 24.6 and 41.7 for 48 h, respectively. On the other hand, compounds **19**, **39** and **48** displayed moderate antiproliferative activity, while nearly all of the other compounds demonstrated poor activity against K562 cells. Herewith, compounds **8**, **15** and **34**, which revealed out cell viability% values less than 50.0% on K562 cells, were selected for further biological studies. These three compounds were also tested for the following IC_{50} calculations, apoptosis studies, cell cycle experiments, and DNA damage assay on K562 cells.

Afterward, to determine IC_{50} values of the compounds **8**, **15** and **34**, K562 cells were treated with 0.1, 1, 10, 25 and 50 µM concentrations of these compounds for 24 and 48 h and cell viability assay was performed. Results obtained from IC_{50} calculations were demonstrated in Fig. 3 and Table 4. As shown in Fig. 3, compound **15** was found to be the most potent compound towards K562 cells (IC_{50} values of 4.86 ± 0.73 and 3.52 ± 0.91 µM for 24 and 48 h, respectively). For 24 and 48 h, the IC_{50} values were calculated as 9.97 ± 1.14 and 8.16 ± 1.27 µM for compound **34**, 8.79 ± 1.09 and 5.26 ± 1.03 µM for compound **8** in K562 cells, respectively. IC_{50} values of imatinib as positive control were calculated as 0.73 ± 0.17 for 24 h and 0.59 ± 0.11 µM for 48 h in K562 cells. These IC_{50} values were used in subsequent apoptosis and cell cycle assays.

Later on, the most active compounds **8**, **15** and **34** were evaluated against mouse subcutaneous connective tissue cells (L929) to determine whether the synthesized compounds display selective cytotoxicity between normal and tumor cells or not. Assertively, compounds **8**, **15** and **34**

Table 1 Cell viability % ± SD values of synthesized compounds **5**–**49** on K562 cells for 24 and 48 h



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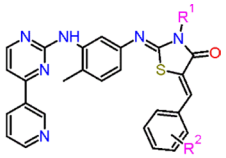


8-49

R^2	R^1								
	–H			–Me			–Et		
	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%
–	**5**	97.4 ± 5.32	83.1 ± 4.56	**6**	101.1 ± 4.22	91.3 ± 5.11	**7**	101.1 ± 3.33	95.7 ± 3.99
2-F	**8**	**34.1 ± 3.46**	**31.8 ± 5.46**	**22**	77.9 ± 3.04	63.4 ± 4.16	**36**	57.3 ± 3.47	49.3 ± 4.13
3-F	**9**	77.4 ± 4.96	68.1 ± 6.11	**23**	97.4 ± 3.78	89.5 ± 1.07	**37**	85.1 ± 1.35	73.4 ± 3.48
4-F	**10**	82.6 ± 1.99	63.4 ± 4.93	**24**	112.8 ± 4.12	101.3 ± 3.79	**38**	77.3 ± 2.34	70.1 ± 1.79
2,6-F_2	**11**	63.5 ± 5.13	52.3 ± 1.89	**25**	83.7 ± 5.13	66.7 ± 5.12	**39**	54.5 ± 3.19	46.9 ± 4.11
2-Cl	**12**	75.2 ± 3.74	67.5 ± 4.19	**26**	94.3 ± 5.63	83.9 ± 4.13	**40**	61.5 ± 5.79	59.4 ± 4.19
4-Cl	**13**	86.1 ± 6.11	70.6 ± 5.98	**27**	98.8 ± 3.15	92.7 ± 5.09	**41**	71.8 ± 1.59	63.9 ± 3.46
4-Br	**14**	74.8 ± 3.12	62.2 ± 3.47	**28**	92.6 ± 3.89	85.8 ± 5.93	**42**	73.5 ± 4.65	68.1 ± 6.15
4-CF_3	**15**	**31.1 ± 3.97**	**24.6 ± 2.45**	**29**	83.8 ± 4.29	67.9 ± 3.49	**43**	57.3 ± 4.16	47.9 ± 4.78
4-OCF_3	**16**	81.1 ± 4.19	73.2 ± 1.79	**30**	85.5 ± 1.07	79.2 ± 3.97	**44**	59.3 ± 4.69	50.2 ± 5.05
2-OH	**17**	56.5 ± 4.17	49.3 ± 3.47	**31**	67.2 ± 4.17	60.4 ± 4.37	**45**	73.4 ± 3.48	62.5 ± 5.09
2-OMe	**18**	61.1 ± 1.95	55.9 ± 5.13	**32**	73.2 ± 5.11	66.6 ± 4.78	**46**	85.7 ± 1.99	73.9 ± 3.31
3-OMe	**19**	54.7 ± 3.47	48.1 ± 2.44	**33**	68.6 ± 4.13	59.7 ± 2.33	**47**	81.5 ± 2.11	72.7 ± 3.89
4-OMe	**20**	58.6 ± 3.11	50.1 ± 4.19	**34**	**49.8 ± 5.97**	**41.7 ± 5.11**	**48**	55.6 ± 3.12	44.6 ± 3.21
4-$N(Me)_2$	**21**	66.9 ± 1.11	57.1 ± 3.47	**35**	91.9 ± 1.02	83.2 ± 1.03	**49**	63.1 ± 3.19	51.6 ± 4.13
		24 h Viab%	48 h Viab%						
Imatinib		22.1 ± 4.13	16.7 ± 2.76						

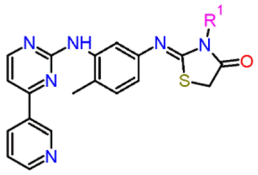
Compounds were implemented at 10 µM concentration on K562 cell line

exhibited a weak cytotoxic effect on L929 cells with IC_{50} values of 87.3 ± 4.96, 73.7 ± 5.13 and 69.1 ± 3.49 µM, respectively, in comparison with imatinib (IC_{50} = 19.6 ± 1.33 µM). Results that are presented in Table 2 suggest that these compounds possess relatively low cytotoxicity towards L929 cells. It is noteworthy to emphasize that compounds **8**, **15** and **34** had lower toxicity according to imatinib on L929 cells.

Imatinib constitutively eliminates K562 cells by inducing apoptosis [54]. In our study, it was also investigated whether the three most effective compounds have apoptotic effects on K562 cells. Before apoptosis experiments, the cells were treated with IC_{50} concentrations of the most active compounds and imatinib for 24 and 48 h. Later, Annexin V binding assay was performed to evaluate the apoptotic effects of the compounds and imatinib on K562 cells. As shown in Fig. 4, step-up of incubation time from 24 to 48 h increased the amount of total apoptotic cells in all groups, especially for compounds **8** and **15** when compared to control cells. Percentages of total apoptotic cells were 27.27%, 19.18%, 14.59% and 24.88% for compounds **8**, **15**, **34** and imatinib at 24 h, respectively. For 48 h, total apoptotic cell amount was significantly increased to 50.24%, 57.72%, 19.70%, and 62.67% for compounds **8**, **15**, **34** and imatinib, respectively. These data indicate that compounds **8** and **15** induce mainly early and late apoptosis in K562 cells in a time-dependent manner.

Above-mentioned studies exhibited that the three compounds inhibited cell proliferation and significantly induced apoptosis compared to control cells. In resuming studies, to investigate whether the compounds induce growth inhibition of cells via alterations in cell cycle arrest, we determined the effect of the compounds on cell cycle distribution by the cell cycle kit. The cell cycle results showed that, after

Table 2 Cell viability % ± SD values of synthesized compounds **5**–**49** on PC-3 cells for 24 and 48 h



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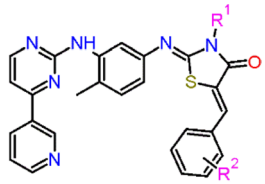

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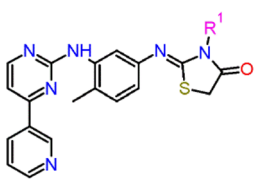
R^2	R^1								
	–H			–Me			–Et		
	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%
–	**5**	87.4 ± 2.72	80.1 ± 2.94	**6**	79.7 ± 1.02	77.1 ± 2.81	**7**	59.4 ± 3.92	50.3 ± 5.13
2-F	**8**	45.6 ± 3.23	40.9 ± 1.05	**22**	83.0 ± 3.27	78.1 ± 2.09	**36**	59.0 ± 2.24	53.8 ± 3.12
3-F	**9**	68.8 ± 3.56	60.1 ± 3.12	**23**	92.1 ± 1.09	88.9 ± 4.22	**37**	98.3 ± 1.91	92.4 ± 1.76
4-F	**10**	50.7 ± 2.46	43.7 ± 1.78	**24**	76.3 ± 4.95	69.3 ± 1.09	**38**	69.4 ± 2.13	63.3 ± 2.78
2,6-F_2	**11**	55.2 ± 4.07	47.9 ± 2.94	**25**	98.9 ± 4.01	89.9 ± 5.01	**39**	52.7 ± 2.17	47.6 ± 1.97
2-Cl	**12**	73.4 ± 1.08	67.1 ± 2.36	**26**	99.5 ± 1.25	95.5 ± 3.13	**40**	79.2 ± 3.08	76.5 ± 2.91
4-Cl	**13**	80.6 ± 4.18	78.6 ± 1.96	**27**	73.4 ± 2.13	67.1 ± 1.57	**41**	68.9 ± 2.28	63.8 ± 3.11
4-Br	**14**	86.2 ± 4.53	78.2 ± 2.04	**28**	75.2 ± 2.98	66.1 ± 2.39	**42**	65.1 ± 1.89	59.6 ± 4.96
4-CF_3	**15**	46.1 ± 1.21	40.1 ± 1.08	**29**	62.7 ± 1.83	57.1 ± 4.16	**43**	58.9 ± 3.07	52.9 ± 2.77
4-OCF_3	**16**	59.2 ± 1.12	56.7 ± 2.07	**30**	94.6 ± 3.90	91.7 ± 4.19	**44**	55.7 ± 3.11	48.7 ± 5.07
2-OH	**17**	87.0 ± 3.94	77.8 ± 2.97	**31**	55.0 ± 4.87	51.6 ± 3.45	**45**	63.7 ± 4.15	58.6 ± 3.46
2-OMe	**18**	69.0 ± 1.10	65.2 ± 2.71	**32**	69.9 ± 5.13	62.9 ± 1.54	**46**	68.0 ± 1.78	57.8 ± 1.78
3-OMe	**19**	62.6 ± 2.86	51.9 ± 3.09	**33**	61.4 ± 2.46	58.3 ± 5.29	**47**	52.1 ± 3.09	48.1 ± 2.47
4-OMe	**20**	63.4 ± 5.64	59.7 ± 1.01	**34**	81.0 ± 2.19	74.5 ± 2.09	**48**	55.3 ± 4.26	49.1 ± 2.12
4-$N(Me)_2$	**21**	93.7 ± 2.11	89.2 ± 5.10	**35**	88.9 ± 3.09	81.4 ± 2.23	**49**	72.6 ± 1.47	67.2 ± 1.01
		24 h Viab%	48 h Viab%						
Imatinib		74.3 ± 2.69	67.1 ± 4.12						

Compounds were implemented at 10 µM concentration on PC-3 cell line

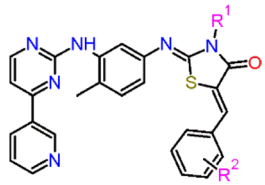
48 h, G0/G1 phase population of control cells was 32.8% and the percentages significantly increased to 52.0%, 47.5% and 57.6% for compounds **15**, **34** and positive control imatinib, respectively. As several studies have reported, imatinib arrests K562 and different cells at G0/G1 phase of the cell cycle [55, 56] and our findings showed consistency with these works of literature. Assertively, compound **8** dramatically induced G2/M phase arrest (69.2%) compared with control cells (33.0%). After the treatment of K562 cells with compounds **8**, **15** and **34**, the cell cycle results demonstrated that the growth inhibition was observed in all groups mainly associated with G0/G1 (compound **15** and **34**) and G2/M phase arrest (compound **8**). Figure 5 represents the cell cycle analysis of K562 cells exposed to compounds **8**, **15**, **34**, and imatinib.

It is well known that DNA damage and apoptosis are closely related processes and in most cases, DNA damage in the cells results in apoptosis. Therefore, DNA is the major target of most cytotoxic anticancer drugs and inducing DNA damage is an important anticancer strategy [57]. Many studies have reported that inducing DNA damage results in apoptotic cell death in many types of cancer [58]. In this study, having compared to the control cells, while compound **34** exhibited low DNA damage response, compounds **8** and **15** significantly promoted DNA damage response (increased phosphorylation of ATM and H2AX) in K562 cells. As given in Fig. 6, total DNA damage percentage of control cells was 8.70% and the percentages were increased to 32.90%, 37.20%, and 10.40%, after treatment of compounds **8**, **15** and **34**, respectively. For the

Table 3 Cell viability % ± SD values of synthesized compounds **5**–**49** on SHSY-5Y cells for 24 and 48 h



5-7



8-49

R^2	R^1								
	–H			–Me			–Et		
	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%	Comp. no.	24 h Viab%	48 h Viab%
–	**5**	83.7 ± 1.09	72.4 ± 4.02	**6**	73.9 ± 3.78	65.1 ± 3.21	**7**	63.3 ± 5.96	54.3 ± 3.12
2-F	**8**	42.3 ± 3.08	33.9 ± 1.56	**22**	81.3 ± 4.25	72.9 ± 3.14	**36**	46.8 ± 2.02	43.1 ± 2.28
3-F	**9**	75.5 ± 2.48	62.9 ± 3.19	**23**	101.1 ± 4.12	93.1 ± 2.91	**37**	109.1 ± 3.28	97.1 ± 2.76
4-F	**10**	47.7 ± 3.24	42.1 ± 4.21	**24**	69.9 ± 4.19	61.9 ± 3.12	**38**	46.8 ± 4.75	42.8 ± 3.12
2,6-F_2	**11**	49.0 ± 2.76	42.3 ± 2.75	**25**	54.1 ± 2.19	41.8 ± 5.21	**39**	49.2 ± 5.47	41.8 ± 3.17
2-Cl	**12**	58.6 ± 1.09	46.4 ± 2.09	**26**	55.5 ± 1.07	42.5 ± 3.09	**40**	52.2 ± 1.79	42.2 ± 2.08
4-Cl	**13**	83.0 ± 5.15	70.1 ± 3.17	**27**	77.8 ± 2.24	59.8 ± 1.23	**41**	66.7 ± 2.18	56.2 ± 1.92
4-Br	**14**	87.0 ± 3.45	74.3 ± 4.13	**28**	63.0 ± 2.78	71.8 ± 3.27	**42**	71.8 ± 3.27	60.9 ± 1.86
4-CF_3	**15**	50.4 ± 2.91	42.6 ± 1.09	**29**	66.6 ± 4.56	53.1 ± 3.02	**43**	59.9 ± 3.48	47.9 ± 2.67
4-OCF_3	**16**	63.1 ± 4.12	50.9 ± 3.56	**30**	59.5 ± 5.24	47.4 ± 2.24	**44**	57.8 ± 2.97	43.7 ± 1.26
2-OH	**17**	84.3 ± 3.58	69.1 ± 4.13	**31**	68.4 ± 1.45	56.4 ± 2.56	**45**	49.3 ± 3.56	42.8 ± 3.87
2-OMe	**18**	70.2 ± 4.18	59.2 ± 1.76	**32**	72.2 ± 2.09	61.2 ± 1.43	**46**	65.4 ± 3.47	51.4 ± 3.86
3-OMe	**19**	51.8 ± 4.10	42.8 ± 1.25	**33**	63.6 ± 2.47	50.6 ± 2.76	**47**	54.6 ± 2.85	41.9 ± 2.09
4-OMe	**20**	67.8 ± 1.08	51.6 ± 3.72	**34**	78.5 ± 3.25	64.5 ± 4.07	**48**	67.6 ± 2.18	53.1 ± 5.13
4-$N(Me)_2$	**21**	104.2 ± 5.02	91.2 ± 3.02	**35**	98.5 ± 2.19	86.4 ± 2.12	**49**	76.8 ± 5.03	64.8 ± 2.99
		24 h Viab%	48 h Viab%						
Imatinib		93.2 ± 1.25	89.3 ± 2.19						

Compounds were implemented at 10 µM concentration on SHSY-5Y cell line

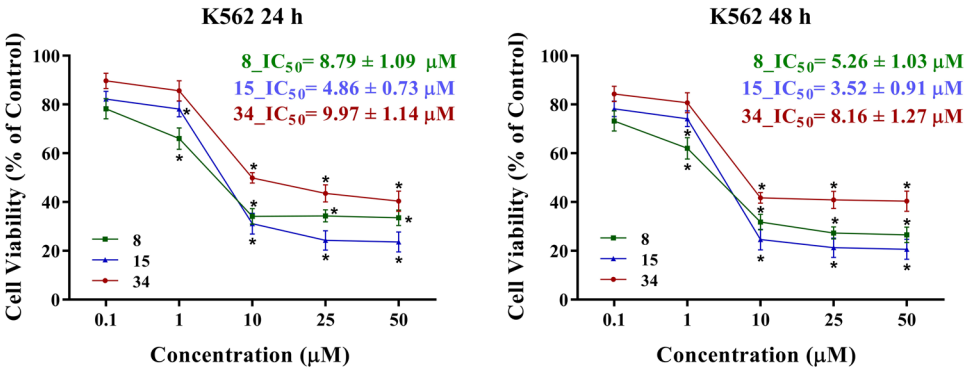


Fig. 3 The antiproliferative activity of compounds **8**, **15** and **34** on K652 cells (The cells were treated with the compounds at various concentrations (0.1–50 µM) for 24 and 48 h and the cell viability was evaluated using the XTT assay. All data are expressed as mean ± SD in three replicates. The differences are identified as $^*p < 0.05$ from the control cells.)

