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Original article

One pot synthesis of thiazolodihydropyrimidinones and evaluation of their anticancer activity

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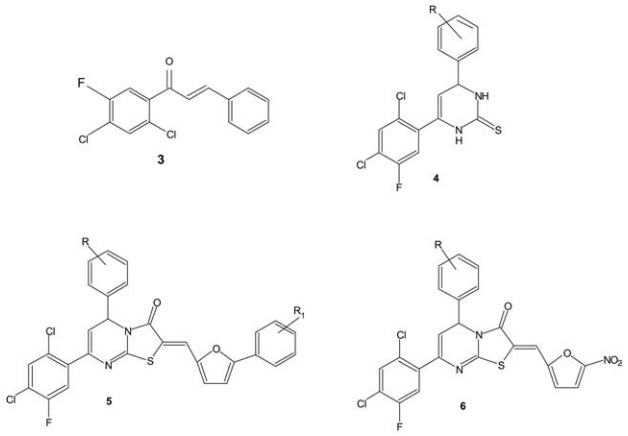
Abstract

2-(5-Arylfurfurylidene/5-nitrofurfurylidene)-5-aryl-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]-pyrimidin-2(1*H*)-ones **5** and **6** are synthesized by a novel three component reaction of 4,6-diarylpyrimidino-2(1*H*)-thiones **4**, monochloroacetic acid, arylfurfuraldehydes and 5-nitro-2-furfuraldenediacetate, respectively. The newly synthesized compounds are characterized by elemental analysis, IR, ^{1}H NMR and mass spectral studies. These compounds exhibited in vitro antitumour activity with moderate to excellent growth inhibition against a panel of 60 cell lines of leukemia, non-small cell lung cancer melanoma, ovarian cancer, prostate cancer and breast cancer.



Keywords: MCR; Three component reaction; Thiazolodihydropyrimidinones; Arylfurfural; 5-nitrofurfuraldiacetate; Anticancer activity

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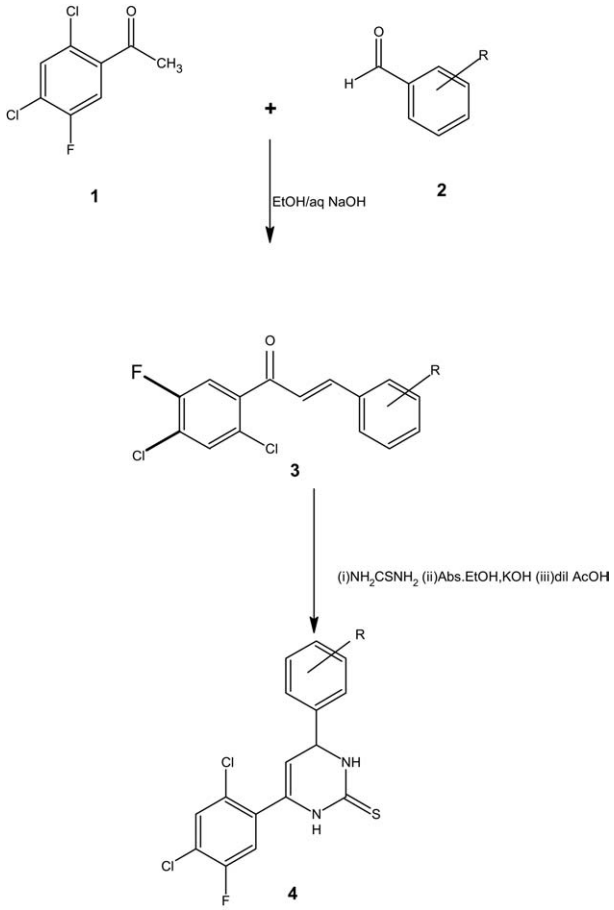
1. Introduction

Multi-component reactions (MCR) are special types of theoretically useful organic reactions in which three or more starting materials react to give a product [1]. Convergent synthesis pathways generally show advantages over linear or divergent approaches with respect to time, speed, yield and reproducibility. Among organic reactions, MCR with more than two starting materials are assembled to afford a complex product. Therefore, they constitute a superior tool for diversity oriented and complexity-generating synthesis for drug discovery [2,3]. Well known examples of MCR, which form heterocycles, are Bignilli reaction [4] and Hantzsch dihydropyridine synthesis [5].

