Original Article

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Synthesis and structure-activity relationship study of 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one derivatives as anticancer agents

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ABSTRACT: The synthesis and structure-activity relationship (SAR) study of a series of 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (4a-4j) derivatives as anticancer agents are described. This series of thiazolopyrimidines were synthesized by the reaction of 7-(4-fluoro phenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*] pyrimidin-3(7*H*)-one (3) with appropriate substituted aldehydes in the presence of anhydrous sodium acetate and glacial acetic acid. Their structures were confirmed by IR, ^{1}H-NMR, mass, and elemental analyses. These novel thiazolopyrimidine derivatives were screened for their anticancer activity on the U937 human histocytic lymphoma cell line by 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. The comparison of anticancer activity of thiazolopyrimidine was performed considering their structures. This study was done using 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (4a-4j) as a basic model, showing that *i*) presence of a hydrogen donor/ acceptor domain [thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one] on the thiazolopyrimidine ring; *ii*) presence of a hydrophobic [(4-fluorophenyl)] aryl ring system on the thiazolopyrimidine ring; *iii*) presence of an electron donor moiety [5-(furan-2-yl)] on the thiazolopyrimidine ring; *iv*) *ortho* and *para* substitution of the distal aryl ring [2-(substituted benzylidene)] function strongly influenced anticancer activity. Among these compounds (4a-4j) *para* substituted derivatives 4c, 4e, 4f, 4g, 4h, and 4j showed significant anticancer activity.

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1. Introduction

Thiazole, pyrimidine and related pyrimidines are classes of fused heterocycles that are of considerable interest because of the diverse range of their biological properties. These are among a wide variety of nitrogen heterocycles that have been explored for developing pharmaceutically important molecules. Thiazolopyrimidine and related fused heterocycles are of interest as potential bioactive molecules, which can be considered as thia-analogues of the natural purine bases such as adenine and guanine, and have acquired a growing importance in the field of medicinal chemistry because of their biological potential. They are known to exhibit pharmacological activities such as analgesic, antiinflammatory, antiarrhythmic, antiparkinsonian, and anticancer activities (*1-8*).

Cancer is a collection of different life threatening diseases characterized by uncontrolled growth of cells leading to invasion of surrounding tissue and often spreading to other parts of the body. When it comes to understanding and controlling cancer scientists are now working from a position of strength because a foundation of knowledge about cancer has been built over the past 50 years. There is an urgent need for novel effective drug regimens for the treatment of cancer because the current chemotherapy suffers from a slim therapeutic index, with significant toxicity from effective drug doses or tumor recurrence at low drug doses. The new anticancer chemotherapeutic agents search continues to be an active area of research at many companies and research centers (*9*,*10*). Searching for new anticancer agents having heterocyclic nucleus continues worldwide at various laboratories (*11-13*).

In the last several decades, fused pyrimidine

derivatives are a class of heterocyclic compounds that have attracted significant interest in medicinal chemistry because they have a wide range of pharmaceutical and pharmacological applications including potential anti-tumor, antimycobacterial, and antiviral activities. Moreover, in recent years, it was reported that many fused pyrimidine analogues were reported to be inhibitors of tyrosine kinase and cyclin-dependent kinases, which are involved in mediating the transmission of mitogenic signals and numerous other cellular events (*14-19*), including, cell proliferation, migration, differentiation, metabolism, and immune responses. It was also found that many of these derivatives may block proliferation of various cancer cell lines (*20*).

Led by the above facts on pyrimidine chemistry, we have synthesized new 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one derivatives (**4a-4j**). The main objective of the present investigation is a modified experimental approach to evaluate position of substitutions in synthesized compounds and validation with reference drugs such as gefitinib. The introduction of the substituted benzylidene at the 2nd position of the thiazolopyrimidine scaffold led to significant anticancer activity.