Table 4 IC_{50} values of compounds **8**, **15**, **34** and imatinib on K562 and L929 cell lines

Compound	IC_{50} (μM)		
	K562_24 h	K562_48 h	L929_48 h
8	8.79 ± 1.09	5.26 ± 1.03	73.7 ± 5.13
15	4.86 ± 0.73	3.52 ± 0.91	69.1 ± 3.49
34	9.97 ± 1.14	8.16 ± 1.27	87.3 ± 4.96
Imatinib	0.73 ± 0.17	0.59 ± 0.11	19.6 ± 1.33

positive control imatinib, total DNA damage was measured as 17.20%. These results demonstrate that compounds **8** and **15** possess more genotoxic effect than imatinib.

Molecular modeling studies

To gain more molecular insight on the activity of the three compounds, molecular modeling studies were conducted with Abl enzyme. It is well known that imatinib owes its clinical success against CML by inhibiting Abl kinase protein [59]. Figure 7 represents the binding of imatinib and hydrogen bond interactions in the active site of Abl kinase. Evaluating possible binding mechanisms of the most active compounds is crucial in this respect. Imatinib makes hydrogen bond contacts with Glu286, Thr315, Met318, Ile360, His361, and Asp381 amino acid residues.

Binding poses of the three most active compounds were studied as representative conformations after docking calculations. Firstly, it is substantial to indicate that PAP cores did not place in the Abl binding site as it places in the structure

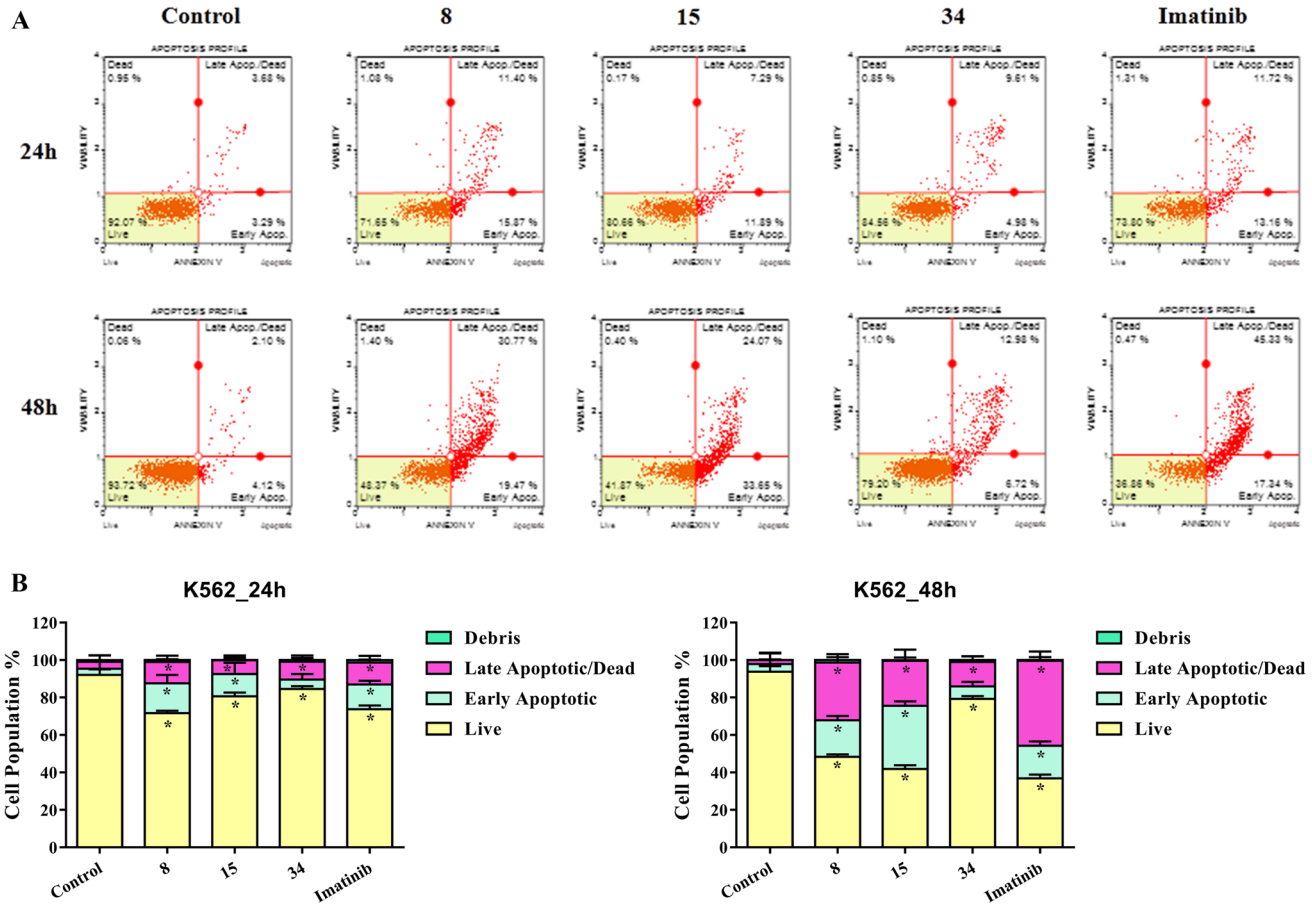


Fig. 4 **a** Apoptotic effects of compound **8**, **15**, **34** and imatinib, on K562 cells (The cells were treated with the IC_{50} concentrations of compounds **8**, **15**, **34** and imatinib for 24 and 48 h. After the incubation time, the cells were stained with Muse Annexin V & Dead Cell reagent and then % gated values evaluated by Muse Cell Analyzer (Merck Millipore). Quantitative data demonstrate that total apoptosis significantly increased in compound **8** and compound **15**-treated cells in a time-dependent manner compared with control.) **b** Early and late apoptotic cell percentages significantly ($p < 0.05$) increased following compounds treatment (All experiments were carried out in triplicate and obtained similar results. Results are expressed as the mean ± SD. Statistically significant differences are $^*p < 0.05$ from values compared to control cells.)

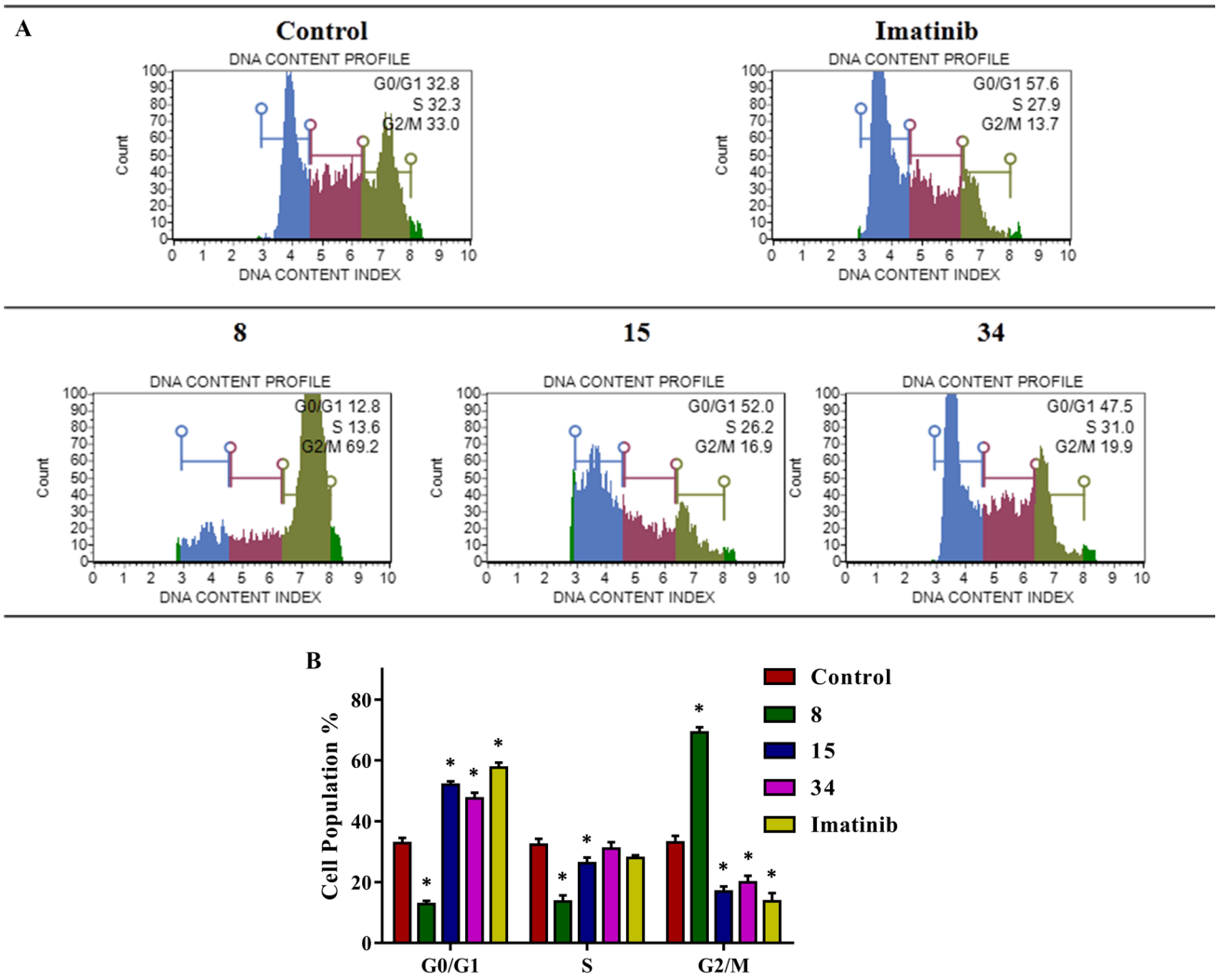


Fig. 5 **a** Effects of compounds **8**, **15**, **34** and imatinib on cell cycle distribution in K562 cells (K562 cells were treated with their IC_{50} concentrations of compounds and imatinib for 48 h. While, compounds **15** and **34** arrest G0/G1 phase similar to imatinib, compound **8** induces cell cycle arrest G2/M phase.) **b** Histogram display the percentage of cell cycle phases of K562 cells (Results are expressed as the mean ± SD. The differences are given as compared to the control cells, $*p < 0.05$.)

of imatinib after 5-arylidene-2-imino-4-thiazolidinone introduction to the PAP core.

In detail, amino-imino tautomerization of 2-amino(/imino)-4-thiazol(idin)one rings were needed to be considered for molecular modeling studies when R^1 is hydrogen in the chemical structure of synthesized compounds [49]. As stated in the chemistry part and supporting file, amino-imino tautomerization of compounds **5** and **8**–**21** was demonstrated by spectral studies. According to NMR results, compounds **5** and **8**–**21** possess both amino and imino isomers. For docking calculations, both configurations of the compounds were considered.

Out of the most active three compounds, **8** and **15** have H at R^1 substitution. Tautomeric forms of compound **8** and **15** were evaluated during docking studies. It was detected that while amino tautomers of **8** and **15** replace binding region of imatinib, imino tautomers of **8** (Fig. 8) and **15** (Fig. 9) replaces allosteric sites away from hinge region. It was also observed that amino tautomers of ring systems form hydrogen bonds with Lys271 and Glu286. Amongst the most active compounds, the amino group of 2-amino-4-thiazolidinone ring behaves as a hydrogen bond donor making hydrogen bond interaction with Glu286. Oxygen atom as hydrogen bond acceptor interacts with Lys271. Moreover, CF_3 substitution of compound **15** interacts with Met318. This interaction is rather important since Met318 amino acid residue takes place hinge region of Abl kinase.

Due to the bulky substitution, an additional methyl group to the nitrogen atom of 3-methyl-2-imino-4-thiazolidinone ring results with another conformational

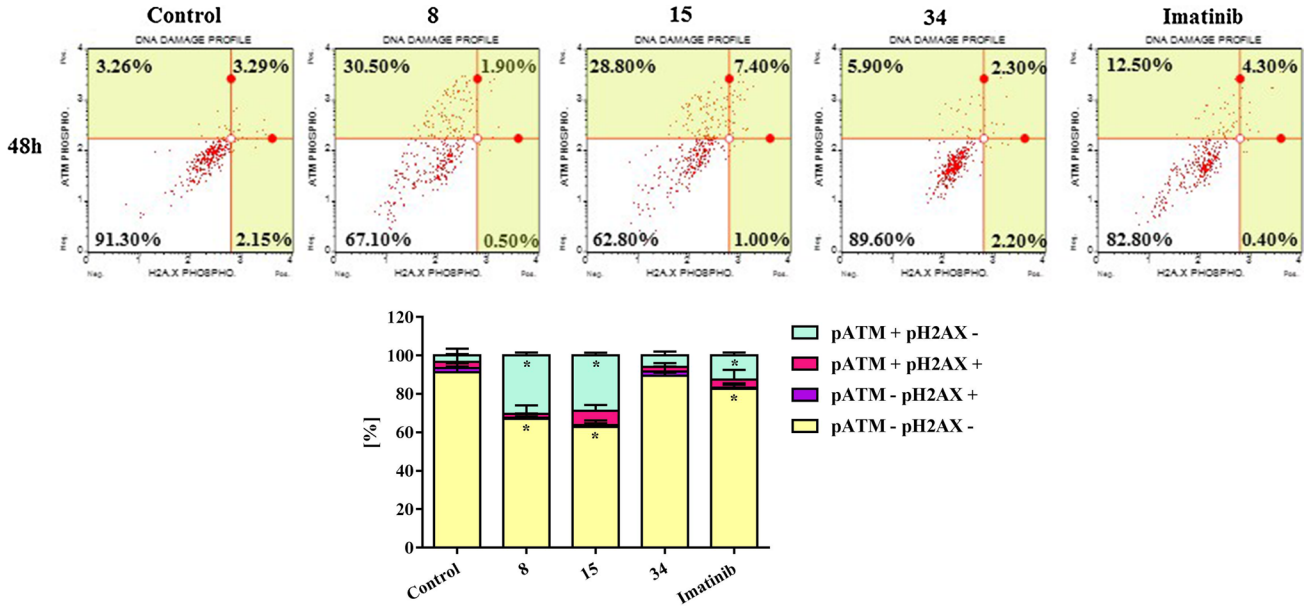


Fig. 6 K562 cells were treated with the IC_{50} concentrations of compounds **8**, **15**, **34** and positive control imatinib for 48 h to induce DNA damage (Activation of ATM and H2AX were determined using the Muse™ Cell Analyzer (Merck Millipore). Bars represent mean ± SD, $*p < 0.05$ compared to the control cells.)