Pyrimidine derivatives are known for their varied biological properties. Brugnatelli was the first scientist to isolate "Alloxan", a pyrimidine derivative in 1818, and later this compound was found to possess antineoplastic properties [6]. Nucleosides of pyrimidine bases have been used extensively as antiviral and anticancer agents [7]. Recently, fluoropyrimidines- and flurouracil-based combination therapy is used in the treatment of gastrointestinal cancer and solid tumors [8]. Fluropyrimidinones are found to be metabolites of dihydropyrimidinones that are subtype-selective antagonists of the α_{1a}-adreginic receptor antagonists [9]. Thiazoles and their derivatives are found to be associated with various biological activities such as antibacterial, antifungal and anti-inflammatory activities [10–13]. Presence of fluorine in a molecule enhances drug persistence and lipid solubility [14] and also groups like 5-nitrofuran, arylfurfurans contribute to the biological activities [15]. Based on these reports and also in continuation of our search for bioactive molecules, possessing anticancer activity [16], it was contemplated to synthesize thiazolodihydropyrimidinones in a one-pot reaction. Results of such studies are discussed in this paper.

2. Chemistry

1,3-Diaryl-2-propen-1-ones (chalcones) **3** were prepared by condensing substituted benzaldehydes **2** with 2,4-dichloro-5-fluoroacetophenones **1** in the presence of sodium hydroxide under the Clasein–Schmidt reaction conditions. These chalcones provided suitable building blocks for the synthesis of 4,6-diaryl-3,4-dihydropyrimidin-2(1*H*)-thiones **4**. These compounds were prepared in good yields by refluxing 1,3-diaryl-2-propen-1-ones **3** and thiourea in the presence of ethanolic potassium hydroxide (Scheme 1). The characterization data of these compounds are given in Table 1. These pyrimidin-2-thiones **4** were further used for the synthesis of *N*-bridged heterocycles. From the previous study, it is clear that the preparation of 2-arylidene-5,6-diaryl-5*H*-thiazolo[2,3-*b*]-pyrimidin-3-ones starting from pyrimidine-2-thione involves two steps. In the first step, pyrimidine-2-thione was condensed with monochloroacetic acid in the presence of anhydrous sodium acetate to yield



Scheme 1. Synthesis of 4-aryl-6-(2,4-dichloro-5-fluorophenyl)-3,4-dihydropyrimidin-2(1*H*)-thiones(**4a-e**).

5,6-disubstituted-5*H*-thiazolo[2,3-*b*]-pyrimidin-3-ones. This compound was then converted into arylidene derivatives by condensing the same with aromatic aldehydes in the presence of piperidine. In the present investigation, it was contemplated to carry out the synthesis of the title compounds **5,6** in a one-pot synthesis. Thus, 2-(5-arylfurylidene/5-nitrofurfurylidene)-5-(aryl)-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]-pyrimidin-2(1*H*)-ones were prepared in good yields by refluxing 4,6-diaryl-3,4-dihydropyrimidin-2(1*H*)-thiones, monochloroacetic acid, anhydrous sodium acetate with the corresponding arylfurfuraldehyde/5-nitro-2-furfural diacetate in acetic acid–acetic anhydride medium (Tables 2 and 3, Scheme 2). The structures of these compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral studies.

3. Results and discussion

The IR spectrum of the compound **4a** showed an absorption band at 3414 cm^{-1} corresponding to the –NH stretching frequency of the pyrimidin-2-thione. An absorption band was seen at 1182 cm^{-1} due to the stretching frequency of C═S group. The absence of characteristic absorption bands due to carbonyl and S–H groups of the starting materials clearly confirmed the formation of pyrimidin-2-thiones. The absorp-

Table 1
Characterization data of compounds 4-aryl-6-(2,4-dichloro-5-fluorophenyl)-3,4-dihydropyrimidin-2(1*H*)-thiones (**4a–e**)