2. Materials and Methods

2.1. *Chemicals and reagents*

The chemicals and reagents used were obtained from various chemical units including Aldrich Co. (Powai, Mumbai, India), E. Merck India Ltd. (Ponda, Goa, India), CDH (Daryaganj, New Delhi, India), and SD Fine Chem (Worli Road, Mumbai, India). These solvents used were of laboratory research (LR) grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Melting points were measured in open capillary tubes on a Boetius apparatus (Carl Zeiss Jena) and are uncorrected. ^{1}H-NMR spectra were taken on a 300 MHz NMR spectrometer (model Ultra Shield, Bruker, Rheinstetten, Germany) in (d_6-DMSO) using tetramethylsilane [$(CH_3)_4Si$] as internal standard. Chemical shifts (δ) are expressed in ppm. Mass spectra were obtained on an instrument (JEOL-SX-102, Japan) using electron impact ionization. IR spectra were recorded in KBr pellets on a Fourier-transform infrared spectrometer (FT-IR 410, Jasco Corporation, Tokyo, Japan). Elemental analyses were performed on an elemental analyzer (Model 240c, Perkin Elmer, Thane, Maharashtra, India) and were within ± 0.4% of the theoretical values.

2.2. *General procedure for the synthesis of title compounds (**4a-4j**)*

2.2.1. *Preparation of 3-(4-fluorophenyl)-1-(furan-2-yl)prop-2-en-1-one (**1**)*

The key intermediates were synthesized by a previously reported method (*21*). 3-(4-Fluorophenyl)-1-(furan-2-yl) prop-2-en-1-one (**1**) prepared by the mixture of KOH (0.055 mol), water (20 mL), ethanol (15 mL), 2-acetyl furan (0.043 mol), and *p*-fluorobenzaldehyde (0.043 mol) was stirred at 30-40°C for 2 h and kept overnight. It was then filtered, washed with water and with ethanol, dried and refluxed with glacial acetic acid (10 mL) for 2 h. The crystals separated after cooling were filtered and washed with water, dried and used in further reactions. Yield 79%, Mp 212°C; IR (KBr) cm^{-1}: 2,991 (Ar-CH_{str}), 1,733 (C=O), 1,631 (C=C), 1,030 (cyclic C–O–C_{str}), 823 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δppm): 7.51 (d, J = 8.2 Hz, 2H, ArH), 7.23 (dd, J_1 = 7.7 Hz, J_2 = 1.83 Hz, 2H, ArH), 6.53-7.21 (m, 3H, –CH-furan), 6.10-7.14 (d, 2H, =CH); MS (EI) m/z 216 [M]$^+$; Anal. Calcd. for $C_{13}H_9FO_2$: C, 72.22; H, 4.20; Found: C, 72.23; H, 4.22.

2.2.2. *4-(4-Fluorophenyl)-6-(furan-2-yl)-3,4-dihydropyrimidin-2(1H)-thione (**2**)*

A mixture of 3-(4-fluorophenyl)-1-(furan-2-yl)prop-2-en-1-one (**1**) (0.039 mol) thiourea (0.03 mol) and potassium hydroxide (2.5 g) in 95% ethanol (100 mL) was heated under reflux for 3 h. The reaction mixture was concentrated to half of its volume, diluted with water, then acidified with dilute acetic acid and kept overnight. The solid thus obtained, was filtered, washed with water and recrystallized from ethanol to give 4-(4-fluorophenyl)-6-(furan-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione (**2**). Yield 72%, Mp 231°C; IR (KBr) cm^{-1}: 3,361 (NH_{str}), 3,021 (Ar-CH_{str}), 1,531 (C=C), 1,034 (cyclic C–O–C_{str}), 843 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.39 (dd, J_1 = 6.4 Hz, J_2 = 1.8 Hz, 2H, ArH), 7.41 (d, J = 8.2 Hz, 2H, ArH), 6.61-6.81 (m, 3H, –CH-furan), 6.14 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, pyrimidine H), 4.70 (dd, J_1 = 12.0 Hz, J_2 = 2.0 Hz, 1H, pyrimidine H), 3.27 (s, 2H, –NH); MS (EI) m/z 274 [M]$^+$; Anal. Calcd. for $C_{14}H_{11}FN_2OS$: C, 61.30; H, 4.04; N, 10.21; Found: C, 61.33; H, 4.06; N, 10.24.