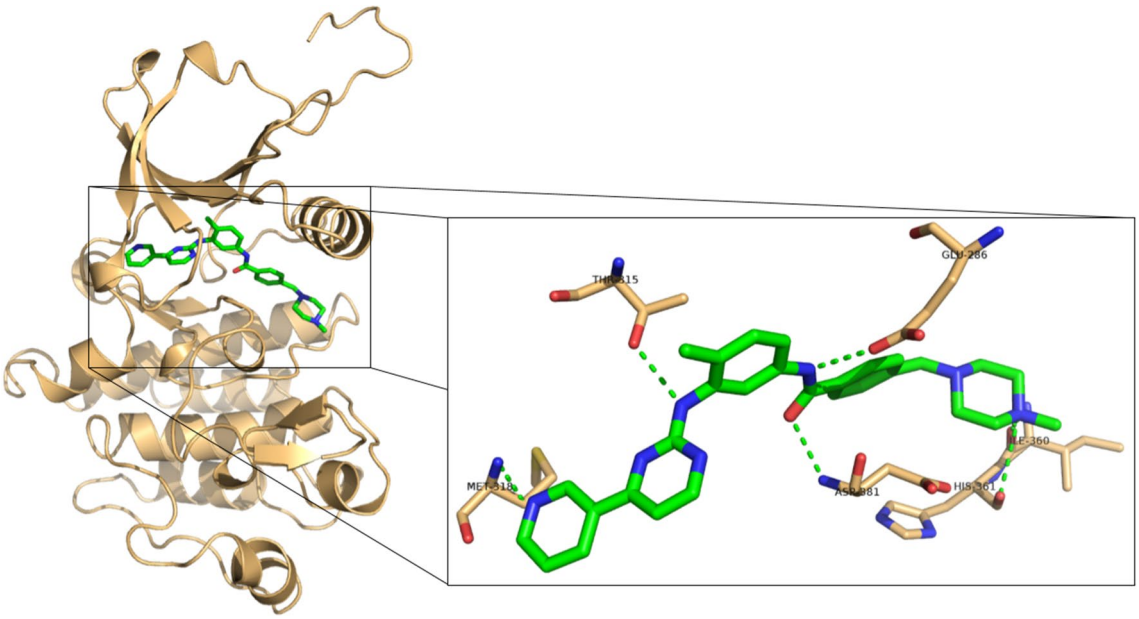


Fig. 7 Imatinib in the Abl binding region and hyrdogen bond interactions between Abl and imatinib

replacement in the Abl binding site. Fig 10 depicts the possible conformation of compound **34** in Abl binding region. Compound **34** also forms hydrogen bond interactions with Asp381 and His361.

Apart from docking calculations, biological results revealed out that compounds **8** and **15** are more active than **34**. Coordination of compounds **8** and **15** in Abl binding site seems more reasonable and rational compared to **34**. Taking into account all these biological and molecular findings, biological data and docking studies support each other.

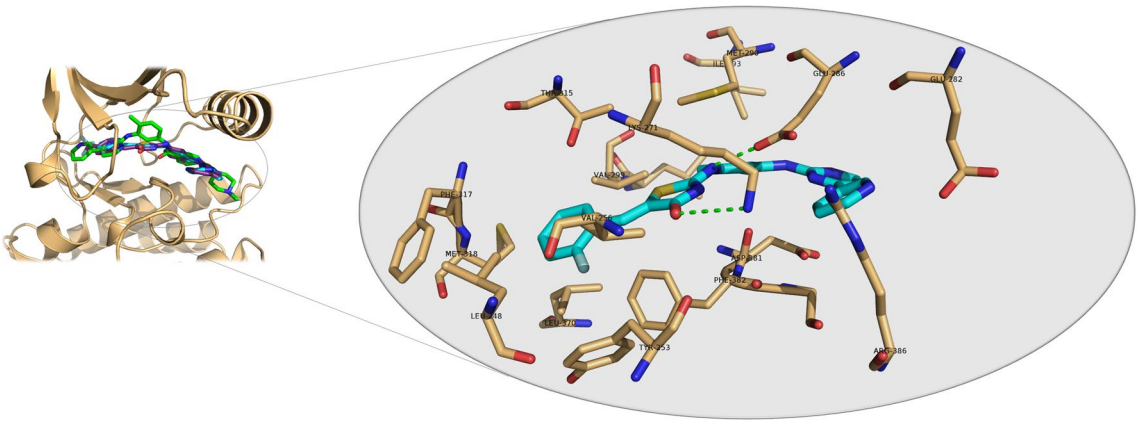


Fig. 8 Interactions of amino tautomer of compound **8** in the Abl binding region

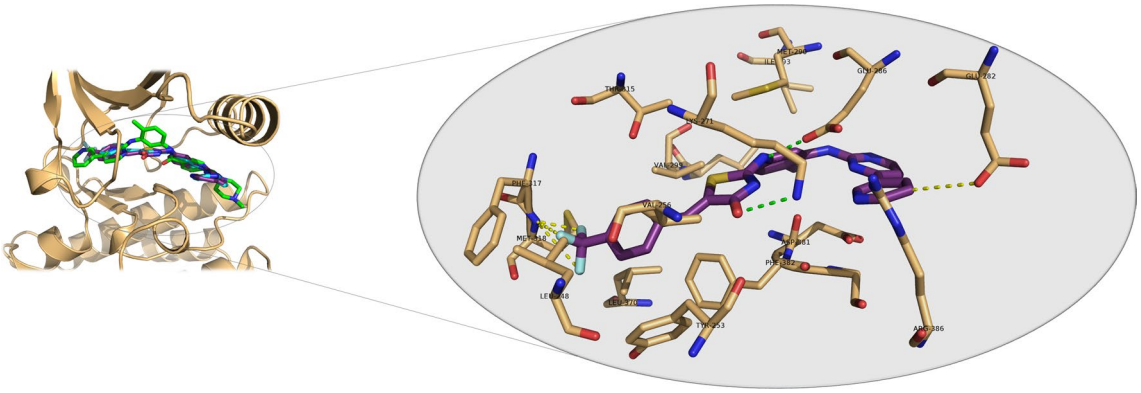


Fig. 9 Interactions of amino tautomer of compound **15** in the Abl binding region

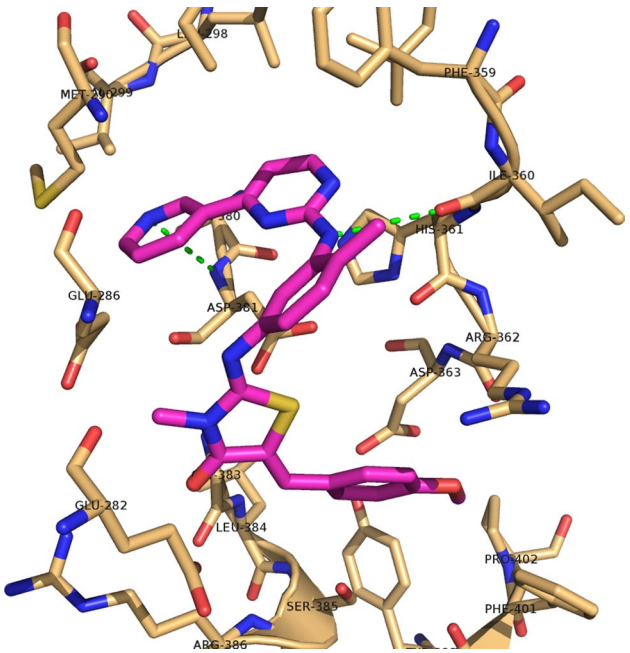


Fig. 10 Interactions of compound **34** in the Abl binding region

Conclusion

In conclusion, starting from 4-methyl-N^3-[4-(pyridin-3-yl)pyrimidin-2-yl]benzene-1,3-diamine, a novel series of PAP core containing 5-benzylidene-2-arylimino-4-thiazolidinone derivatives were designed and synthesized in this study. For the synthesis of designed compounds, starting material was first converted to thiourea derivatives **1**–**4**. Thiourea compounds were later reacted with α-chloroacetic acid to obtain 2-arylimino-4-thiazolidinone compounds **5**–**7**. 5-Substituted benzylidene-2-arylimino-4-thiazolidinone compounds **8**–**49** were achieved from related 2-arylimino-4-thiazolidinones and benzaldehydes in the presence of sodium methoxide via *Knoevenagel* condensation. After the confirmation of the structures of the compounds with spectral studies, compounds were tested for their anticancer activity on CML cell line. Based on the viability assay, the introduction of arylmethylene group to 2-arylimino-4-thiazolidinones prominently increased inhibitory activity on K562 cells. Compounds inhibiting

50.0% of the cells at 10 μM concentration were selected for follow-up studies to analyze induction of apoptosis, cell cycle arrest and DNA damage profiles. Prior to these further studies, IC_{50} values were found as 5.26, 3.52 and 8.16 μM for compound **8**, **15** and **34**, respectively. After IC_{50} calculation, it was detected that compounds **8** and **15** induced early and late apoptosis in a time-dependent manner. In depth, 48 h after the implementation of the compounds to K562 cells, the total apoptotic cell amount was 50.24%, 57.72%, 19.70% and 62.67% for **8**, **15**, **34** and imatinib, respectively. In addition, cell cycle studies revealed out that compound **8** dramatically induced G2/M phase arrest (69.2%) compared with control cells (33.0%), while compound **15**, **34** and imatinib caused an increment of the cell population to 52.0, 47.5 and 57.6%, respectively, at G0/G1 phase. Moreover, DNA damage assay results which are notably connected with cellular apoptotic results executed that compound **8** (32.90%) and **15** (37.20%) possess more genotoxicity than imatinib (17.20%). It is better to state that inhibition of Abl protein results with apoptosis. From this point of view, in furtherance compounds have PAP structure as so imatinib, molecular modeling studies were held with Abl protein kinase. Finally, in light of biological results as well as docking studies, we purpose that our compounds may induce programmed cell death by inhibiting Abl kinase in CML cells.

Materials and methods

Chemistry

Solvents, reagents and starting materials except from 4-methyl-N^3-[4-(pyridin-3-yl)pyrimidin-2-yl]benzene-1,3-diamine were purchased from Sigma-Aldrich or Merck. To monitor reactions, TLC studies were run on silica gel 60 F254 plates. The RP-HPLC was used to prove the purity of the compounds. Agilent technologies 1100 series instrument equipped with a quaternary solvent delivery system, a model Agilent series G1315, a photodiode array detector, a Rheodyne syringe loading sample injector with a 50-μL sample loop and Agilent ChemStation Plus software was used for chromatographic analysis. All synthesized compounds were significantly separated from the starting materials and chromatographic purities of the compounds were found above 95% based on the peak area values obtained from chromatograms. Chromatographic systems (CS_{1-7}) which were used in RP-HPLC studies and chromatograms were presented in supplementary data. Melting points (°C) of the compounds were measured with Schmelzpunktbestimmer SMP II basic model melting point apparatus. Elemental analysis studies were held with LECO CHNS-932 instrument. Infrared spectra were performed on a Shimadzu FTIR 8400S and data were implied by wavenumber ν (cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were recorded on Brüker AVANCE-DPX instrument. DMSO-d_6 or $CDCl_3$ was used as a solvent and chemical shifts were specified in δ (ppm) downfield from tetramethylsilane (TMS). Low-resolution mass spectra were acquired with AB SCIEX API 2000 LC–MS/MS instrument.

Documented spectral and chromatographic data of the compounds are presented as the supplementary file.

General procedure for the synthesis of thiourea derivatives (1–4)

Synthesis of thiourea derivatives (**1**–**4**) and structure characterization of the compounds was published in our previous study [12].

*N-(Benzoyl)-N′-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)thiourea **1***
White solid. M.p.: 204–205 °C [12].
*N-(4-Methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)thiourea **2***
White solid. M.p.: 210–212 °C [12].
*N-(4-Methyl)-N′-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)thiourea **3***
White solid. M.p.: 201–202 °C [12].
*N-(4-Ethyl)-N′-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)thiourea **4***
White solid. M.p.: 187–188 °C [12].

General procedure for the synthesis of thiazolidin-4-one derivatives (5–7)

Corresponding thiourea derivative compounds **1**–**4** (0.01 mol) were dissolved in ethanol and heated in the presence of sodium acetate (0.03 mol) and α-chloroacetic acid (0.02 mol) for 8–10 h. Ethanol was evaporated under vacuum at the end of the reaction. For the purification, the precipitate was washed with water first. The solid material obtained was washed with petroleum ether later and crystallized from ethanol.

2-[(4-Methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 5 Salmon color solid. Yield 42%, 1.52 g. TLC R_f: 0.43 (S_1). HPLC t_R (min): 3.19 (CS_1). M.p.: 200–201 °C. IR υ_{max} (cm^{-1}): 3435, 3229 (N–H str), 3031 (aromatic C–H str), 2981–2897 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1647, 1585, 1553 (C=N str, N–H bending, C=C str), 1406 (aliphatic C–H bending), 810–798 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 and 9.24 (s and s, 1H), 9.02 and 8.90 (s and s, 1H), 8.69 (s, 1H), 8.52 (d, 1H, J = 4.8 Hz), 8.42 (d, 1H, J = 7.8 Hz), 7.99 (s, 0.5H), 7.54 (m, 1H), 7.43 (d, 1H, J = 5.1 Hz), 7.39 (m, 1H), 7.25 (m,

1H), 6.75 (d, 0.5H, $J = 7.8$ Hz), 3.98 and 3.92 (s and s, 2H, SC**H**$_2$CO), 2.24 and 2.22 (s and s, 3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 188.08 and 177.04 (thiazolidin-4-one **C**=O), 161.68, 160.94, 159.43, 151.33, 148.14, 138.44, 136.79, 134.39, 132.15, 130.44, 128.66, 127.92, 123.77, 117.52, 116.34, 107.83, 35.26 (thiazolidin-4-one **C**H$_2$) 17.63. (Ar**C**H$_3$). LC/MS ESI$^+$ m/z (%): 399.154 ([M+Na]$^+$, 77), 377.162 ([M+H]$^+$, 100); ESI$^-$ m/z (%): 375.394 ([M−H]$^-$, 7), 97.027 (100). Elemental analysis cald for $C_{19}H_{16}N_6OS{\cdot}5/4H_2O$, C 57.20, H 4.67, N 21.07, S 8.04; found C 57.21, H 4.64, N 21.18, S 7.61.

3-Methyl-2-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 6 Yellow-orange solid. Yield 80%, 3.12 g. TLC R_f: 0.49 (S_1). HPLC t_R (min): 6.12 (CS_1). M.p.: 182–184 °C. IR υ_{max} (cm^{-1}): 3352 (N–H str), 3031 (aromatic C–H str), 2945–2915 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1627, 1581, 1519 (C=N str, N–H bending, C=C str), 1415, 1400 (aliphatic C–H bending), 802 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.30 (d, 1H, $J = 1.5$ Hz), 8.90 (s, 1H, sec. N**H**), 8.70–8.68 (dd, 1H, J = 1.5 Hz, $J = 3.2$ Hz, J = 1.5 Hz), 8.53 (d, 1H, $J = 5.3$ Hz), 8.43 (d, 1H, $J = 8.5$ Hz), 7.55–7.51 (q, 1H), 7.44 (d, 1H, $J = 5.1$ Hz), 7.28 (1H, d, $J = 2.1$ Hz), 7.21–7.19 (1H, d, $J = 8.1$ Hz), 6.68–6.64 (dd, 1H, $J = 2.1$ Hz, $J = 6.0$ Hz, $J = 2.3$ Hz), 4.01 (s, 2H, SC**H**$_2$CO), 3.16 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.25 (s, 3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 171.93 (thiazolidin-4-one **C**=O), 161.53, 160.89, 159.41, 155.48, 151.41, 148.18, 146.00, 138.40, 134.26, 132.03, 130.81, 127.18, 123.75, 116.76, 116.62, 107.72, 32.60 (thiazolidin-4-one **C**H$_2$), 29.14 (thiazolidin-4-one N**C**H$_3$), 17.58 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 390.927 ([M+H]$^+$, 100); APCI$^-$ m/z (%): 389.285 ([M−H]$^-$, 100), 347.083 (87), 318,059 (27). Elemental analysis cald for $C_{20}H_{18}N_6OS$, C 61.52, H 4.65, N 21.52, S 8.21; found C 61.33, H 4.63, N 21.35, S 7.95.