Compound no.	R	M.p. (°C)	Yield (%)	Molecular formula	*N*-Analysis found [Calc.]	*C*-Analysis found [Calc.]
4a	H	98–100	84	$C_{16}H_{11}Cl_2FN_2S$	7.90 [7.93]	54.50 [54.34]
4b	4-OCH_3	86–88	86	$C_{17}H_{13}Cl_2FN_2OS$	7.34 [7.31]	53.28 [53.26]
4c	3,4-$(OCH_3)_2$	112–14	81	$C_{18}H_{15}Cl_2FN_2O_2S$	6.79 [6.77]	52.38 [52.30]
4d	3,4-O-CH_2O-	95–97	76	$C_{17}H_{11}Cl_2FN_2O_2S$	7.02 [7.05]	51.30 [51.38]
4e	4-Cl	103–105	79	$C_{16}H_{10}Cl_3FN_2S$	7.26 [7.22]	49.50 [49.54]

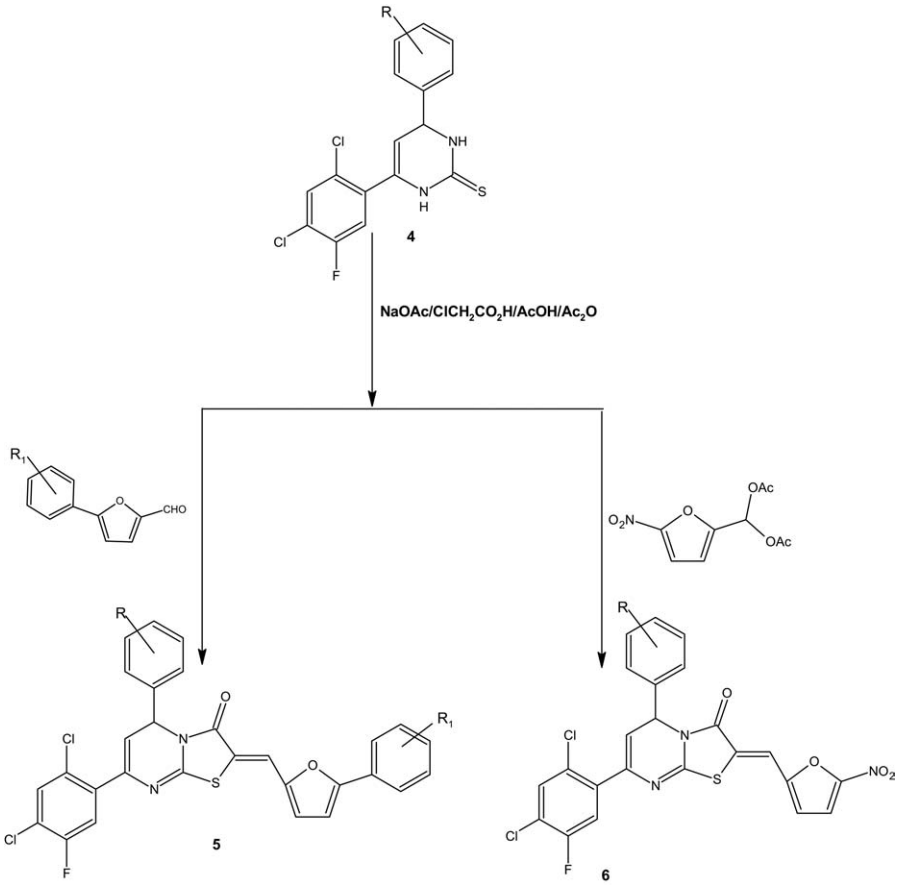
Solvent of crystallization: benzene and methanol
IR (KBr, γ_{max} cm^{-1}): **4a**: 3414 (NH), 1563 (C=C), 1083 (C–F), 726 (C–Cl); **4b**: 3419 (N–H), 2933(C=H), 1610 (C=N), 732 (C–Cl); **4c**: 3420 (N–H), 2935 (C–H), 1565 (C=C), 1084 (C–F), 726 (C–Cl);
^{1}H NMR ($CDCl_3$ + DMSO-d_6): **4a**: δ 5.06 (d, 1H, methine H J = 4.6 Hz), 5.26 (d, 1H, olefinic H, J = 4.6 Hz), 7.01–7.53 (m, 6H, Ar–H); 7.72 (s, 1H, NH); 7.81(s, 1H, NH); 8.05 (d, Ar–H, J = 9.3 Hz). 4b: δ 3.9(s, 3H, OCH_3), 5.07(d, 1H, methine H J = 4.8 Hz), 5.23 (d, 1H, olefinic H, J = 4.8 Hz), 7.02–7.6 (m, 6H, Ar–H) 8.05(d, 1H, Ar–H, J = 9.2 Hz); 7.7 (bs, 1H, N–H); 7.8 (s, 1H, N–H); **4c**: δ 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.07 (d, 1H, methine H J = 4.5 Hz), 5.22 (d, 1H, olefinic H, J = 4.5 Hz), 6.91(m, 2H, Ar–H) 7.21(d, 1H, Ar–H, J = 8.6 Hz); 7.52 (m, 2H, Ar–H), 7.62 (bs, 1H, N–H); 7.83 (s, 1H, N–H); **4d**: δ 5.04 (d, 1H, methine H, J = 4.8 Hz), 5.12 (d, 1H, olefinic H, J = 4.8 Hz), 6.01(s, 2H, -OCH_2O-), 6.78–7.43 (m, 5*H*, Ar–H), 7.83 (bs, 1H, N–H). **4e**: δ 4.9 (d, 1H, methine H J = 5 Hz), 5.2 (d, 1H, olefinic H, J = 5 Hz), 7.2–7.8(m, H, Ar–H), 7.8 (bs, 1H, N–H); 8.03 (s, 1H, N–H);
MS: *m/z* (% abundance): **4a**: 352 (96%, M^+), 318 (24%, M^+-H_2S), 275 (38% M^+-C_6H_5), 189 (100%, M^+-2,4-dichlorophenyl radical), 77 (51%, phenyl cation) **4b**: 382 (100%, M^+), 348 (13%, M^+-H_2S), 276 (15%, M^+-4-$MeOC_6H_4$), 219 (34%, M^+-2,4-dichlorophenyl radical), **4c**: 412 (80%, M^+), 378 (15% M^+-H_2S), 275 (36% M^+-163), 138 (15% 3,4-$(OCH_3)_2C_6H_3$); **4d**: 396 (49%, M^+), 362 (29%, M^+-H_2S), 275 (35%, 3,4-methylenedioxy phenyl radical), 234 (15%, M^+-163); **4e**: 386 (88%, M^+), 352 (52%, M^+-H_2S), 275 (28% M^+-111), 223 (100%, M^+-163), 163(17%, 2,4-dichloro-5-fluorophenyl cation), 111(33%, 4-chlorophenyl cation).