2.2.3. *7-(4-Fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**3**)*

The chloroacetic acid (0.096 mol) was melted on a water bath and (**2**) (0.009 mol) added to it portion wise to maintain its homogeneity. The homogeneous mixture was further heated on a water bath for 30 min and kept overnight. The solid thus obtained was washed with water and recrystallized from ethanol to give 7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (**3**). Yield 69%, Mp 227°C; IR (KBr) cm^{-1}: 3,354 (NH_{str}), 3,047 (Ar-CH_{str}), 1,721 (C=O), 1,512 (C=C), 1,021 (cyclic C–O–C_{str}), 822 (C-Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.31 (dd, J_1 = 7.1 Hz, J_2 = 2.2 Hz, 2H, ArH), 7.74 (d, J = 7.9 Hz, 2H, ArH), 6.71-7.12 (m, 3H, –CH-furan), 5.32 (dd, J_1 = 7.5 Hz, J_2 = 2.1 Hz, 1H, thiazolopyrimidine H), 4.13 (dd, J_1 = 12.1 Hz, J_2 = 1.8 Hz, 1H, thiazolopyrimidine H), 3.61 (s,

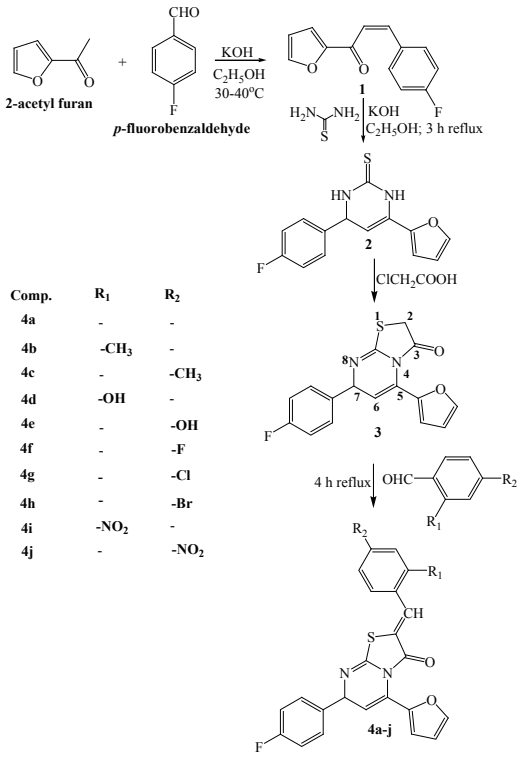
2H, –CH_2 thiazole); MS (EI) m/z 314 $[M]^+$; Anal. Calcd. for $C_{16}H_{11}FN_2O_2S$: C, 61.14; H, 3.53; N, 8.91; Found: C, 61.12; H, 3.51; N, 8.94.

2.2.4. *General procedure for the synthesis of 2-(substitutedbenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4a-4j**)*

A mixture of (**3**) (0.002 mol), substituted benzaldehyde (0.002 mol), and anhydrous sodium acetate (0.002 mol) in 100% glacial acetic acid (10 mL) was heated under reflux for 4 h. The reaction mixture was kept overnight and the solid, thus separated, was filtered, washed with water and recrystallized from ethanol to furnish 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (**4a-4j**) (Scheme 1).