3-Ethyl-2-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 7 Orange solid; yield 51%, 2.06 g. TLC R_f: 0.52 (S_1). HPLC t_R (min): 6.03 (CS_1). M.p.: 159–160 °C. IR υ_{max} (cm^{-1}): 3340 (N–H str), 2983–2947 (aliphatic C–H str), 1712 (thiazolidin-4-one C=O str), 1635, 1606, 1587, 1519 (C=N str, N–H bending, C=C str), 1402, 1386 (aliphatic C–H bending), 1230 (C–N str), 896, 786, 702 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.30 (d, 1H, $J = 1.8$ Hz), 8.90 (s, 1H, sec. N**H**), 8.70–8.68 (dd, 1H, $J = 1.8$, $J = 3.0$ Hz, $J = 4.8$ Hz), 8.53 (d, 1H, $J = 5.7$ Hz), 8.42 (d, 1H, $J = 8.4$ Hz), 7.55–7.50 (q, 1H), 7.45 (d, 1H, $J = 5.1$ Hz), 7.31 (1H, d, $J = 2.1$ Hz), 7.22 (1H, d, $J = 8.4$ Hz), 6.67–6.64 (dd, 1H, $J = 2.3$ Hz, $J = 5.6$ Hz, $J = 2.1$ Hz), 4.01 (s, 2H, -SC**H**$_2$CO), 3.79–3.72 (q, 2H, NC**H**$_2$CH$_3$), 2.25 (s, 3H, ArC**H**$_3$), 1.19–1.15 (t, 3H, NCH$_2$C**H**$_3$, $J = 6.9$ Hz, $J = 7.2$ Hz). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 172.24 (thiazolidin-4-one **C**=O), 162.02, 161.43, 160.05, 155.17, 152.02, 148.78, 146.57, 138.98, 134.78, 132.61, 131.38, 127.66, 124.31, 117.24, 117.10, 108.29, 39.81 (thiazolidin-4-one N**C**H$_2$CH$_3$), 33.10 (thiazolidin-4-one **C**H$_2$), 18.17 (Ar**C**H$_3$), 12.83 (thiazolidin-4-one NCH$_2$**C**H$_3$). LC/MS APCI$^+$ m/z (%): 419.155 ([M+CH$_3$]$^+$, 100), 405.259 ([M+H]$^+$, 29); APCI$^-$ m/z (%): 403.275 ([M−H]$^-$, 6), 97.034 (100). Elemental analysis cald for $C_{21}H_{20}N_6OS{\cdot}1/2EtOH$, C 61.81, H 5.42, N 19.66, S 7.50; found C 61.54, H 5.03, N 19.99, S 6.82.

General procedure for the synthesis of 5-substituted benzylidene-1,3-thiazolidin-4-one derivatives (8–49)

Compounds **2**–**4** (1.0 mmol) were dissolved in methanolic solution of sodium methoxide (1.0 mmol). Corresponding aldehyde (1.1 mmol) was added into the reaction medium. The mixture was refluxed for 4–24 h. At the end of the reaction, the product was cooled, washed with ice-cold water, and neutralized by 10% acetic acid. The precipitate was filtered and the crude product was washed with hot ethanol to obtain pure compounds (**8**–**49**).

5-(2-Fluorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 8 Yellow solid. Yield 80%, 0.385 g. TLC R_f: 0.49 (S_1). HPLC t_R (min): 6.12 (CS_1). M.p.: 183–186 °C. IR υ_{max} (cm^{-1}): 3352 (N–H str), 3031 (aromatic C–H str), 2945–2915 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1627, 1581, 1519 (C=N str, N–H bending, C=C str), 1415, 1400 (aliphatic C–H bending), 802 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.30 (d, 1H, $J = 1.5$ Hz), 8.90 (s, 1H, sec. N**H**), 8.70–8.68 (dd, 1H, $J = 1.5$ Hz, $J = 3.2$ Hz, $J = 1.5$ Hz), 8.53 (d, 1H, $J = 5.3$ Hz), 8.42 (d, 1H, $J = 8.5$ Hz), 7.55–7.51 (q, 1H), 7.44 (d, 1H, $J = 5.1$ Hz), 7.28 (1H, d, $J = 2.1$ Hz), 7.21–7.19 (1H, d, $J = 8.1$ Hz), 6.68–6.64 (dd, 1H, $J = 2.1$ Hz, $J = 6.0$ Hz, $J = 2.3$ Hz), 4.01 (s, 2H, SC**H**$_2$CO), 3.16 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.25 (s, 3H, ArC**H**$_3$). LC/MS APCI$^+$ m/z (%): 390.927 ([M+H]$^+$, 100); APCI$^-$ m/z (%): 389.285 ([M−H]$^-$, 100), 347.083 (87), 318,059 (27). Elemental analysis cald for $C_{20}H_{18}N_6OS$, C 64.14, H 4.39, N 16.62, S 6.34; found C 63.78, H 4.06, N 16.68, S 6.28.

5-(3-Fluorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 9 Yellow solid. Yield 47%, 0.226 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 4.30 (CS_1). M.p.: 254–256 °C. IR υ_{max} (cm^{-1}): 3437 (N–H str), 2978 (aliphatic C–H str), 1691 (thiazolidin-4-one C=O str), 1654, 1602, 1581, 1564, 1523 (C=N str, N–H bending, C=C str), 1446, 1415, 1402 (aliphatic C–H bending), 1224 (C–N str), 1147 (C–F str), 794, 777, 700 (aromatic C–H

bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.80–11.52 (bs, 3H-thiazolidin-4-one), 9.26 (s, 1H), 9.10 and 8.97 (s and s, 1H, sec. N**H**), 8.69–8.68 (dd, 0.5H, J = 1.5 Hz, J = 4.8 Hz, J = 3.3 Hz), 8.65–8.63 (dd, 0.5H, J = 1.5 Hz, J = 4.8 Hz, J = 3.3 Hz), 8.54–8.50 (q, 1H), 8.47–8.39 (m, 1H), 8.12 (s, 0.5H), 7.71 and 7.63 (s and s, 1H, =C**H**–Ar), 7.61–7.22 (m, 8H), 6.81–6.79 (d, 0.5H, J = 7.5 Hz), 2.28 and 2.26 (s, s, 3H, ArC**H$_3$**). LC/MS APCI$^+$ m/z (%): 482.892 ([M+H]$^+$ (100), 376.084 (48), 278.108 (22); APCI$^-$ m/z (%): 481.136 ([M−H]$^-$, 100). Elemental analysis cald for $C_{26}H_{19}FN_6OS$, C 64.72, H 3.97, N 17.42, S 6.65; found C 64.13, H 4.09, N 17.30, S 6.37.

5-(4-Fluorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 10 Yellow solid. Yield 43%, 0.207 g. TLC R_f: 0.52 (S_1). HPLC t_R (min): 5.48 (CS_2). M.p.: 240–243 °C. IR υ_{max} (cm^{-1}): 3452 (N–H str), 3071 (aromatic C–H str), 2918 (aliphatic C–H str 1653 (thiazolidin-4-one C=O str), 1577, 1539, 1516 (C=N str, N–H bending, C=C str), 1456, 1417 (aliphatic C–H bending), 1261 (C–N str), 1159 (C–F str), 887, 827, 788, 771 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.50–11.40 (bs, 3H-thiazolidin-4-one), 9.26 (s, 1H), 9.09 and 8.98 (s, 1H, sec. N**H**), 8.69–8.67 (d, 0.5H, J = 3.3 Hz), 8.65–8.64 (d, 0.5H, J = 4.8 Hz), 8.54–8.51 (t, 1H, J = 4.8 Hz, J = 4.2 Hz), 8.46–8.40 (m, 1H), 8.11 (s, 0.5H), 7.46 and 7.44 (s and s, 1H, =C**H**–Ar), 7.61–7.22 (m, 8H), 6.80–6.77 (d, 0,5H, J = 8.4 Hz), 3.83–3.77 (s, s, 2H), 2.28 and 2.26 (s, s, 3H, ArC**H$_3$**). LC/MS ESI$^+$ m/z (%): 505.192 ([M+Na]$^+$, 100), 483.101 ([M+H]$^+$, 77), ESI$^-$ m/z (%): 481.268 ([M−H]$^-$, 7), 89.203 (100). Elemental analysis cald for $C_{26}H_{19}FN_6OS$·MeOH, C 63.02, H 4.51, N 16.33, S 6.32; found C 63.42, H 4.35, N 16.74, S 6.03.

5-(2,6-Difluorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 11 Yellow solid. Yield 41%, 0.205 g. TLC R_f: 0.47 (S_1). HPLC t_R (min): 1.56 (CS_6). M.p.: 161–163 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 2935 (aliphatic C–H str), 1639 (thiazolidin-4-one C=O str), 1604, 1573, 1523 (C=N str, N–H bending, C=C str), 1473, 1452, 1423, 1321 (aliphatic C–H bending, C–N str), 1091 (C–F str), 779, 748, 702 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.20 (s, 1H), 8.66 (d, 1H, J = 3.3 Hz), 8.50 (d, 1H, J = 5.1 Hz), 8.34 (d, 1H, J = 8.1), 8.02 (s, 1H), 7.83 and 7.70 (s, 1H, =C**H**–Ar), 7.38–7.17 (m, C**H**Cl$_3$ and 3.5H), 6.90–6.82 (m, 3H), 6.91–6.82 (m, 0.5H), 2.32 and 2.28 (s, s, 3H, ArC**H$_3$**). Elemental analysis cald for $C_{26}H_{18}F_2N_6OS·2H_2O$, C 58.20, H 4.13, N 15.66, S 5.98; found C 58.58, H 4.16, N 15.53, S 5.32.

5-(2-Chlorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 12 Yellow solid. Yield 68%, 0.338 g. TLC R_f: 0.52 (S_1). HPLC t_R (min): 5.68 (CS_1). M.p.: 240–242 °C. IR υ_{max} (cm^{-1}): 3444 (N–H str), 3051 (aromatic C–H str), 2978 (aliphatic C–H str), 1691 (thiazolidin-4-one C=O str), 1643, 1573, 1552, 1533, 1514 (C=N str, N–H bending, C=C str), 1471, 1454, 1435, 1427, 1394 (aliphatic C–H bending), 1222 (C–N str), 1190 (C–Cl str), 881, 808, 756 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (s, 1H), 9.08 and 8.95 (s, 1H, sec. N**H**), 8.68–8.67 (d, 0.5H, J = 3.5 Hz), 8.65–8.63 (d, 0.5H, J = 3.5 Hz), 8.54–8.52 (d, 0.5H, J = 5.0 Hz), 8.49–8.48 (d, 0.5H, J = 5.0 Hz), 8.46–8.38 (m, 1H), 8.12 (s, 0.5H), 7.88 and 7.78 (s, s, 1H, =C**H**–Ar), 7.68–7.24 (m, 8H), 6.75–6.72 (d, 0.5H, J = 7.7 Hz), 2.27 and 2.26 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 179.74 and 170.22, 161.71 and 161.55, 160.91 and 160.80, 159.44 and 159.37, 151.33, 148.12 and 148.08, 138.59 and 138.28, 134.39 and 134.03 (=**C**H–Ar), 134.28 and 134.22, 132.10 and 132.01, 131.44, 131.16 and 131.06, 130.65, 130.22 and 130.13, 128.64, 128.57, 128.00, 127.87, 125.11, 124.40, 123.77 and 123.64, 117.33 and 117.16, 116.96 and 116.68, 107.91, 17.70 and 17.63. LC/MS APCI$^+$ m/z (%): 500.991 ([M+2]$^+$, 62), 498.938 ([M+H]$^+$, 98), 463.087 (54), 376.07 (100); APCI$^-$ m/z (%): 497.103 ([M−H]$^-$, 100), 461.311 (54), 139.17 (47). Elemental analysis cald for $C_{26}H_{19}ClN_6OS·1/2H_2O$, C 61.47, H 3.97, N 16.54, S 6.31; found C 61.77, H 4.05, N 16.77, S 6.43.

5-(4-Chlorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 13 Yellow solid. Yield 13%, 0.065 g. TLC R_f: 0.54 (S_1). HPLC t_R (min): 2.08 (CS_3). M.p.: 227–231 °C. IR υ_{max} (cm^{-1}): 3443 (N–H str), 1691 (thiazolidin-4-one C=O str), 1637, 1612, 1571, 1564, 1523, 1518 (C=N str, N–H bending, C=C str), 1454, 1445, 1415, 1398 (aliphatic C–H bending), 1089 (C–Cl str), 783, 700 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.09 (bs, 3H-thiazolidin-4-one 0.6H), 9.26 (s, 1H), 9.02 and 8.97 (s, s 1H, sec. N**H**), 8.69–8.65 (dd, 1H, J = 3.3 Hz, J = 6.6 Hz, J = 1.5 Hz), 8.54–8.51 (t, 1H, J = 4.5 Hz, J = 5.1 Hz), 8.44–8.40 (m, 1H), 8.09 (s, 0.4H), 7.69–7.25 (m, 9H), 6.79–6.77 (d, 0.6H, J = 7.8 Hz), 2.28 and 2.25 (s, s, 3H, ArCH$_3$). LC/MS APCI$^+$ m/z (%): 521.127 ([M+Na]$^+$, 100), 523.066 ([M+Na+2]$^+$, 40), 499.099 ([M+H]$^+$, 37), 343.18 (37), 303.133 (40), 259.180 (43), 131.173 (83); APCI$^-$ m/z (%): 497.068 ([M−H]$^-$, 7), 181.15 (34), 155.209 (41), 97.039 (46), 59.118 (100). Elemental analysis cald for $C_{26}H_{19}ClN_6OS·2H_2O$, C 58.37, H 4.33, N 15.71, S 5.99; found C 58.94, H 3.98, N 16.25, S 5.58.

5-(4-Bromobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 14 Yellow solid. Yield 59%, 0.320 g. TLC R_f: 0.55 (S_1). HPLC t_R (min): 3.28 (CS_4). M.p.: 198–200 °C. IR υ_{max} (cm^{-1}):

3446 (N–H str), 1649 (thiazolidin-4-one C=O str), 1587, 1548, 1535 (C=N str, N–H bending, C=C str), 1415 (aliphatic C–H bending), 1003 (C–Br str), 812–798 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25–9.23 (dd, 1H, J = 1.5 Hz, J = 5.5 Hz, J = 1.8 Hz), 8.83 (s, 1H, sec. N**H**), 8.68–8.67 (dd, 0.5H, J = 1.5 Hz, J = 4.8 Hz, J = 1.5 Hz), 8.66–8.63 (dd, 0.5 H, J = 1.5 Hz, J = 4.7 Hz, J = 1.8 Hz), 8.50 (d, 1H, J = 5.1 Hz), 8.48–8.42 (m, 1H), 7.64–7.35 (m, 6.5H), 7.18–7.03 (m, 3H), 6.75–6.72 (dd, 0,5H, J = 2.1 Hz, J = 6.0 Hz, J = 8.1 Hz), 2.23 and 2.17 (s, s, 3H, ArC**H$_3$**). LC/MS ESI$^+$ m/z (%): 564.962 ([M+Na]$^+$, 100), 347.165 (47); ESI$^-$ m/z (%): 541.276 ([M−H]$^-$, 100). Elemental analysis cald for $C_{26}H_{19}BrN_6OS$·MeOH, C 63.02, H 4.51, N 16.33, S 6.23; found C 63.42, H 4.35, N 16.74, S 6.03.

5-(4-Trifluoromethylbenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 15 Yellow solid. Yield 71%, 0.377 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 11.94 (CS_1). M.p.: 257–262 °C (dec.). IR υ_{max} (cm^{-1}): 3441 (N–H str), 3055 (aromatic C–H str), 1699 (thiazolidin-4-one C=O str), 1639, 1612, 1583, 1537, 1514 (C=N str, N–H bending, C=C str), 1456, 1438 (aliphatic C–H bending), 1224 (C–N str), 1114 (C–F str), 810, 783, 752 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 13.05–11.20 (bs, 3**H**-thiazolidin-4-one), 9.26 (s, 1H), 9.10 and 8.97 (s, 1H, sec. N**H**), 8.68 (d, 1H, J = 3.4 Hz), 8.63 (d, 1H, J = 3.4 Hz), 8.55–8.51 (t, 1H, J = 5.1 Hz, J = 9.1 Hz, J = 6.0 Hz), 8.47–8.39 (m, 1H), 8.12 (s, 0.5H), 7.93–7.71 (m, 5H), 7.53–7.26 (m, 4H), 6.81–6.79 (d, 0.5H, J = 7.7 Hz), 2.29 and 2.26 (s, s, 3H, ArC**H$_3$**). LC/MS APCI$^+$ m/z (%): 532.659 ([M+H]$^+$, 100), 338.199 (17); APCI$^-$ m/z (%): 531.087 ([M−H]$^-$, 100). Elemental analysis cald for $C_{27}H_{19}F_3N_6OS$·3/2H_2O, C 57.95, H 3.96, N 15.02, S 5.73; found C 58.17, H 3.07, N 15.00, S 5.82.