Table 2
Characterization data of compounds 2-(5-arylfurfurylidene)-5-(aryl)-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-b]pyrimidin-3-ones (**5a–j**)

Compound No	R	R^1	M.p. (°C)	Yield (%)	Molecular formula	*N*-Analysis found [cald.]	*C*-Analysis found [cald.]
5a	H	4-NO_2-2-CH_3	145–47	78	$C_{30}H_{18}Cl_2FN_3O_2S$	6.95 [6.93]	59.44 [59.40]
5b	4-OCH_3	4-NO_2-2-CH_3	116–18	81	$C_{31}H_{20}Cl_2FN_3O_5S$	6.64 [6.60]	58.52 [58.49]
5c	3,4-$(OCH_3)_2$	4-NO_2-2-CH_3	126–28	76	$C_{32}H_{22}Cl_2FN_3O_4S$	6.31 [6.30]	57.68 [57.65]
5d	3,4-O- CH_2-O-	4-NO_2-2-CH_3	119–21	79	$C_{31}H_{18}Cl_2FN_3O_6S$	6.48 [6.46]	57.28 [57.23]
5e	4-Cl	4-NO_2-2-CH_3	138–40	83	$C_{30}H_{12}Cl_3FN_3O_4S$	6.60 [6.55]	56.26 [56.20]
5f	H	2,4-dichloro	132–34	71	$C_{29}H_{15}Cl_4FN_2O_2S$	4.56 [4.54]	56.47 [56.49]
5g	4-OCH_3	2,4-dichloro	128–30	74	$C_{30}H_{17}Cl_4FN_2O_3S$	4.30 [4.33]	55.70 [55.72]
5h	3,4-$(OCH_3)_2$	2,4-dichloro	142–44	78	$C_{31}H_{19}Cl_4FN_2O_2S$	4.10 [4.14]	55.10 [55.02]
5i	3,4- O- CH_2-O-	2,4-dichloro	126–28	68	$C_{30}H_{15}Cl_4FN_2O_2S$	4.21 [4.24]	54.52 [54.54]
5j	4-Cl	2,4-dichloro	133–35	73	$C_{29}H_{14}Cl_5FN_2O_2S$	4.27 [4.30]	53.53 [53.49]