2.3. *Cell proliferation assay*

The U937 human histocytic lymphoma cell line was obtained from cell line bank of National Center for Cellular Sciences (NCCS), Pune, India. These cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C, in a CO_2 incubator in the presence or absence of test compounds. The anticancer property of the compounds was measured by MTT assay (*22*). The cells were plated in a 96-well plate at a density of 5,000 cells/well. After 24 h, cell culture media was replaced with DMEM containing 10% FBS and the cells were treated with different concentrations of the compounds



Comp.	R_1	R_2
4a	-	-
4b	$-CH_3$	-
4c	-	$-CH_3$
4d	-OH	-
4e	-	-OH
4f	-	-F
4g	-	-Cl
4h	-	-Br
4i	$-NO_2$	-
4j	-	$-NO_2$

Scheme 1. Synthetic protocols of target compounds (4a-4j).

(0.01-50 mM). The cells were later incubated for 72 h. Cytotoxicity was measured by adding 5 mg/mL of MTT to each well and incubating for another 3 h. The purple formazan crystals were dissolved by adding 100 µL of DMSO to each well. The absorbance was read at 570 nm in a spectrophotometer. Cell death was calculated as follows: Cell death percentage = 100 – [test absorbance/ control absorbance] × 100. The test result is expressed as the concentration of a test compound which inhibits the cell growth by 50% (IC_{50}).

3. Results and Discussion

3.1. *Chemistry*

The chemical structures of the synthesized compounds were confirmed by infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, mass spectrometry, and elemental analysis. The presence of the carbonyl and olefinic group in compound (**1**) is characterized by the presence of two strong bands in its IR spectrum at 1,733 and 1,631 cm^{-1}. The formations of compound (**2**) were confirmed by NH stretching, bending peaks in the range of 3,382, 1,621 cm^{-1}, and appearance of a singlet peak 3.27 for two protons in its ^{1}H-NMR spectra which might be assigned to NH group connecting the pyrimidine. The conversion of thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (**3**) can be recognized by a strong absorption peak at 1,721cm^{-1} in IR due to the carbonyl group in the thiazole ring. The title compounds (**4a-4j**) showed a singlet at δ 7.12, 7.35, 7.32, 7.31, 7.14, 7.22, 7.25, 7.23, 7.19, and 7.32 ppm due to the benzylidine ring proton in ^{1}H-NMR confirms the formation of (**4a-4j**) respectively. Further mass spectra confirmed their purity and molecular weight.

3.2. *Biological activity*

All the selected compounds 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*] pyrimidin-3(7*H*)-one (**4a-4j**) were evaluated for cytotoxic properties on the U937 human histocytic lymphoma cell line with gefitinib as a standard positive control. Inhibition of cell-proliferation was measured by MTT assay. The inhibitory potency (IC_{50}) of compounds (**4a-4j**) are given in Table 1. The fact that a majority of clinically active anticancer drugs possess a nitrogen hetero atomic system with one or two phenyl rings, at least one carbonyl group in their structure and the presence of hydrogen donor/ acceptor unit is noted. In all of the pioneering experiments important core fragments (*23*) are defined by the presence of a hydrogen donor/acceptor unit (HAD), a hydrophobic domain (A) (aryl ring substituted/unsubstituted) and an electron donor atom (D). These common features were found in the structures of well-established anticancer drugs such as gefitinib, erlotinib, lapatinib, and dasatinib as well as synthesized compounds (Figure 1). In general,

Table 1. Anticancer study of synthesized compounds (4a-4j) on U937 human histocytic lymphoma cell line

Compounds	R_1	R_2	IC_{50} (μM) ± SEM[a]
4a	-	-	10.04 ± 0.52
4b	$-CH_3$	-	12.07 ± 0.31
4c	-	$-CH_3$	5.37 ± 0.12
4d	-OH	-	15.12 ± 0.22
4e	-	-OH	5.81 ± 0.17
4f	-	-F	3.04 ± 0.26
4g	-	-Cl	4.04 ± 0.32
4h	-	-Br	3.51 ± 0.43
4i	$-NO_2$	-	20.12 ± 0.26
4j	-	$-NO_2$	6.42 ± 0.21
Gefitinib	-	-	1.00 ± 1.00