5-(4-Trifluoromethoxybenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 16 Yellow-orange solid. Yield 47%, 0.260 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 4.30 (CS_1). M.p.: 250–256 °C (dec.). IR υ_{max} (cm^{-1}): 3444 (N–H str), 3063 (aromatic C–H str), 2978 (aliphatic C–H str), 1697 (thiazolidin-4-one C=O str), 1641, 1583, 1541, 1516 (=N str, N–H bending, C=C str), 1458, 1438, 1400 (aliphatic C–H bending), 1249 (C–O str), 1224 (C–N str), 1157 (C–F str), 810, 783, 702 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.90–11.40 (bs, 3H-thiazolidin-4-one), 9.26 (d, 1H, J = 1.8 Hz), 9.10 and 8.98 (s, 1H, sec. N**H**), 8.69–8.75 (dd, 0.5 H, J = 1.5 Hz, J = 4.8 Hz, J = 1.5 Hz), 8.70–8.62 (dd, 0.5 H, J = 1.5 Hz, J = 4.7 Hz, J = 1.8 Hz), 8.55–8.50 (t, 1H, J = 5.2 Hz, J = 11.4 Hz, J = 5.2 Hz), 8.47–8.39 (m, 1H), 8.12 (s, 0.5H), 7.78–7.25 (m, 9H), 6.75–6.72 (d, 0.5 H, J = 5.4 Hz), 2.28 and 2.25 (s, s, 3H, ArC**H$_3$**). LC/MS APCI$^+$ m/z (%): 548.663 (M$^+$, 100); APCI$^-$ m/z (%): 547,095 ([M−H]$^-$, 100). Elemental analysis cald for $C_{27}H_{19}F_3N_6O_2S$, C 59.12, H 3.49, N 15.32, S 5.85; found C 58.52, H 3.72, N 15.09, S 5.80.

5-(2-Hydroxybenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 17 Yellow solid. Yield 27%, 0.130 g. TLC R_f: 0.49 (S_1). HPLC t_R (min): 2.61 (CS_2). M.p.: 217–218 °C (dec.). IR υ_{max} (cm^{-1}): 3450–3100 (O–H str, N–H str), 3066 (aromatic C–H str), 2974 (aliphatic C–H str), 1680 (thiazolidin-4-one C=O str), 1573, 1525 (C=N str, N–H bending, C=C str), 1444, 1417, 1402 (aliphatic C–H bending, C–N str), 1193 (C–O str), 800, 758 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26–6.77 (14H, ArH), 2.35–2.15 (3H, ArC**H$_3$**). Elemental analysis cald for $C_{26}H_{20}N_6O_2S$·3/2EtOH, C 63.37, H 5.32, N 15.29, S 5.83; found C 62.74, H 4.56, N 14.40, S 3.83.

5-(2-Methoxybenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 18 Yellow-orange solid. Yield 29%, 0.145 g. TLC R_f: 0.49 (S_1). HPLC t_R (min): 1.56 (CS_5). M.p.: 228–230 °C. IR υ_{max} (cm^{-1}): 3443 (N–H str), 2902 (aliphatic C–H str), 1687 (thiazolidin-4-one C=O str), 1645, 1581, 1521 (C=N str, N–H bending, C=C str), 1479, 1442, 1415 (aliphatic C–H bending), 1330 (C–N str), 1209 (C–O str), 833, 798, 723 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, D_2O Exchange, DMSO-d_6): 9.25 (s, 1H), 8.69–8.65–6.80 (m, 14H), 3.86 (D_2O), 3.90–3.86 (s, 3H, OC**H$_3$**), 2.29 and 2.27 (s, 3H, ArC**H$_3$**). Elemental analysis cald for $C_{27}H_{22}N_6O_2S$·EtOH, C 64.97, H 4.87, N 16.24, S 6.19; found C 64.86, H 4.60, N 16.47, S 6.14.

5-(3-Methoxybenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 19 Yellow-orange solid. Yield 56%, 0.275 g. TLC R_f: 0.49 (S_1). HPLC t_R (min): 1.69 (CS_3). M.p.: 160–161 °C. IR υ_{max} (cm^{-1}): 3453 (N–H str), 2962 (aliphatic C–H str), 1691 (thiazolidin-4-one C=O str), 1649, 1606, 1564, 1552, 1523 (C=N str, N–H bending, C=C str), 1446, 1415 (aliphatic C–H bending), 1398 (C–N str), 1147 (C–F str), 877, 785, 763, 752 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, $CDCl_3$): 9.24 and 9.20 (s, s, 1H), 8.63–8.33 (m, 3H), 8.00 (s, 1H), 7.77–6.57 (m, C**H**Cl$_3$ and 9H), 3.87–3.75 (m, 3H, OC**H$_3$**), 2.37 and 2.33 (s, s, 3H, ArC**H$_3$**). Elemental analysis cald for $C_{27}H_{22}N_6O_2S$·H_2O·3/2H_2O, C 62.17, H 5.41, N 15.04, S 6.15; found C 62.15, H 4.95, N 15.84, S 5.66.

5-(4-Methoxybenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 20 Yellow-orange solid. Yield 61%, 0.300 g. TLC R_f: 0.49

(S_1). HPLC t_R (min): 1.47 (CS_6). M.p.: 235–238 °C. IR υ_{max} (cm^{-1}): 3450 (N–H str), 3012 (aromatic C–H str), 2972 (aliphatic C–H str), 1710 (thiazolidin-4-one C=O str), 1649, 1600, 1573, 1537, 1514 (C=N str, N–H bending, C=C str), 1454, 1417, 1402, 1336 (aliphatic C–H bending, C–N str), 1180, 1157 (C–O str), 825, 790, 769, 700 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.5–11.5 (bs), 9.26 (d, 1H, J = 1.5 Hz), 9.08 and 8.96 (s, and s, 1H, sec. N**H**), 8.68–8.64 (m, 1H), 8.52 (d, 1H, J = 5,1 Hz), 8.46–8.41 (m, 1H), 8.11 (s, 0.4H), 7.67–7.38 (m, 6H), 7.31–7.25 (t, 1H, J = 7.5 Hz, J = 7.8 Hz), 7.13 (d, 1H, J = 8.4 Hz), 7.03 (d, 1H, J = 8.7 Hz), 6.80 (d, 1H, J = 7.2 Hz), 6.77 (d, 0.6H, J = 7.2 Hz), 3.83–3.78 (s, s, 3H, OC**H$_3$**), 2.28 and 2.25 (s, s, 3H, ArC**H$_3$**). Elemental analysis cald for $C_{27}H_{22}N_6O_2S$, C 65.57, H 4.48, N 16.99, S 6.47; found C 65.12, H 4.53, N 16.82, S 5.77.

5-(N,N-Dimethylaminobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 21 Yellow solid. Yield 43%, 0.220 g. TLC R_f: 0.45 (S_1). HPLC t_R (min): 3.28 (CS_3). M.p.: 246–251 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 2899 (aliphatic C–H str), 1649 (thiazolidin-4-one C=O str), 1620, 1573, 1548 (C=N str, N–H bending, C=C str), 1473, 1444, 1413 (aliphatic C–H bending), 1338 (C–N str), 1195 (C–Cl str), 802, 754, 705 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 12.12 and 11.51 (bs and bs 0.9 H, 3H-thiazolidin-4-one), 9.27 (s, 1H, sec. N**H**), 9.08 and 8.97 (s, s 1H), 8.66 (s, 1H), 8.53–8.52 (d, 1H, J = 5.1 Hz), 8.44–8.40 (m, 1H), 8.11 (s, 0.5H), 7.60–7.26 (m, 7H), 6.85–6.72 (m, 2.5H), 3.01 and 3.95 (s and s, 6H), 2.28 and 2.26 (s and s, 3H, ArC**H$_3$**). LC/MS APCI$^+$ m/z (%): 482.892 [M+H]$^+$, 100), 376.084 (48), 278.108 (22); APCI$^-$ m/z (%): 481.136 ([M−H]$^-$, 100). Elemental analysis cald for $C_{28}H_{25}N_7OS{\cdot}3/2H_2O$, C 62.90, H 5.28, N 18.34, S 6.00; found C 62.65, H 5.00, N 18.56, S 5.63.

5-(2-Fluorobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 22 Yellow solid. Yield 65%, 0.322 g. TLC R_f: 0.56 (S_1). HPLC t_R (min): 13.47 (CS_6). M.p.: 210–213 °C. IR υ_{max} (cm^{-1}): 3410 (N–H str), 3030 (aromatic C–H str), 2912 (aliphatic C–H str), 2858 (aliphatic C–H str sim.), 1703 (thiazolidin-4-one C=O str), 1631, 1604, 1583, 1556, 1516 (C=N str, N–H bending, C=C str), 1435, 1411, 1398, 1363 (aliphatic C–H bending), 1232 (C–N str), 1118 (C–F str,), 825, 806, 752, 700 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 (d, 1H, J = 1.5 Hz), 8.95 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.6 Hz), 8.51 (d, 1H, J = 5.0 Hz), 8.42 (d, 1H, J = 8.5 Hz), 7.75 (s, 1H, =C**H**–Ar), 7.52–7.26 (m, 6H), 7.37 (d, J = 2.7 Hz, 1H), 7.28 (d, 1H, J = 8.1 Hz), 6.76–6.73 (dd, 1H, J = 2.1 Hz, J = 5.8 Hz, J = 2.1 Hz), 3.35 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.28 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.15 (thiazolidin-4-one **C**=O), 162.11, 161.42, 159.94, 159.57, 151.93, 150.34, 148.68, 146.17, 139.16, 134.79 (=**C**H–Ar), 132.73, 132.58, 131.66, 129.20, 128.53, 125.83, 125.00, 124.25, 121.80, 121.28, 117.45, 117.18, 116.76, 116.55, 108.42, 30.24 (thiazolidin-4-one N**C**H$_3$), 18.22 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 496.757 (M$^+$, 100), 498.002 ([M+H]$^+$), 338.187 (38); LC/MS APCI$^-$ m/z (%): 495.228 ([M−H]$^-$, 100), 219.358 (26). Elemental analysis cald for $C_{27}H_{21}FN_6OS{\cdot}1/2MeOH$, C 64.44, H 4.52, N 16.40, S 6.26; found C 64.60, H 4.44, N 16.64, S 6.43.

5-(3-Fluorobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 23 Yellow solid. Yield 56%, 0.277 g. TLC R_f: 0.60 (S_1). HPLC t_R (min): 12.49 (CS_6). M.p.: 184–185 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 3010 (aromatic C–H str), 1720 (thiazolidin-4-one C=O str), 1641, 1577, 1533 (C=N str, N–H bending, C=C str), 1415 (aliphatic C–H bending), 1120 (C–F str), 792–783 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.64–8.62 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.27 Hz), 8.41 (d, 1H, J = 7.9 Hz), 7.75 (s, 1H, =C**H**–Ar), 7.54–7.23 (m, 8H), 6.77 (d, 1H, J = 5.6 Hz), 3.37 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.29 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 165.66 (thiazolidin-4-one C=O), 163.77, 161.61, 160.87, 160.53, 159.33 151.33, 149.71, 148.09, 145.59, 138.58, 135.77, 135.66, 134.22 (=**C**H–Ar), 132.03, 131.15, 128.40, 127.92, 125.20, 125.16, 123.65, 123.29, 116.82, 116.68, 116.27, 107.86, 29.62 (thiazolidin-4-one N**C**H$_3$), 17.62 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 498.081 ([M+H]$^+$, 100), 496.897 (M+, 70). LC/MS APCI$^-$ m/z (%): 495.312 ([M−H]$^-$, 100), 347.148 (24). Elemental analysis cald for $C_{27}H_{21}FN_6OS$, C 65.31, H 4.26, N 16.92, S 6.46; found C 65.20, H 4.26, N 16.89, S 6.29.

5-(4-Fluorobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 24 Yellow solid. Yield 65%, 0.320 g. TLC R_f: 0.60 (S_1). HPLC t_R (min): 14.17 (CS_6). M.p.: 217–219 °C. IR υ_{max} (cm^{-1}): 3410 (N–H str), 3053 (aromatic C–H str), 2912 (aliphatic C–H str); 1699 (thiazolidin-4-one C=O str), 1629, 1581, 1558, 1519,1508 (C=N str, N–H bending, C=C str), 1437, 1402, 1363 (aliphatic C–H bending), 1116 (C–F str), 802 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (d, 1H, J = 2.0 Hz), 8.94 (s, 1H, sec. N**H**), 8.63–8.61 (dd, 1H, J = 1.6 Hz, J = 3.2 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.27 Hz), 8.43–8.39 (d, 1H, J = 7.9 Hz), 7.74 (s, 1H, =C**H**–Ar), 7.36 (d, 1H, J = 2.0 Hz), 7.60–7.25 (m, 8H), 6.76–6.72 (dd, 1H, J = 2.0 Hz, J = 5.9 Hz, J = 2.1 Hz), 3.33 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.28 (s,

3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.43 (thiazolidin-4-one **C**=O), 162.14, 161.79, 161.42, 159.95, 151.93, 150.58, 148.68, 146.30, 139.16, 134.81 (=**C**H–Ar), 132.85, 132.66, 132.59, 131.65, 130.59, 129.33, 128.42, 124.25, 121.86, 117.45, 117.13, 117.03, 116.81, 108.43, 29.66 (thiazolidin-4-one N**C**H$_3$), 18.22 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 519.242 ([M+Na]$^+$, 100); LC/MS APCI$^-$ m/z (%): 495.398 ([M−H]$^-$, 92), 219, 369 (71), 78.972 (100). Elemental analysis cald for $C_{27}H_{21}FN_6OS{\cdot}H_2O$, C 63.02, H 4.51, N 16.33, S 6.24; found C 63.06, H 4.37, N 16.26, S 6.24.

5-(2,6-Difluorobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 25 Yellow solid. Yield 64%, 0.330 g. TLC R_f: 0.55 (S_1). HPLC t_R (min): 11.94 (CS_6). M.p.: 172–174 °C. IR υ_{max} (cm^{-1}): 3429 (N–H str), 3041, 3022 (aromatic C–H str), 2939 (aliphatic C–H str), 1710 (thiazolidin-4-one C=O str), 1629, 1602, 1583, 1519 (C=N str, N–H bending, C=C g.b), 1452, 1437, 1415, 1398, 1369 (aliphatic C–H bending), 1257 (C–N str), 1114 (C–F str), 877, 792, 740 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.5 Hz), 8.92 (s, 1H, sec. N**H**), 8.66–8.64 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.6 Hz), 8.48 (d, 1H, J = 5.1 Hz), 8.40 (d, 1H, J = 8.5 Hz), 7.60 (s, 1H, =C**H**–Ar), 7.57–7.43 (m,3H), 7.32 (d, 1H, J = 2.1 Hz), 7.25–7.16 (m, 3H), 6.72–6.69 (dd, J = 2.1 Hz, 1H, J = 5.8 Hz, J = 8.1 Hz), 3.34 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.26 (s, 3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.59 (thiazolidin-4-one **C**=O), 162.11, 161.40, 161.05, 159.91, 158.56, 151.91, 150.31, 148.67, 146.19, 139.10, 134.76 (=**C**H–Ar), 133.14, 132.55, 131.55, 129.54, 128.52, 124.20, 117.83, 117.40, 117.18, 112.89, 112.79, 111.39, 108.36, 30.27 (thiazolidin-4-one N**C**H$_3$), 18.17 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 515.893 ([M+H]$^+$, 100), 514.655 (M$^+$, 90), APCI$^-$ m/z (%): 513.128 ([M−H]$^-$, 100), 219.338 (17). Elemental analysis cald for $C_{27}H_{20}F_2N_6OS{\cdot}H_2O$, C 60.89, H 4.16, N 15.78, S 6.02; found C 60.83, H 4.05, N 15.80, S 6.17.