Solvent of crystallization: glacial acetic acid
Spectral data: IR (KBr, γ_{max} cm^{-1}): **5a**: 1684 (C=O), 1610 (C=N), 1516 (NO_2), 1347 (NO_2), 1075 (C–F), 736 (C–Cl); **5f**: 1684 (C=O), 1586 (C=N), 732 (C–Cl); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) : **5a**: δ 2.68 (s, 3H, CH_3), 5.92 (d, 1H, methine H, J = 4.4 Hz), 5.81 (d, 1H, olefinic H, J = 4.4 Hz), 6.91–7.53 (m, 9H, Ar–H and furan H), 8.01 (d, 1H, Ar–H, J = 8.6Hz), 8.15 (m, 2H Ar–H), 9.75 (s, 1H, exocyclic vinylic H). **5b**: δ 2.68 (s, 3H, CH_3), 3.9 (s, 3H, –OCH_3) 5.8 (d, 1H, methine H, J = 4.4 Hz), 5.91(d, 1H, olefinic H, J = 4.4 Hz), 6.91–7.63 (m, 9H, Ar–H and furan H), 8.0 (d, 1H, Ar–H, J = 8.6 Hz), 8.15 (m, 2H Ar–H), 9.65 (s, 1H, exocyclic vinylic H). **5c**: δ 2.91 (s, 3H, CH_3), 3.6 (s, 3H, –OCH_3), 3.9 (s, 3H, OCH_3), 5.7 (d, 1H, methine H, J = 4.5 Hz), 5.85 (d, 1H, olefinic H, J = 4.5 Hz), 7.09–7.50 (m, 8H, Ar–H), 7.9 (d, 1H, J = 9 Hz) 8.1 (d, 1H, J = 9 Hz), 9.8 (s, 1H, exocyclic vinylic H); **5d**: δ 2.7 (s, 3H, CH_3), 3.9 (s,3H, –OCH_3), 6.05(s, 2H, methylene dioxy), 5.6 (d, 1H, methine H, J = 5 Hz), 5.7 (d, 1H, olefinic H, J = 5 Hz), 7.05–7.65 (m, 8H, Ar–H and furan H), 7.95 (d, 1H, Ar–H, J = 8.5 Hz), 8.0(d, 1H J = 8.5 Hz), 9.65 (s, 1H, exocyclic vinylic H). **5e**: δ 2.6 (s, 3H, CH_3), 5.8 (d, 1H, methine H, J = 4.4 Hz), 5.9 (d, 1H, olefinic H, J = 4.4 Hz), 6.98–7.71 (m, 8H, Ar–H and furan H), 7.95 (d, 1H, Ar–H, J = 8.7 Hz), 8.0 (d, 1H J = 8.7 Hz), 9.68 (s, 1H, exocyclic vinylic H). **5f**: δ, 5.85 (d, 1H, methine H, J = 4.6 Hz), 5.91 (d, 1H, olefinic H, J = 4.6 Hz), 6.66–7.40 (m, 10H, Ar–H and furan H), 7.9 (d, 1H, Ar–H, J = 8.9 Hz), 8.1 (d, 1H J = 8.9 Hz), 9.67(s, 1H, exocyclic vinylic H). **5g**: 3.9 (s, 3H, –OCH_3) 5.85 (d, 1H, methine H, J = 4.4 Hz), 5.91 (d, 1H, olefinic H, J = 4.4 Hz), 6.7–7.43 (m, 9H, Ar–H and furan H), 7.9 (d, 1H, Ar–H, J = 9 Hz), 8.0 (d, 1H, Ar–H, J = 9 Hz), 9.60 (s, 1H, exocyclic vinylic H). **5h**: δ 3.85 (s, 3H, –OCH_3), 3.86 (s, 3H, OCH_3), 5.9 (d, 1H, methine H, J = 4.8 Hz), 5.76 (d, 1H, olefinic H, J = 4.8 Hz), 6.82 (d, 1H, furan-H, J = 3.7 Hz), 6.68–6.71 (m, 7H, Ar–H and furan H), 7.79–7.96 (m, 2H, Ar–H), 9.69 (s, 1H, exocyclic vinylic H); **5i**: 3.9 (s, 3H, –OCH_3), 6.05 (s, 2H, methylene dioxy), 5.8 (d, 1H, methine H, J = 5 Hz), 5.9 (d, 1H, olefinic H, J = 5 Hz), 7.05–7.65 (m, 8H, Ar–H and furan H), 7.95 (d, 1H, Ar–H, J = 8.5 Hz), 8.1 (d, 1H J = 8.5 Hz), 9.70 (s, 1H, exocyclic vinylic H). **5j**: δ 6.27 (d, 1H, methine H J = 4.5 Hz), 5.93 (d, 1H, olefinic H, J = 4.5 Hz), 6.91–7.93 (m, 10H, Ar–H and furan H), 7.96(d, 1H, Ar–H J = 8.8 Hz), d, 1H,
Ar–H J = 8.6 Hz). MS: *m/z* (% abundance): **5b**[Fab Mass]: 636 (100%, MH^+), 635 (13%, MH^+-CH_3), 527 (10%, MH^+-107), 163 (10% ,2,4-dichloro-5-fluorophenyl cation), 107 (30%, 4-methoxyphenyl cation). **5d**[Fab Mass]: 650 (100%, MH^+), 528 (18%, M^+-121), 487,(MH^+-163), 163 (11%, 2,4-dichloro-5-fluorophenyl cation), 121 (20%, 3,4-methoxyphenyl cation); **5f**[FabMass]: 616 (22%, M^++2), 538 (10%, M^+-C_6H_5), 163 (8%, 2,4-dichloro-5-fluorophenyl cation); **5j**[FabMass]: 649 (100%, MH^+), 651(100%, MH^++2).