[a] Mean of three independent experiments ± mean standard error.

lipophilicity is one of the most important parameters because it is mainly involved in pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) and in ligand-target interactions (*24*). Lipophilicity is the molecular parameter of choice in numerous quantitative structure-activity relationships (QSAR) of different classes of compounds (*25*). The promising activity of the compounds may be attributed to the substitutions on the hydrophobic domain. These compounds contain methyl, hydroxy, nitro, and halogens at the *para* position of the benzylidene aryl ring. Moreover, observed data showed that the *para* substituted derivatives exhibited better activity than other *ortho* and unsubstituted derivatives. Compounds **4f** and **4h** were found to be most potent and showed IC_{50} values of 3.04 and 3.51 mM, respectively. When the *ortho* group was on the benzylidene aryl ring of compounds, we found a decrease in inhibitory activity. For example, compounds **4d** and **4i** were 3-fold less potent than **4e** and **4j**. Among the ten compounds synthesized, we found that compounds **4f** and **4h** showed comparable activity to that of gefitinib. Moreover, *p*-CH_3, *p*-OH, and *p*-NO_2 substituted compounds (**4c**, **4e**, and **4j**) had slighter lower activity than (**4f-4h**). However, the unsubstituted and *ortho* substituted (*o*-methyl, *o*-hydroxyl, and *o*-nitro) compounds (**4a**, **4b**, **4d**, and **4i**) exhibited lesser activity. The anticancer activity of test compounds with decreasing order is shown in Figure 2 and is tabulated in Table 1.

3.3. *Structure activity relationships (SAR) study*

SAR studies give insights into molecular properties causing receptor affinity and selectivity. The promising nature of the compounds may be attributed to the substitutions on the hydrophobic domain (benzylidene aryl ring). These compounds had electron withdrawing and donating groups at the *ortho* and *para* positions of the hydrophobic aryl ring. In general it was observed that the *para* substituted derivatives were more active than the other derivatives. This may be because of the fact that the *para* substituted derivatives fit better into the receptor site.

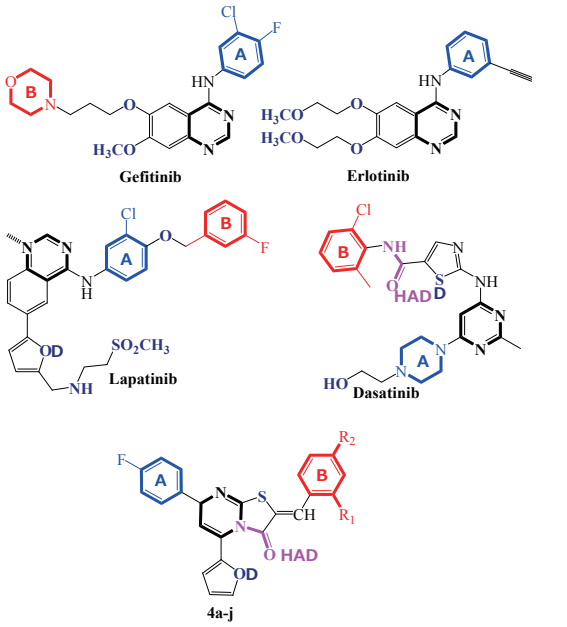


Figure 1. Vital core fragments of wellknown anticancer drugs and synthesized compounds with its important structural features: (HAD) hydrogen bond acceptor/donor domain. (A) hydrophobic aryl ring system, **(B)** distal aryl ring system, and **(D)** electron donor moiety.

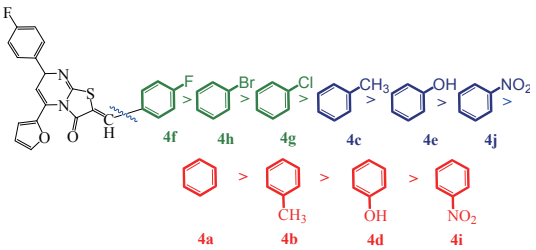


Figure 2. Decreasing order of anticancer activity of thiazolopyrimidine derivatives.