5-(2-Chlorobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 26 Yellow solid. Yield 55%, 0.282 g. TLC R_f: 0.56 (S_1). HPLC t_R (min): 4.08 (CS_1). M.p.: 205-210 °C. IR υ_{max} (cm^{-1}): 3443 (N–H str), 3070 (aromatic C–H str), 2976 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1663, 1600, 1583, 1518 (C=N str, N–H bending, C=C str), 1437, 1417, 1398, 1365 (aliphatic C–H bending), 1259 (C–N str), 1105 (C–Cl str), 881, 798, 759, 702 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.7 Hz), 8.95 (s, 1H, sec. N**H**), 8.66–8.63 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.7 Hz), 8.50 (d, 1H, J = 5.0 Hz), 8.41 (d, 1H, J = 8.5 Hz), 7.78 (s, 1H, =C**H**–Ar), 7.62–7.60 (m, 1H),7.49–7.38 (m, 5H), 7.35 (d, 1H, J = 2.0 Hz), 7.27 (d, 1H, J = 8.3 Hz), 6.75–6.72 (dd, 1H, J = 2.3 Hz, J = 5.6 Hz, J = 2.1 Hz), 3.34 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.27 (s, 3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.05 (thiazolidin-4-one **C**=O), 162.13, 162.02, 161.41, 159.94, 151.93, 150.38, 148.69, 146.16, 139.15, 134.79 (=**C**H–Ar), 134.59, 132.81, 132.59, 131.96, 131.87, 130.77, 129.33, 128.55, 125.95, 125.50, 124.23, 117.44, 117.17, 108.43, 30.23 (thiazolidin-4-one N**C**H$_3$), 18.21 (Ar**C**H$_3$). LC/MS ESI$^+$ m/z (%): 513.125 ([M+H]$^+$, 34), 515.129 ([M+H+2]$^+$, 29), 325.376 (69), 288.347 (100). LC/MS ESI$^-$ m/z (%): 78.95 (100). Elemental analysis cald for $C_{27}H_{21}ClN_6OS{\cdot}3/4H_2O$, C 61.59, H 4.31, N 15.96, S 6.09; found C 61.98, H 4.28, N 16.06, S 6.08.

5-(4-Chlorobenzylidene)-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 27 Yellow solid. Yield 63%, 0.323 g. TLC R_f: 0.60 (S_1). HPLC t_R (min): 3.44 (CS_4). M.p.: 190–193 °C. IR υ_{max} (cm^{-1}): 3435 (N–H str), 3093, 3049 (aromatic C–H str), 1712 (thiazolidin-4-one C=O str), 1643, 1606, 1575, 1548, 1523 (C=N str, N–H bending, C=C str), 1413 (aliphatic C–H bending), 1093 (C–Cl str), 798 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (d, 1H, J = 2.0 Hz), 8.94 (s, 1H, sec. N**H**), 8.63–8.61 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.7 Hz), 8.50 (d, 1H, J = 5.3 Hz), 8.41 (d, 1H, J = 8.1 Hz), 7.71 (s, 1H, =C**H**–Ar), 7.53–7.41 (m, 6H), 7.36 (d, 1H, J = 2.1 Hz), 7.27 (d, 1H, J = 8.1 Hz), 6.75–6.72 (dd, 1H, J = 2.3 Hz, J = 5.8 Hz, J = 2.1 Hz), 3.33 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.28 (s, 3H, ArC**H**$_3$). Elemental analysis cald for $C_{27}H_{21}ClN_6OS$, C 63.21, H 4.13, N 16.38, S 6.25; found C 64.24, H 4.42, N 16.72, S 6.55.

5-(4-Bromobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 28 Yellow solid. Yield 58%, 0.325 g. TLC R_f: 0.58 (S_1). HPLC t_R (min): 14.17 (CS_6). M.p.: 205–207 °C. IR υ_{max} (cm^{-1}): 3433 (N–H str), 3095(aromatic C–H str), 1714 (thiazolidin-4-one C=O str), 1641 1604, 1575, 1548, 1523 (C=N str, N–H e.b), (C=C str), 1413 (aliphatic C–H bending), 1003 (C–Br str), 798 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (d, 1H, J = 1.8 Hz), 8.94 (s, 1H, sec. N**H**), 8.63–8.61 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.7 Hz), 8.51 (d, 1H, J = 5.3 Hz), 8.41 (d, 1H, J = 8.1 Hz), 7.69 (s, 1H, =C**H**–Ar), 7.65 (d, 2H, J = 8.4 Hz), 7.45–7.42 (m, 3H), 7.36 (d, 1H, J = 2.1 Hz), 7.28 (d, 1H, J = 8.1 Hz), 6.75–6.72 (dd, 1H, J = 2.1 Hz, 1H, J = 5.8 Hz, J = 2.3 Hz), 3.33 (s, 3H, thiazolidin-4-one NC**H**$_3$), 2.28 (s, 3H, ArC**H**$_3$). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.37 (thiazolidin-4-one **C**=O), 162.14, 161.42, 159.96, 151.96, 150.43, 148.69, 146.26, 139.19, 134.80 (=**C**H–Ar), 133.11, 132.86, 132.59, 132.74, 132.56, 132.10, 131.66, 129.17, 128.43, 124.25, 123.86, 123.01, 117.45, 117.06, 108.43, 30.20 (thiazolidin-4-one N**C**H$_3$), 18.23 (Ar**C**H$_3$). LC/MS ESI$^+$ m/z

(%): 580.097 ([M+Na]$^+$, 100); ESI$^-$ m/z (%): 75.12 (100). Elemental analysis cald for $C_{27}H_{21}BrN_6OS{\cdot}H_2O$, C 56.35, H 4.03, N 14.60, S 5.57; found C 56.92, H 3.86, N 14.80, S 5.74.

5-(4-Trifluoromethylbenzylidene)-3-methyl--[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 29 Yellow solid. Yield 40%, 0.218 g. TLC R_f: 0.58(S_1). HPLC t_R (min): 4.49 (CS_4). M.p.: 196–200 °C. IR υ_{max} (cm^{-1}): 3435 (N–H str), 3030 (aromatic C–H str), 2945 (aliphatic C–H str), 1703 (thiazolidin-4-one C=O str), 1639, 1602, 1579, 1566, 1554, 1523 (C=N str N–H bending, C=C str), 1448, 1415, 1400, 1319 (aliphatic C–H bending), 1263 (C–N str), 1114 (C–F str), 794, 702 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 (d, 1H, J = 1.5 Hz), 8.95 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.2 Hz, J = 1.6 Hz), 8.51 (d, 1H, J = 5.0 Hz), 8.42 (d, 1H, J = 8.5 Hz), 7.75 (s, 1H, =C**H**–Ar), 7.52–7.26 (m, 6H), 7.37 (d, 1H, J = 2.7 Hz), 7.29 (d, 1H, J = 8.1 Hz), 6.76–6.73 (dd, J = 2.1 Hz, 1H, J = 5.8 Hz, J = 8.1 Hz), 3.35 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.28 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.20 (thiazolidin-4-one **C**=O), 162.15, 162.42, 159.94, 151.93, 150.25, 148.68, 146.19, 139.20, 137.88, 134.77 (=**C**H–Ar), 132.58, 131.66, 130.74, 129.86, 129.54, 128.56, 128.49, 126.52, 125.75, 125.21, 124.20, 123.03, 117.42, 117.02, 108.43, 30.24 (thiazolidin-4-one N**C**H$_3$), 18.23 (Ar**C**H$_3$). LC/MS APCI$^+$ m/z (%): 547.860 ([M+H]$^+$, 100), 546.547 (M$^+$, 94), 514.953 (21), 338.200 (22); APCI$^-$ m/z (%): 545.146 ([M−H]$^-$, 100), 219.351 (11). Elemental analysis cald for $C_{28}H_{21}F_3N_6OS{\cdot}H_2O$, C 61.53, H 3.87, N 15.38, S 5.87; found C 61.26, H 3.90, N 15.27, S 5.93.

5-(4-Trifluoromethoxybenzylidene)-3-methyl--[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 30 Yellow solid. Yield 53%, 0.296 g. TLC R_f: 0.58 (S_1). HPLC t_R (min): 4.59 (CS_1). M.p.: 184–187 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 3038 (aromatic C–H str), 1713 (thiazolidin-4-one C=O str), 1644, 1581, 1567, 1556, 1529 (C=N str, N–H bending, C=C str), 1412 (aliphatic C–H bending), 1153 (C–F str), 794 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.64–8.62 (dd, 1H, J = 1.7 Hz, J = 3.2 Hz, J = 1.7 Hz), 8.52 (d, 1H, J = 5.1 Hz), 8.42 (d, 1H, J = 8.5 Hz), 7.78 (s, 1H, =C**H**–Ar), 7.68–7.64 (m, 2H), 7.47–7.42 (m, 4H), 7.37 (d, 1H, J = 2.0 Hz), 7.28 (d, 1H, J = 8.3 Hz), 6.76–6.73 (dd, 1H, J = 2.3 Hz, J = 5.8 Hz, J = 2.1 Hz), 3.35 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.29 (s, 3H, ArC**H$_3$**). LC/MS APCI$^+$ m/z (%): 585.155 ([M+Na]$^+$, 23), 563.111 ([M+H]$^+$, 100), 360.380 (53); APCI$^-$ m/z (%): 97.036 (100). Elemental analysis cald for $C_{28}H_{21}F_3N_6O_2S$, C 59.78, H 3.76, N 14.94, S 5.70; found C 59.49, H 3.89, N 14.84, S 5.62.

5-(2-Hydroxybenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 31 Yellow solid. Yield 32%, 0.158 g. TLC R_f: 0.53 (S_1). HPLC t_R (min): 1.72 (CS_7). M.p.: 195–198 °C. IR υ_{max} (cm^{-1}): 3387 (O–H str, N–H str), 3041 (aromatic C–H str), 2951 (aliphatic C–H str), 1716 (thiazolidin-4-one C=O str), 1633, 1593, 1560, 1527 (C=N str, N–H bending, C=C str), 1444, 1415 (aliphatic C–H bending, C–N str), 1122 (C–O str), 896, 758, 704 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 10.47 (s, 0.7 H), 9.27 (d, 1H, J = 1.5 Hz), 8.98 (s, sec. N**H**), 8.66–8.64 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.1 Hz), 8.44–8.40 (m, 1H), 7.98 (s, 1H, =C**H**–Ar), 7.47–7.43 (m, 2H), 7.33 (d, 1H, J = 2.1 Hz), 7.27–7.23 (m, 3H), 7.21 (d, 1H, J = 8.1 Hz), 6.87 (t, 1H, J = 7.8 Hz, J = 7.5 Hz), 6.76–6.73 (dd, 1H, J = 2.1 Hz, J = 6.0 Hz, J = 2.1 Hz), 3.34 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.27 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.68 (thiazolidin-4-one **C**=O), 162.11, 161.46, 159.94, 151.93, 151.17, 148.68, 146.45, 139.12, 134.80 (=**C**H–Ar), 132.61, 132.65, 131.60, 128.74, 128.39, 125.65, 124.26, 120.85, 120.55, 120.05, 117.59, 117.17, 116.52, 110.33, 108.35, 30.03 (thiazolidin-4-one N**C**H$_3$), 18.21 (Ar**C**H$_3$). Elemental analysis cald for $C_{27}H_{22}N_6O_2S{\cdot}H_2O$, C 63.27, H 4.72, N 16.40, S 6.26; found C 62.97, H 4.44, N 15.97, S 6.04.

5-(2-Methoxybenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 32 Yellow solid. Yield 67%, 0.340 g. TLC R_f: 0.59 (S_1). HPLC t_R (min): 3.09 (CS_3). M.p.: 177–180 °C. IR υ_{max} (cm^{-1}): 3450 (N–H str), 3051, 3016 (aromatic C–H str), 2897, 2845(aliphatic C–H str), 1716 (thiazolidin-4-one C=O str), 1643, 1612, 1577, 1558, 1523 (C=N str, N–H bending, C=C str), 1454, 1410, 1396 (aliphatic C–H bending, C–N str), 1128 (C–O str), 879, 796, 746 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.66–8.63 (dd, 1H, J = 1.5 Hz, J = 3.3 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.1 Hz), 8.43–8.40 (m, 1H), 7.95 (s, 1H, =C**H**–Ar), 7.45–7.27 (m, 6H), 7.13 (d, 1H, J = 8.4 Hz), 7.02–6.97 (t, 1H, J = 7.5 Hz, J = 7.8 Hz), 6.76–6.72 (dd, 1H, J = 2.1 Hz, J = 3.0 Hz, J = 2.1 Hz), 3.87 (s, 3H, OC**H$_3$**), 3.34 (s, 3H, thiazolidin-4-one NC**H$_3$**, covered by DMSO peak), 2.28 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.50 (thiazolidin-4-one **C**=O), 162.12, 161.45, 159.93, 158.33, 151.96, 150.93, 148.67, 146.35, 139.12, 134.79 (=**C**H–Ar), 132.60, 132.42, 131.61, 128.62, 128.44, 124.81, 124.24, 122.36, 122.05, 121.37, 117.54, 117.28, 112.29, 108.37, 56.33 (2-O**C**H$_3$), 30.07 (thiazolidin-4-one N**C**H$_3$), 18.21 (Ar**C**H$_3$). Elemental analysis cald for $C_{28}H_{24}N_6O_2S{\cdot}1/2MeOH$, C

65.25, H 5.00, N 16.02, S 6.11; found C 65.41, H 4.53, N 16.38, S 5.57.

5-(3-Methoxybenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 33 Yellow solid. Yield 56%, 0.285 g. TLC R_f: 0.59 (S_1). HPLC t_R (min): 3.02 (CS_3). M.p.: 158–161 °C. IR υ_{max} (cm^{-1}): 3448 (N–H str), 3043, 3001 (aromatic C–H str), 2893, 2978 (aliphatic C–H str), 1712 (thiazolidin-4-one C=O str), 1641, 1608, 1579, 1558, 1554, 1525 (C=N str, N–H bending, C=C str), 1475, 1410 (aliphatic C–H bending), 1396, 1300 (C–N str), 1147 (C–F str), 875, 790, 705 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, $CDCl_3$): 9.21 (d, 1H, J = 1,8 Hz), 8.65–8.63 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, J = 1.8 Hz), 8.51 (d, 1H, J = 5.1 Hz), 8.38–8.34 (m, 1H), 8.01 (m, 1H), 7.72 (s, 1H, =C**H**–Ar), 7.35–7.22 (m, 3H), 7.17 (d, 1H, J = 8.1 Hz), 7.08–6.86 (m, 3H), 6.73–6.70 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.77 (s, 3H, OC**H$_3$**), 3.49 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.39 (s, 3H, ArC**H$_3$**), 1.70 (**H$_2$**O). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.38 (thiazolidin-4-one **C**=O), 162.14, 161.45, 160.04, 159.93, 151.93, 150.67, 148.67, 146.24, 139.13, 135.25, 134.76 (=**C**H–Ar), 132.60, 131.63, 130.88, 130.33, 128.51, 124.24, 122.54, 121.85, 117.48, 117.31, 116.39, 115.88, 108.38, 55.75 (3-O**C**H$_3$), 30.12 (thiazolidin-4-one N**C**H$_3$), 18.23 (Ar**C**H$_3$). Elemental analysis cald for $C_{28}H_{24}N_6O_2S{\cdot}1/2H_2O$, C 64.97, H 4.87, N 16.24, S 6.19; found C 65.25, H 4.49, N 16.27, S 5.64.

5-(4-Methoxybenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 34 Yellow solid. Yield 51%, 0.260 g. TLC R_f: 0.56 (S_1). HPLC t_R (min): 2.28 (CS_3). M.p.: 195–198 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 3010 (aromatic C–H str), 2947 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1635, 1577, 1533, 1508 (C=N str, N–H bending, C=C str), 1435, 1410, 1402, 1365 (aliphatic C–H bending, C–N str), 1101 (C–O str), 885, 785, 700 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.5 Hz), 8.95 (s, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, J = 1.5 Hz), 8.52 (d, 1H, J = 5.4 Hz), 8.45–8.41 (m, 1H), 7.70 (s, 1H, =C**H**–Ar), 7.49–7.42 (m, 4H), 7.29 (d, 1H, J = 8.1 Hz), 7.04 (d, 2H, J = 8.7 Hz), 6.77–6.74 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.77 (s, 3H, OC**H$_3$**), 3.33 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.28 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.62 (thiazolidin-4-one **C**=O), 162.11, 161.45, 160.06, 159.95, 151.94, 150.94, 148.67, 146.46, 143.49, 139.14, 134.76 (=**C**H–Ar), 132.59, 132.27, 132.25, 131.62, 130.38, 128.36, 126.37, 124.24, 118.97, 117.55, 117.25, 115.31, 108.37, 55.92 (4-O**C**H$_3$), 30.04 (thiazolidin-4-one N**C**H$_3$), 18.32 (Ar**C**H$_3$). Elemental analysis cald for $C_{28}H_{24}N_6O_2S$, C 66.12, H 4.76, N 16.52, S 6.30; found C 65.68, H 4.50, N 16.43, S 5.39.