tion bands due to (C–F) and (C–Cl) were seen at 1083 cm^{-1} and 726 cm^{-1}, respectively. The 300 MHz ^{1}H NMR spectrum of compound **4a** showed two doublets at δ 5.06 (J = 4.6 Hz) and 5.26 (J = 4.6 Hz), respectively, due to the methine and olefinic protons of dihydropyrimidine ring. The two –NH protons of the pyrimidine ring were seen as two broad singlets at δ 7.72 and 7.81, respectively. The aromatic protons resonated as a complex multiplet in the region δ 6.85–



Scheme 2. Synthesis of 2-(5-aryfufurylidene/nitrofufurylidene)-5-(aryl)-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-b]pyrimidin-3-ones(**5a-j, 6a-e**).

7.57 integrating for seven protons. The mass spectrum of the compound **4d** showed a molecular ion peak at *m/z* 396, which is consistent with its molecular formula, $C_{17}H_{11}Cl_2FN_2O_2S$. A peak observed at *m/z* 362 is attributed to an ion obtained by the loss of H_2S from the molecular ion. A peak at *m/z* 121 corresponds to the formation of 3,4-methylenedioxyphenyl cation during the fragmentation process. A peak at *m/z* is attributable to the radical cation of 2,4-dichloro-5-fluoro-benzonitrile obtained during fragmentation. A peak seen at *m/z* 275 was attributed to the cation obtained by the loss of 3,4-dimethoxyphenyl radical from the molecular ion. The base peak seen at *m/z* 44 is due to the formation of radical cation of carbon monosulphide during the fragmentation process. The spectroscopic data of some other compounds **4** are given in experimental section.