Based on these general concepts we planned to prepare different modifications in title compounds, namely, *ortho* and *para* substitution in the thiazolopyrimidine ring. These modifications showed insight into the dependency of the receptor binding efficacy. Furthermore the *para* substituted thiazolopyrimidine skeletal structure conserved the good receptor binding results. Finally *para* substituted compounds **4c**, **4e**, **4f**, **4g**, **4h**, and **4j** exhibited significant anticancer activity.

4. Conclusions

The literature survey revealed that *para* substitution on the phenyl ring appeared to greatly influence pharmacological activity. This research examined anticancer properties of a novel series of 2-(substituted benzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one (**4a-4j**) compounds. Results revealed that para substituted derivatives exhibited better anticancer activity.

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Appendix

Spectral data of the synthesized compounds

*2-Benzylidene-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4a**)*

Yield 73%, Mp 284°C; IR (KBr) cm^{-1}: 3,061 (Ar-CH_{str}), 1,642 (C=O), 1,514 (C=C benzylidine), 1,032 (cyclic C–O–C_{str}), 811 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.41 (dd, J_1 = 7.3 Hz, J_2 = 1.8 Hz 2H, ArH), 7.53 (d, J = 6.2 Hz, 2H, ArH), 7.56-7.87 (m, 5H, Ar-H), 7.12 (s, 1H, –CH benzylidine), 6.51-6.89 (m, 3H, –CH-furan), 5.16 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz 1H, thiazolopyrimidine H), 3.21 (dd, J_1 = 17.6 Hz, J_2 = 1.8 Hz 1H, thiazolopyrimidine H); MS (EI) m/z 402 $[M]^+$; Anal. Calcd. for $C_{23}H_{15}FN_2O_2S$: C, 68.64; H, 3.76; N, 6.96; Found: C, 68.69; H, 3.73; N, 6.92.

*2-(2-Methylbenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4b**)*

Yield 76%, Mp 291°C; IR (KBr) cm^{-1}: 3,047 (Ar-CH_{str}), 1,623 (C=O), 1,521 (C=C), 1,031 (cyclic C–O–C_{str}), 837 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.24 (dd, J_1 = 6.4 Hz, J_2 = 1.4 Hz 2H, ArH), 7.24 (d, J = 8.2 Hz, 2H, ArH), 7.31-7.54 (m, 4H, Ar-H), 7.35 (s, 1H, –CH benzylidine), 6.14-6.66 (m, 3H, –CH-furan), 5.43 (dd, J_1 = 8.6 Hz, J_2 = 2.9 Hz 1H, thiazolopyrimidine H), 3.24 (dd, J_1 = 11.6 Hz, J_2 = 1.6 Hz 1H, thiazolopyrimidine H), 2.71 (m, 3H, –CH_3); MS (EI) m/z 416 $[M]^+$; Anal. Calcd. for $C_{24}H_{17}FN_2O_2S$: C, 69.21; H, 4.11; N, 6.73; Found: C, 69.24; H, 4.13; N, 6.71.

*2-(4-Methylbenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4c**)*

Yield 82%, Mp 297°C; IR (KBr) cm^{-1}: 3,057 (Ar-CH_{str}), 1,632 (C=O), 1,543 (C=C), 1,031 (cyclic C–O–C_{str}), 811 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.33 (dd, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 2H, ArH), 7.54 (d, J = 7.7 Hz, 2H, ArH), 7.67-7.71 (m, 4H, Ar-H), 7.32 (s, 1H, –CH benzylidine), 6.23-6.74 (m, 3H, –CH-furan), 5.43 (dd, J_1 = 8.1 Hz, J_2 = 1.7 Hz, 1H, thiazolopyrimidine H), 3.14 (dd, J_1 = 13.2 Hz, J_2 = 2.2 Hz, 1H, thiazolopyrimidine H), 2.25 (m, 3H, –CH_3); MS (EI) m/z 416 $[M]^+$; Anal. Calcd. for $C_{24}H_{17}FN_2O_2S$: C, 69.21; H, 4.11; N, 6.73; Found: C, 69.18; H, 4.14; N, 6.75.