5-(N,N-Dimethylaminobenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 35 Yellow solid. Yield 55%, 0.285 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 3.35, 3.95 (CS_3). M.p.: 203–205 °C. IR υ_{max} (cm^{-1}): 3448 (N–H str), 3024 (aromatic C–H str), 2893, 2818 (aliphatic C–H str), 1699 (thiazolidin-4-one C=O str), 1641, 1612, 1579, 1554, 1523 (C=N str, N–H bending, C=C str), 1442, 1400, 1402, 1363 (aliphatic C–H bending, C–N str), 877, 790 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.28 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.66–8.64 (dd, 1H, J = 1.5 Hz, J = 3.3 Hz, J = 1.5 Hz), 8.53 (d, 1H, J = 5.1 Hz), 8.45–8.41 (m, 1H), 7.62 (s, 1H, =C**H**–Ar), 7.45–7.36 (m, 2H), 7.36–7.34 (m, 3H), 7.28–7.25 (d, 1H, J = 8.1 Hz), 6.77–6.72 (m, 3H), 3.32 (s, 3H, thiazolidin-4-one NC**H$_3$**), 2.96 (s, 6H, N(C**H$_3$**)$_2$), 2,28 (s, 3H, ArC**H$_3$**). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.84 (thiazolidin-4-one **C**=O), 162.07, 161.49, 159.97, 151.96, 151.58, 151.44, 151.40, 148.67, 146.72, 139.11, 134.76 (=**C**H–Ar), 132.61, 132.23, 131.56, 131.47, 128.27, 124.26, 120.83, 117.68, 117.39, 114.63, 112.50, 108.30, 59.38 (4-N(**C**H$_3$)$_2$), 29.91 (thiazolidin-4-one N**C**H$_3$), 18.22 (Ar**C**H$_3$). Elemental analysis cald for $C_{29}H_{27}N_7OS{\cdot}1/2MeOH$, C 65.90, H 5.44, N 18.24, S 5.96; found C 65.82, H 4.92, N 18.68, S 5.55.

5-(2-Fluorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiaz olidin-4-one 36 Yellow solid. Yield 75%, 0.380 g. TLC R_f: 0.59 (S_1). HPLC t_R (min): 4.42 (CS_5). M.p.: 170–171 °C. IR υ_{max} (cm^{-1}): 3443 (N–H str), 3036, 2976, 2933 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1639, 1608, 1581, 1558, 1554, 1518 (C=N str, N–H bending, C=C str), 1431, 1390, 1371, 1340 (aliphatic C–H bending, C–N str), (1118 (C–F str), 875, 796, 769, 707 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, D_2O Exchange, DMSO-d_6): 9.24 (d, 1H, J = 1.5 Hz), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.3 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.1 Hz), 8.43–8.39 (m, 1H), 7.72 (s, 1H, =C**H**–Ar), 7.50–7.21 (m, 8H), 6.77–6.74 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.98 (q, 2H, thiazolidin-4-one NC**H$_2$**CH$_3$), 2.56 (H_2O), 2.29 (s, 3H, ArC**H$_3$**), 1.30–1.25 (t, 3H, thiazolidin-4-one NCH$_2$C**H$_3$**, J = 6.9 Hz, J = 7.2 Hz). Elemental analysis cald for $C_{28}H_{23}FN_6O_2S{\cdot}H_2O$, C 63.62, H 3.59, N 15.90, S 6.07; found C 63.69, H 4.30, N 15.85, S 5.94.

5-(3-Fluorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 37 Yellow solid. Yield 56%, 0.285 g. TLC R_f: 0.62 (S_1). HPLC t_R (min): 4.49 (CS_4). M.p.: 184 °C. IR υ_{max} (cm^{-1}): 3446 (N–H str), 3068 (aromatic C–H str), 2970 (aliphatic C–H str), 1712 (thiazolidin-4-one C=O str), 1647, 1606, 1573, 1529 (C=N str, N–H bending, C=C str), 1438, 1413, 1386 (aliphatic C–H bending), 1249 (C–N str), 1120 (C–F

str), 840, 808, 783, 707 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6):): 9.26 (d, 1H, J = 1.8 Hz), 8.96 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.7 Hz), 8.52 (d, 1H, J = 5.4 Hz), 8.43–8.39 (d, 1H, J = 8.4 Hz), 7.75 (s, 1H, =C**H**–Ar), 7.54–7.22 (m, 6H), 7.41 (d, 1H, J = 2.1 Hz), 7.29 (d, 1H, J = 8.3 Hz), 6.78–6.74 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.98–3.91 (q, 2H), 2.29 (s, 3H, ArC**H$_3$**), 1.29–1.25 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). ^{13}C-NMR δ ppm (75 MHz, DMSO-d_6): 166.89 (thiazolidin-4-one **C**=O), 163.93, 162.11, 161.50, 161.40, 159.96, 151.95, 149.42, 148.68, 146.14, 139.16, 136.26, 134.73 (=**C**H–Ar), 132.59, 131.63, 129.06, 128.44, 125.73, 124.19, 123.81, 117.33, 117.17, 117.12, 116.97, 108.43, 38.55 (thiazolidin-4-one N**C**H$_2$CH$_3$), 18.22 (Ar**C**H$_3$), 12.99 (thiazolidin-4-one NCH$_2$**C**H$_3$). LC/MS APCI$^+$ m/z (%): 511.997 ([M+H]$^+$, 95), 510.786 (M$^+$, 100); APCI$^-$ m/z (%): 509.275 ([M−H]$^-$, 100). Elemental analysis cald for $C_{28}H_{23}FN_6OS$, C 65.87, H 4.54, N 16.46, S 6.28; found C 65.51, H 4.67, N 16.42, S 6.11.

5-(4-Fluorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 38 Yellow solid. Yield 55%, 0.280 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 3.98, 4.30 (CS_4). M.p.: 139 °C. IR υ_{max} (cm^{-1}): 3437 (N–H str), 3026 (aromatic C–H str), 2974 (aliphatic C–H str) 1707 (thiazolidin-4-one C=O str), 1637, 1575, 1533, 1506 (C=N str, N–H bending, C=C str), 1442, 1417, 1386, 1367, 1338 (aliphatic C–H bending), 1228 (C–N str), 1124 (C–F str), 885, 825, 796 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.8 Hz), 8.96 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.7 Hz), 8.52 (d, 1H, J = 5.4 Hz), 8.43–8.39 (d, 1H, J = 8.4 Hz), 7.75 (s, 1H, =C**H**–Ar), 7.60–7.56 (m, 2H), 7.45–7.43 (m, 1H), 7.40 (d, 1H, J = 2.1 Hz), 7.33–7.25 (m, 3H), 6.76–6.73 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.2 Hz), 3.97–3.91 (q, 2H), 2.29 (s, 3H, ArC**H$_3$**), 1.29–1.24 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). LC/MS APCI$^+$ m/z (%):510.877 ([M+H]$^+$, 100); APCI$^-$ m/z (%): 509.250 ([M−H]$^-$, 100). Elemental analysis cald for $C_{28}H_{23}FN_6OS$·1/2 MeOH, C 65.00, H 4.79, N 15.96, S 6.09; found C 64.84, H 4.77, N 16.09, S 6.07.

5-(2,6-Difluorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 39 Yellow solid. Yield 27%, 0.142 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 4.03 (CS_4). M.p.: 151–153 °C. IR υ_{max} (cm^{-1}): 3431 (N–H str), 3095, 3730 (aromatic C–H str), 2976, 2947 (aliphatic C–H str), 1708 (thiazolidin-4-one C=O str), 1633, 1604, 1581, 1554, 1519 (C=N str, N–H bending, C=C str), 1435, 1390, 1371, 1342 (aliphatic C–H bending), 1253 (C–N str), 1118 (C–F g.b), 792, 744, 707 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (d, 1H, J = 1.5 Hz), 8.92 (s, 1H, sec. N**H**), 8.63–8.63 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.6 Hz), 8.48 (d, 1H, J = 5.1 Hz), 8.41–8.37 (d, 1H, J = 6.3 Hz), 7.58 (s, 1H, =C**H**–Ar), 7.56–7.42 (m, 3H), 7.35 (d, 1H, J = 2.1 Hz), 7.25–7.16 (m, 3H), 6.72–6.69 (dd, 1H, J = 2.4 Hz, J = 5.7 Hz, J = 2.3 Hz), 3.97–3.90 (q, 2H), 2,25 (s, 3H, ArC**H$_3$**), 1.30–1.25 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). LC/MS APCI$^+$ m/z (%): 529.896 ([M+H]$^+$, 66), 528.564 (M$^+$, 100); APCI$^-$ m/z (%): 527.271 ([M−H]$^-$, 100), 219.363 (18). Elemental analysis cald for $C_{28}H_{22}F_2N_6OS$, C 63.62, H 4.20, N 15.90, S 6.07; found C 63.36, H 4.31, N 15.80, S 5.75.

5-(2-Chlorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 40 Yellow solid. Yield 60%, 0.315 g. TLC R_f: 0.55 (S_1). HPLC t_R (min): 6.00 (CS_4). M.p.: 160–162 °C. IR υ_{max} (cm^{-1}): 3444 (N–H str), 3036 (aromatic C–H str), 2974, 2933 (aliphatic C–H str), 1705 (thiazolidin-4-one C=O str), 1633, 1581, 1554, 1518 (C=N str, N–H bending, C=C str), 1431, 1390, 1371, 1342 (aliphatic C–H bending), 1284 (C–N str), 1093 (C–Cl str), 796, 763, 704 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.8 Hz), 8.95 (s, 1H, sec. N**H**), 8.65–8.64 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.7 Hz), 8.50 (d, 1H, J = 5.1 Hz), 8.43–8.39 (d, 1H, J = 8.5 Hz), 7.88 (s, 1H, =C**H**–Ar), 7.62–7.59 (m, 1H), 7.49–7.41 (m, 5H), 7.38 (d, 1H, J = 2.1 Hz), 7.27 (d, 1H, J = 8.4 Hz), 6.76–6.72 (dd, 1H, J = 2.4 Hz, J = 5.7 Hz, J = 2.3 Hz), 3.99–3.91 (q, 2H), 2.27 (s, 3H, ArC**H$_3$**), 1.30–1.25 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). LC/MS APCI$^+$ m/z (%): 528.789 ([M+2]$^+$, 75), 526.787 ((M + H]$^+$, 100); APCI$^-$ m/z (%): 525.172 ([M−H]$^-$, 100). Elemental analysis cald for $C_{28}H_{23}ClN_6OS$·MeOH, C 62.30, H 4.87, N 15.03, S 5.74; found C 62.14, H 4.39, N 15.54, S 5.37.

5-(4-Chlorobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 41 Yellow solid. Yield 25%, 0.130 g. TLC R_f: 0.59 (S_1). HPLC t_R (min): 5.35 (CS_4). M.p.: 162–163 °C. IR υ_{max} (cm^{-1}): 3441 (N–H str), 3022 (aromatic C–H str), 2991 (aliphatic C–H str), 1703 (thiazolidin-4-one C=O str), 1641, 1573, 1535 (C=N str, N–H bending, C=C str), 1450, 1423, 1388, 1373, 1333 (aliphatic C–H bending), 1234 (C–N str), 1093 (C–Cl str), 829, 802, 707 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.8 Hz), 8.96 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, J = 1.7 Hz), 8.50 (d, 1H, J = 5.1 Hz), 8.43–8.39 (d, 1H, J = 8.1 Hz), 7.74 (s, 1H, =C**H**–Ar), 7.45–7.39 (m, 6H), 7.40 (d, 1H, J = 2.1 Hz), 7.28 (d, 1H, J = 8.1 Hz), 6.76–6.73 (dd, 1H, J = 2.4 Hz, J = 5.7 Hz, J = 2.4 Hz), 3.98–3.91 (q, 2H), 2.28 (s, 3H, ArC**H$_3$**), 1.29–1.24 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). LC/MS APCI$^+$ m/z (%): 548.917 ([M+Na]$^+$, 34), 526.873 (M$^+$, 100), 363.16 (27); APCI$^-$ m/z (%): 525.189 ([M−H]$^-$, 100). Elemental analysis cald for

$C_{28}H_{23}ClN_6OS$, C 63.81, H 4.40, N 15.95, S 6.08; found C 63.32, H 4.55, N 15.88, S 5.71.

5-(4-Bromobenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 42 Yellow solid. Yield 62%, 0.355 g. TLC R_f: 0.60 (S_1). HPLC t_R (min): 6.98 (CS_4). M.p.: 167–170 °C. IR υ_{max} (cm^{-1}): 3309 (N–H str), 3084 (aromatic C–H str), 2974 (aliphatic C–H str), 1701 (thiazolidin-4-one C=O str), 1635, 1604, 1573, 1548, 1533 (C=N str, N–H bending, C=C str), 1452, 1421, 1384, 1369 (aliphatic C–H bending), 1240 (C–N str), 1114 (C–Br str), 812, 794, 705 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.8 Hz), 8.96 (s, 1H, sec. N**H**), 8.65–8.63 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.8 Hz), 8.52 (d, 1H, J = 5.4 Hz), 8.43–8.39 (d, 1H, J = 8.5 Hz), 7.71 (s, 1H, =C**H**–Ar), 7.76–7.73 (d, 2H, J = 9.0 Hz), 7.47–7.41 (m, 4H), 7.40 (d, 1H, J = 4.2 Hz), 7.28 (d, 1H, J = 8.1 Hz), 6.76–6.73 (dd, 1H, J = 2.4 Hz, J = 5.7 Hz, J = 2.3 Hz), 3.98–3.91 (q, 2H), 2.28 (s, 3H, ArC**H$_3$**), 1.29–1.24 (t, 3H, J = 6.9 Hz, J = 7.2 Hz). LC/MS APCI$^+$ m/z (%): 572.796 ([M+2]$^+$, 96), 570.700 (M^+, 100); APCI$^-$ m/z (%): 569.078 ([M−H]$^-$, 100), 219.352 (21). Elemental analysis cald for $C_{28}H_{23}BrN_6OS$, C 57.93, H 4.17, N 14.48, S 5.52; found C 58.05, H 4.06, N 14.59, S 4.92.

5-(4-Trifluoromethylbenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 43 Yellow solid. Yield 22%, 0.125 g. TLC R_f: 0.62 (S_1). HPLC t_R (min): 6.91 (CS_4). M.p.: 152–153 °C. IR υ_{max} (cm^{-1}): 3267 (N–H str), 2997, 2972 (aliphatic C–H str), 1707 (thiazolidin-4-one C=O str), 1637, 1579, 1556, 1525 (C=N str, N–H bending, C=C str), 1417, 1386, 1373 (aliphatic C–H bending), 1253 (C–N str), 1165 (C–F str), 798, 786, 705 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.64-8.62 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.8 Hz), 8.52 (d, 1H, J = 5.1 Hz), 8.43–8.39 (d, 1H, J = 6.3 Hz), 7.78 (s, 1H, =C**H**–Ar), 7.68–7.63 (d, 2H), 7.47–7.42 (m, 4H), 7.41 (d, 1H, J = 2.4 Hz), 7.29 (d, 2H, J = 8.4 Hz), 6.77–6.74 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.99–3.92 (q, 2H), 2.28 (s, 3H, ArC**H$_3$**), 1.30–1.25 (t, 3H, J = 6.9 Hz, J = 7.8 Hz). LC/MS APCI$^+$ m/z (%): 560.584 (M^+, 100), 562.870 ([M+2]$^+$, 66); APCI$^-$ m/z (%): 559.241 ([M−H]$^-$, 100). Elemental analysis cald for $C_{29}H_{23}FN_6O_2S$, C 60.41, H 4.02, N 14.58, S 5.56; found C 61.25, H 4.29, N 14.75, S 5.48.