Similarly the structure of the compounds **5** and **6** is established on the basis of spectral data. IR spectrum of **5f** showed an absorption band at 1684 cm^{-1} indicating the presence of amide carbonyl group in the molecule. Absence of the characteristic absorption bands due to NH stretch clearly showed that –NH protons were involved in the cyclization. An absorption band appearing at 1586 cm^{-1} is attributed to C═C stretching frequency. The absorption bands due to (C–F) and (C–Cl) were seen at 1083 cm^{-1} and 726 cm^{-1}, respectively. Further support for the formation of condensed products was obtained by recording ^{1}H NMR spectra of a few compounds. The 300 MHz ^{1}H NMR spectrum of compound **5h** showed two doublets at δ 5.90 (J = 4.8 Hz) and 5.76 (J = 4.8 Hz), which are assigned to the methine protons. The shift of the signals to higher δ values is due to the deshielding effect of the carbonyl group of thiazolidinone ring. The two methoxy protons resonated as two closely packed singlets at δ 3.85 and 3.86, respectively. The signal due to the exocyclic vinyl protons was seen as a singlet at δ 9.69. The signals of the protons of the furan ring appeared as two sharp doublets at δ 6.82 (J = 3.7 Hz) and 7.43 (J = 3.7 Hz), respectively. The aromatic protons resonated as a complex multiplet in the region δ 6.81–7.98 integrating for eight protons. The mass spectral data of these compounds further confirmed the formation of condensed products **5** and **6**. The FAB mass spectrum of the compound showed a molecular ion peak at 636, which is consistent with the assigned molecular formula, $C_{31}H_{20}Cl_2FN_3O_5S$. A peak seen at *m/z* 107 was due to 4-methoxyphenyl cation. The mass spectrum of compound **6b** showed a peak due to the protonated molecular ion at *m/z* 547, which is in agreement with the molecular formula $C_{24}H_{14}Cl_2FN_3O_5S$ assigned for this compound. A peak seen at *m/z* 189 is due to the formation of molecular ion of 2,4-dichloro-5-fluorobenzonitrile. Loss of CN radical from this ion produced 2,4-dichloro-5-fluorophenyl cation as evidenced by the presence of peak at *m/z* 163. The spectral data of the other newly synthesized compounds are given in the experimental section.

4. Pharmacology

Eight newly synthesized compounds were screened for their antitumour activities at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI according to the procedure suggested by Boyd and Paull [17] in a primary three cell line—one dose antitumour assay against NCI-H (lung), MCF-7 (breast) and SF 268 (CNS). In the current protocol, each cell line is inoculated on a preincubated microtiter plate. Test agents are added at a single concentration and the culture is incubated for 48 h. End point determinations are made with sulpharhodamine B, a protein binding dye. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in a panel of 60 cell lines over a 5-log dose range (Table 3). In the present screening programme, (Table 4) almost all the compounds showed moderate to good antiproliferative activity on the whole panel of 60 cell lines (Table 5) derived from seven cancer types namely lung, colon, melonoma, renal, ovarian, CNS and leukamia. Their GI_{50}, TGI and LC_{50} values were determined.

5. Conclusion

All the compounds tested showed moderate to good anticancer activity. Among them compound **5j**, 2-[5-(2,4-dichlorophenylfurfurylidene)]-5-(4-chlorophenyl)-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]-pyrimidin-2(1*H*)-one showed highest activity against leukemia HL-60(TB) cell line[GI_{50} = <0.001 μM, TGI = <0.001 μM, LC_{50} = <0.001 μM]. The compounds 2-(5-nitrofurfurylidene)-5-[4-(3,4-methylenedioxyphenyl)]-7-(2.4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]-pyrimidin-2(1*H*)-one (**6d**) and 2-(5-nitrofurfurylidene)-5-[(4-chlorophenyl)]-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]-pyrimidin-2(1*H*)-one (**6e**) showed moderate activity against all the tested cell lines and highest against leukemia SR cell line [GI_{50} = 3.34 μM], CCRP-CEM Cell Line [GI_{50} = 3.24 μM] and coloncancer HCT-116 cell line [GI_{50} = 3.56 μM]. Hence, the presence of 5-nitro-2-furfuryl moiety and 4-chlorphenyl groups as substituents tends to increase the anticancer property in these molecules, although they did not prove cytotoxic or cytostatic at the maximum tested concentration (100 μM).