*2-(2-Hydroxybenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4d**)*

Yield 82%, Mp 276°C; IR (KBr) cm^{-1}: 3,412 (phenolic OH), 3,043 (Ar-CH_{str}), 1,643 (C=O), 1,512 (C=C), 1,021 (cyclic C–O–C_{str}), 819 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 9.93 (s, 1H; Ar-OH), 7.36 (dd, J_1 = 7.6 Hz, J_2 = 2.0 Hz, 2H, ArH), 7.51 (d, J = 7.5 Hz, 2H, ArH), 7.63-7.74 (m, 4H, Ar-H), 7.31 (s, 1H, –CH benzylidine) 6.23-6.65 (m, 3H, –CH-furan), 5.41 (dd, J_1 = 8.7 Hz, J_2 = 1.8 Hz, 1H, thiazolopyrimidine H), 3.13 (dd, J_1 = 12.4 Hz, J_2 = 1.9 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 418 $[M]^+$; Anal. Calcd. for $C_{23}H_{15}FN_2O_3S$: C, 66.02; H, 3.61; N, 6.69; Found: C, 66.06; H, 3.64; N, 6.67.

*2-(4-Hydroxybenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4e**)*

Yield 79%, Mp 294°C; IR (KBr) cm^{-1}: 3,471 (phenolic OH), 3,037 (Ar-CH_{str}), 1,624 (C=O), 1,511 (C=C), 1,037 (cyclic C–O–C_{str}), 817 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 9.71 (s, 1H, Ar-OH), 7.16 (dd, J_1 = 7.2 Hz, J_2 = 2.2 Hz, 2H, ArH), 7.26 (d, J = 7.1 Hz, 2H, ArH), 7.35-7.48 (m, 4H, Ar-H), 7.14 (s, 1H, –CH benzylidine), 6.26-6.59 (m, 3H, –CH-furan), 5.36 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, thiazolopyrimidine H), 3.17 (dd, J_1 = 11.8 Hz, J_2 = 2.0 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 418 $[M]^+$; Anal. Calcd. for $C_{23}H_{15}FN_2O_3S$: C, 66.02; H, 3.61; N, 6.69; Found: C, 66.06; H, 3.64; N, 6.71.

*2-(4-Fluorobenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4f**)*

Yield 73%, Mp 289°C; IR (KBr) cm^{-1}: 3,055 (Ar-CH_{str}), 1,636 (C=O), 1,536 (C=C), 1,035 (cyclic C–O–C_{str}), 822 (C-F), 912 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.47 (dd, J_1 = 6.8 Hz, J_2 = 1.4 Hz 2H, ArH), 7.53 (d, J = 7.0 Hz, 2H, ArH), 7.62-7.96 (m, 4H, Ar-H), 7.22 (s, 1H, –CH benzylidine), 6.21-6.76 (m, 3H, –CH-furan), 5.42 (dd, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H, thiazolopyrimidine H), 3.19 (dd, J_1 = 12.5 Hz, J_2 = 2.4 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 420 $[M]^+$; Anal. Calcd. for $C_{23}H_{14}F_2N_2O_2S$: C, 65.71; H, 3.36; N, 6.66; Found: C, 65.74; H, 3.37; N, 6.68.