5-(4-Trifluoromethoxybenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 44 Yellow solid. Yield 36%, 0.205 g. TLC R_f: 0.62 (S_1). HPLC t_R (min): 6.29 (CS_4). M.p.: 191–193 °C. IR υ_{max} (cm^{-1}): 3452 (N–H str), 3034 (aromatic C–H str), 2931, 2872 (aliphatic C–H str), 1701 (thiazolidin-4-one C=O str), 1637, 1602, 1583, 1558, 1523 (C=N str, N–H bending, C=C str), 1413, 1338 (aliphatic C–H bending), 1234 (C–N str), 1163 (C–O str), 1112 (C–F str), 800, 705 (aromatic C–H e.b). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.26 (d, 1H, J = 1.5 Hz), 8.96 (s, 1H, sec. N**H**), 8.64–8.62 (dd, 1H, J = 1.5 Hz, J = 3.0 Hz, J = 1.8 Hz), 8.52 (d, 1H, J = 5.1 Hz), 8.43–8.39 (d, 1H, J = 6.3 Hz), 7.78 (s, 1H, =C**H**–Ar), 7.78–7.70 (dd, 4H), 7.45–7.43 (m, 2H), 7.41 (d, 1H, J = 2.1 Hz), 7.29 (d, 2H, J = 8.1 Hz), 6.77–6.74 (dd, 1H, J = 2.1 Hz, J = 5.7 Hz, J = 2.1 Hz), 3.99–3.92 (q, 2H), 2.29 (s, 3H, ArC**H$_3$**), 1.30–1.26 (t, 3H, J = 6.9 Hz, J = 7.8 Hz). LC/MS APCI$^+$ m/z (%): 576.564 (M^+, 100); APCI$^-$ m/z (%): 575.247 ([M−H]$^-$, 100). Elemental analysis cald for $C_{29}H_{23}FN_6O_2S$, C 60.41, H 4.02, N 14.58, S 5.56; found C 61.25, H 4.29, N 14.75, S 5.48.

5-(2-Hydroxybenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 45 Yellow solid. Yield 40%, 0.202 g. TLC R_f: 0.54 (S_1). HPLC t_R (min): 2.46 (CS_7). M.p.: 250–255 °C. IR υ_{max} (cm^{-1}): 3228 (O–H str, N–H str), 3039 (aromatic C–H str), 2970, 2928 (aliphatic C–H str), 1701 (thiazolidin-4-one C=O str), 1622, 1597, 1587, 1554 (C=N str, N–H bending, C=C str), 1448, 1388, 1371 (aliphatic C–H bending, C–N str), 1244 (C–O str), 788, 704 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 10.49 (s, 0.9 H), 9.27 (d, 1H, J = 1.5 Hz), 8.97 (s, sec. N**H**), 8.66–8.64 (dd, 1H, J = 1.2 Hz, J = 3.3 Hz, J = 1.5 Hz), 8.52 (d, 1H, J = 5.1 Hz), 8.44–8.40 (m, 1H), 7.99 (s, 1H, =C**H**–Ar), 7.46–7.43 (m, 2H), 7.38 (d, 1H, J = 1.8 Hz), 7.27–7.23 (m, 3H), 7.22 (d, 1H, J = 8.1 Hz), 6.87 (t, 1H, J = 7.5 Hz, J = 7.5 Hz), 6.77–6.74 (dd, 1H, J = 2.1 Hz, J = 6.0 Hz, J = 2.1 Hz), 3.97 (q, 2H, thiazolidin-4-one NC**H$_2$**CH$_3$), 3.43 (H_2O), 2.29 (s, 3H, ArC**H$_3$**), 1.29–1.24 (t, 3H, thiazolidin-4-one NCH$_2$C**H$_3$**, J = 6.9 Hz, J = 6.9 Hz). Elemental analysis cald for $C_{28}H_{24}N_6O_2S$, C 66.12, H 4.76, N 16.52, S 6.30; found C 65.97, H 4.58, N 16.07, S 6.07.

5-(2-Methoxybenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 46 Yellow solid. Yield 81%, 0.422 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 4.16 (CS_7). M.p.: 205–208 °C. IR υ_{max} (cm^{-1}): 3221 (N–H str), 3039–2841 (aliphatic C–H str), 1703 (thiazolidin-4-one C=O str), 1637, 1593, 1579, 1529 (C=N str, N–H bending, C=C str), 1419, 1384 (aliphatic C–H bending, C–N str), 1244 (C–O str), 800, 705 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, D_2O Exchange, DMSO-d_6): 9.25 (d, 1H, J = 1.8 Hz), 8.65–8.63 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, J = 1.5 Hz), 8.51 (d, 1H, J = 5.1 Hz), 8.43–8.39 (m, 1H), 7.93 (s, 1H, =C**H**–Ar), 7.46–7.42 (m, 4H), 7.29 (d, 1H, J = 7.8 Hz), 7.13 (d, 1H, J = 8.7 Hz), 7.01 (t, 1H, J = 7.5 Hz, J = 7.5 Hz), 6.77–6.74 (dd, 1H, J = 2.1 Hz,

$J = 5.7$ Hz, $J = 2.1$ Hz), 3.98 (q, 2H, thiazolidin-4-one NC**H**$_2$CH$_3$), 3.86 (D_2O), 3.73 (s, 3H, OC**H**$_3$), 3.18 (MeOH), 2.29 (s, 3H, ArC**H**$_3$), 1.29–1.25 (t, 3H, thiazolidin-4-one NCH$_2$C**H**$_3$, $J = 7.2$ Hz, $J = 6.9$ Hz). Elemental analysis cald for $C_{29}H_{26}N_6O_2S \cdot 1/7$MeOH, C 66.39, H 5.08, N 15.94, S 6.08; found C 65.71, H 5.37, N 16.03, S 5.78.

5-(3-Methoxybenzylidene)-3-methyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 47 Yellow solid. Yield 31%, 0.162 g. TLC R_f: 0.57 (S_1). HPLC t_R (min): 4.08 (CS_7). M.p.: 164–165 °C. IR υ_{max} (cm^{-1}): 3490 (N–H str), 2949, 2901, 2829 (aliphatic C–H str), 1716 (thiazolidin-4-one C=O str), 1639, 1608, 1579, 1554, 1523 (C=N str, N–H bending, C=C str), 1446, 1427, 1386 (aliphatic C–H bending, C–N str), 1242 (C–O str), 781, 682 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.27 (d, 1H, $J = 1.5$ Hz), 8.97 (s, sec. N**H**), 8.66–8.64 (dd, 1H, $J = 1.5$ Hz, $J = 3.3$ Hz, $J = 1.5$ Hz), 8.52 (d, 1H, $J = 5.1$ Hz), 8.44–8.40 (m, 1H), 7.72 (s, 1H, =C**H**–Ar), 7.46–7.36 (m, 4H), 7.29 (d, 1H, $J = 8.1$ Hz), 7.10–6.89 (m, 3H), 6.78–6.74 (dd, 1H, $J = 1.5$ Hz, $J = 5.7$ Hz, $J = 2.1$ Hz), 3.98 (q, 2H, thiazolidin-4-one NC**H**$_2$CH$_3$), 3.74 (s, 3H, OC**H**$_3$) 3.45 (H_2O), 2.29 (s, 3H, ArC**H**$_3$), 1.29–1.25 (t, 3H, thiazolidin-4-one NCH$_2$C**H**$_3$, $J = 7.2$ Hz, $J = 6.9$ Hz). Elemental analysis cald for $C_{29}H_{26}N_6O_2S \cdot 1/2$EtOH, C 66.03, H 5.36, N 15.40, S 5.88; found C 65.69, H 5.19, N 15.78, S 5.98.

5-(4-Methoxybenzylidene)-3-ethyl-[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 48 Yellow solid. Yield 58%, 0.305 g. TLC R_f: 0.56 (S_1). HPLC t_R (min): 3.58 (CS_7). M.p.: 170–174 °C. IR υ_{max} (cm^{-1}): 3295 (N–H str), 2966–2829 (aliphatic C–H str), 1697 (thiazolidin-4-one C=O str), 1637, 1595, 1579, 1525 (C=N str, N–H bending, C=C str), 1421,1384 (aliphatic C–H bending, C–N str), 1249 (C–O str), 798, 705 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.25 (d, 1H, $J = 1.5$ Hz), 8.95 (s, sec. N**H**), 8.64–8.62 (dd, 1H, $J = 1.5$ Hz, $J = 3.3$ Hz, $J = 1.5$ Hz), 8.51 (d, 1H, $J = 5.1$ Hz), 8.43–8.39 (m, 1H), 7.68 (s, 1H, =C**H**–Ar), 7.47–7.38 (m, 4H), 7.28 (d, 1H, $J = 8.1$ Hz), 7.02 (d, 2H, $J = 9.0$ Hz), 6.76–6.73 (dd, 1H, $J = 2.1$ Hz, $J = 5.7$ Hz, $J = 2.1$ Hz), 3.96 (q, 2H, thiazolidin-4-one NC**H**$_2$CH$_3$), 3.76 (s, 3H, OC**H**$_3$), 2.28 (s, 3H, ArC**H**$_3$), 1.27–1.23 (t, 3H, thiazolidin-4-one NCH$_2$C**H**$_3$, $J = 6.9$ Hz, $J = 7.2$ Hz). Elemental analysis cald for $C_{29}H_{26}N_6O_2S$, C 66.65, H 5.01, N 16.08, S 6.14; found C 66.24, H 4.77, N 15.87, S 5.84.

5-(N,N-Dimethylaminobenzylidene)-3-ethyl--[(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)imino]-1,3-thiazolidin-4-one 49 Yellow solid. Yield 40%, 0.215 g. TLC R_f: 0.55 (S_1). HPLC t_R (min): 4.61, 5.34 (CS_5). M.p.: 187–190 °C. IR υ_{max} (cm^{-1}): 3454 (N–H str), 2972, 2893 (aliphatic C–H str), 1697 (thiazolidin-4-one C=O str), 1639, 1610, 1579, 1554, 1523 (C=N str, N–H bending, C=C str), 1452, 1443, 1398 (aliphatic C–H bending), 1365, 1344 (C–N str), 792, 700 (aromatic C–H bending). ^{1}H-NMR δ ppm (300 MHz, DMSO-d_6): 9.28 (d, 1H, $J = 1.8$ Hz), 8.95 (s, 1H, sec. N**H**), 8.66–8.64 (dd, 1H, $J = 1.5$ Hz, $J = 3.3$ Hz, $J = 1.5$ Hz), 8.53 (d, 1H, $J = 5.1$ Hz), 8.45–8.41 (m, 1H), 7.62 (s, 1H, =C**H**–Ar), 7.46–7.41 (m, 2H), 7.38–7.33 (m, 3H), 7.26 (d, 1H, $J = 8.1$ Hz), 6.77–6.72 (m, 3H), 3.95–3.89 (q, 2H, thiazolidin-4-one NC**H**$_2$CH$_3$), 2.95 (s, 6H, N(C**H**$_3$)$_2$), 2.29 (s, 3H, ArC**H**$_3$), 1.28–1.23 (t, 3H, thiazolidin-4-one NCH$_2$C**H**$_3$, $J = 6.9$ Hz, $J = 7.2$ Hz). Elemental analysis cald for $C_{30}H_{29}N_7OS \cdot 1/2$MeOH, C 66.40, H 5.66, N 17.77, S 5.81; found C 66.38, H 5.31, N 18.12, S 5.70.

Anticancer activity

Cell culture conditions and reagents

K562 (CCL-243), PC3 (CRL-1435), SHSY-5Y (CRL-2266) and L929 (CRL-6364) cells were acquired from American Type Culture Collection (Manassas, VA, USA). PC3, SHSY-5Y and L929 cells were cultured in DMEM and K562 cells were grown in RPMI-1640 (Gibco Thermo Fisher Scientific) medium supplemented with 10% (v/v) heat-inactivated FBS (Sigma-Aldrich) and 1% penicillin/streptomycin (Gibco Thermo Fisher Scientific). Cells were cultured in a 25 cm^2 cell culture flask and incubated at 37 °C in a 5% CO_2 humidified atmosphere until they reached approximately 80% confluence.

Cell viability assay

Cell viability was assessed using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) colorimetric assay (Roche Diagnostic, Germany). Imatinib and compounds **5**–**49** were dissolved in dimethyl sulfoxide (DMSO concentration did not exceed 0.1%) and diluted in DMEM or RPMI-1640 prior to treatment. The cells were incubated with 10 µM constant concentration of compounds for 24 and 48 h. At the end of the incubation period, for determination of living cells, 50-µL XTT labeling mixture was added to each well and then the plates were incubated at 37 °C for another 4 h. After mixing, the absorbance of XTT-formazan was measured using an ELISA microplate reader (Thermo, Germany) at 450 nm. All experiments were performed in three independent experiments and the cell viability was expressed in percentage related to control (100% of viability).

Annexin V binding assay

After treatment of K562 cells with IC_{50} concentrations of the compounds (8, 15 and 34) and imatinib, the extent apoptosis was examined using the Muse Annexin V/Dead Cell (Merck Millipore, Germany) assay, as described in the manufacturer's instructions. Briefly, K562 cells were treated with selected compounds and imatinib for 24 and 48 h. After that, the cells were collected, diluted with PBS containing 1% FBS and incubated with Muse™ Annexin V & Dead Cell reagent for 20 min at room temperature in the dark. The events for live, dead, early and late apoptotic cells were analyzed by Muse™ Cell Analyzer (Merck Millipore, Germany). Data of apoptosis induction by compounds and imatinib were calculated from three independent experiments.

Cell cycle analysis

Cell cycle arrest was evaluated by Muse Cell Cycle Assay Kit (Merck Millipore, Germany) according to the user's guide. The cells were treated with IC_{50} concentrations of the compounds (**8**, **15** and **34**) and imatinib first and then incubated for 48 h at 37 °C. After completion of the incubation, the cells were collected by centrifugation (300×*g*, 5 min), washed with cold PBS and fixed in chilled 70% (v/v) ethanol for at least 3 h at − 20 °C before staining. Next, the fixed cells were washed once with PBS and incubated with 200 µL of assay solution for 30 min in the dark at room temperature according to the manufacturer's protocol. After staining, the percentages of the cells at different stages of the cell cycle (G0/G1, S and G2/M phases) were analyzed by the Muse Cell Analyzer (Merck Millipore, Germany).

DNA damage assay

DNA damage induced by the compounds (**8**, **15** and **34**) was evaluated using Muse™ Multi-Color DNA Damage kit (Merck Millipore, Germany) according to the manufacturer's instructions. Initially, K562 cells were treated with IC_{50} concentrations of the compounds and imatinib and then they were incubated for 48 h at 37 °C. Next, the cells were centrifuged, washed once with 1X PBS and fixed in fixation buffer for 10 min on ice, followed by washing and permeabilization in the ice-cold buffer. Ten µL antibody cocktail solution was added to each tube containing the cell suspension and incubated again for 30 min in dark at room temperature. After the final centrifugation and washing steps, the cells were resuspended in 200 µL 1X assay buffer and percentages of negative cells (no DNA damage), percentages of ATM activated cells, percentages of H2AX activated cells and percentages of DNA double-strand breaks were determined using the Muse™ Multi-Color DNA Damage software module.

Statistical analyses

Statistical significances for the assays were assessed using the GraphPad Prism 7.0 (GraphPad Software, Inc.). The data obtained from the experiments were expressed as the mean ± standard deviation (SD) and one-way ANOVA test was performed for multiple comparisons. *p* values less than 0.05 were considered to be statistically significant.

Molecular modeling studies

Molecular modeling studies with Abl kinase protein were carried out to simulate the potential inhibition profile of designed compounds. Protein crystal structure of the Abl enzyme was obtained from protein data bank with the 1IEP PDB ID. Prior to molecular modeling studies, water was removed from the crystal structure and protein was prepared using the Schrödinger Protein Preparation Wizard. Ligands were subsequently prepared as not charged by using the Schrödinger LigPrep tool. Energy minimization was held using the OPLC force field. Prepared ligand structures were docked into protein binding site at pH: 7.0 ± 1.0 using the Schrödinger Glide-Standard Precision docking procedure considering induced fit approach [60]. In the meantime, imatinib which was extracted from the 1IEP structure was re-docked for the validation of the docking procedure. RMSD between co-crystallized and re-docked conformations of imatinib was found less than 3.0 Å that exists under the limitations. Superposition of co-crystallized and re-docked conformations of imatinib is presented in the supplementary file. Detailed analyses of the most active compounds are visualized in Figs. 7, 8, 9 and 10. Figures are prepared using the PyMOL software.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

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