*2-(4-Chlorobenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4g**)*

Yield 75%, Mp 291°C; IR (KBr) cm^{-1}: 3,053 (Ar-CH_{str}), 1,634 (C=O), 1,532 (C=C), 1,031 (cyclic C–O–C_{str}), 834 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.41 (dd, J_1 = 7.1 Hz, J_2 = 1.8 Hz, 2H, ArH), 7.56 (d, J = 6.8 Hz, 2H, ArH), 7.61-7.96 (m, 4H, Ar-H), 7.25 (s, 1H, –CH benzylidine), 6.22-6.77 (m, 3H, –CH-furan), 5.42 (dd, J_1 = 7.9 Hz, J_2 = 1.8 Hz, 1H, thiazolopyrimidine H), 3.11 (dd, J_1 = 14.2 Hz, J_2 = 2.0 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 438 [M+2]; Anal. Calcd. for $C_{23}H_{14}ClFN_2O_2S$: C, 63.23; H, 3.23; N, 6.41; Found: C, 63.26; H, 3.22; N, 6.44.

*2-(4-Bromobenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4h**)*

Yield 72%, Mp 286°C; IR (KBr) cm^{-1}: 3,051 (Ar-CH_{str}), 1,643 (C=O), 1,544 (C=C), 1,041 (cyclic C–

O–C_{str}), 851 (C-F), 621 (C-Br); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.41 (dd, J_1 = 7.9 Hz, J_2 = 2.0 Hz, 2H, ArH), 7.53 (d, J = 6.3 Hz, 2H, ArH), 7.61-7.94 (m, 4H, Ar-H), 7.23 (s, 1H, –CH benzylidine), 6.26-6.75 (m, 3H, –CH-furan), 5.43 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, thiazolopyrimidine H), 3.12 (dd, J_1 = 16.1 Hz, J_2 = 2.2 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 483 [M+2]; Anal. Calcd. for $C_{23}H_{14}BrFN_2O_2S$: C, 57.39; H, 2.93; N, 5.82; Found: C, 57.35; H, 2.91; N, 5.86.

*2-(2-Nitrobenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4i**)*

Yield 79%, Mp 293°C; IR (KBr) cm^{-1}: 3,054 (Ar-CH_{str}), 1,647 (C=O), 1,549 (C=C), 1,047 (cyclic C–O–C_{str}), 842 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.86 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2H, ArH), 7.91 (d, J = 6.9 Hz, 2H, ArH), 8.02-8.35 (m, 4H, Ar-H), 7.19 (s, 1H, –CH benzylidine), 6.32-6.59 (m, 3H, –CH-furan), 5.36 (dd, J_1 = 8.0 Hz, J_2 = 1.8 Hz, 1H, thiazolopyrimidine H), 3.12 (dd, J_1 = 14.2 Hz, J_2 = 2.0 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 447 $[M]^+$; Anal. Calcd. for $C_{23}H_{14}FN_3O_4S$: C, 61.74; H, 3.15; N, 9.39; Found: C, 61.77; H, 3.11; N, 9.37.

*2-(4-Nitrobenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2H-thiazolo[3,2-a]pyrimidin-3(7H)-one (**4j**)*

Yield 81%, Mp 286°C; IR (KBr) cm^{-1}: 3,051 (Ar-CH_{str}), 1,643 (C=O), 1,547 (C=C), 1,041 (cyclic C–O–C_{str}), 887 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.81 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 2H, ArH), 7.94 (d, J = 6.3 Hz, 2H, ArH), 8.12-8.37 (m, 4H, Ar-H), 7.32 (s, 1H, –CH benzylidine), 6.19-6.52 (m, 3H, –CH-furan), 5.47 (dd, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 1H, thiazolopyrimidine H), 3.14 (dd, J_1 = 12.4 Hz, J_2 = 1.8 Hz, 1H, thiazolopyrimidine H); MS (EI) m/z 447 $[M]^+$; Anal. Calcd. for $C_{23}H_{14}FN_3O_4S$: C, 61.74; H, 3.15; N, 9.39; Found: C, 61.71; H, 3.17; N, 9.35.