Table 3
Characterization data of compounds 2-(5-nitrofurfurylidene)-5-(aryl)-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]pyrimidin-3-ones (**6a–e**)

Compound No.	R	M.p. (°C)	Yield (%)	Molecular formula	*N*-Analysis found [cald.]	*C*- Analysis found [cald.]
6a	H	78–80	68	$C_{23}H_{12}Cl_2FN_3O_4S$	8.10 [8.14]	53.46 [52.74]
6b	4-OCH_3	81–83	79	$C_{24}H_{14}Cl_2FN_3O_5S$	7.74 [7.69]	52.70 [52.74]
6c	3,4-$(OCH_3)_2$	109–11	71	$C_{25}H_{16}Cl_2FN_3O_6S$	7.26 [7.29]	52.12 [52.08]
6d	3,4-O-CH_2-O-	85–87	74	$C_{24}H_{12}Cl_2FN_3O_6S$	7.54 [7.50]	57.40 [57.42]
6e	4-Cl	106–108	76	$C_{23}H_{11}Cl_3FN_3O_4S$	7.60 [7.62]	50.16 [50.13]

Solvent of crystallization: glacial acetic acid and DMF
IR (KBr, γ_{max} cm^{-1}): **6a**: 3034(C–H), 1759 (C=O), 1598 (C=N), 1541 (NO_2), 1354 (NO_2), 1077 (C–F), 728 (C–Cl); **6c**: 2935(C–H), 1713 (C=O), 1594 (C=N), 1514 (NO_2), 1348 (NO_2), 1079 (C–F), 729 (C–Cl).
^{1}H NMR ($CDCl_3$ + DMSO-d_6): **6a**: 6.01 (d, 1H, methine H *J* = 4.7 Hz), 5.75 (d, 1H, olefinic H, *J* = 4.7 Hz), 6.725–7.81 (m, 7H, Ar–H and furan H), 7.95 (d, 1H, *J* = 9 Hz) 8.1(d, 1H, *J* = 9 Hz), 9.83 (s, 1H, exocyclic vinylic H); **6b**: δ 3.9 (s, 3H, –OCH_3), 6.05 (d, 1H,methine H *J* = 4.8 Hz), 5.80 (d, 1H, olefinic H, *J* = 4.8 Hz), 6.70–7.6 (m, 6H, Ar–H and furan H), 7.92 (d, 1H, *J* = 9 Hz) 8.05 (d, 1H, *J* = 9 Hz), 9.82 (s, 1H, exocyclic vinylic H); **6c**: δ 3.83 (s, 3H, –OCH_3), 3.84 (s, 3H, OCH_3), 6.18 (d, 1H,methine H *J* = 5.1 Hz), 5.89 (d, 1H, olefinic H, *J* = 5.1 Hz), 6.85–7.85 (m, 7H, Ar–H and furan H), 9.84 (s,1H, exocyclic vinylic H);**6d**: δ 6.01(s, 2H, –OCH_2O–), 6.04 (d, 1H, methine H *J* = 4.6 Hz), 5.71 (d, 1H, olefinic H, *J* = 4.6 Hz), 6.72–7.71 (m, 7H, Ar–H and furan H), 9.89 (s, 1H, exocyclic vinylic H); **6e**: 6.02 (d, 1H,methine H *J* = 4.5 Hz), 5.9 (d, 1H, olefinic H, *J* = 4.5 Hz), 6.85–7.78 (m, 8H, Ar–H and furan H), 9.84 (s, 1H, exocyclic vinylic H);
MS: *m/z* (% abundance): **6b**: 547(16%, M^++2), 502 (96%, M^+-NO_2), 275 (38%, M^+–C_6H_5), 163 (8%, 2,4-dichloro-5-fluorophenyl cation), 83 (33%, 1-nitrocyclopropenyl cation). **6c**: ($M^+$is not observed), 340 (50% M^+-2,4-dicloro-5-fluorobenzonitrile & NO_2), 163 (8%, 2,4-dichloro-5-fluorophenyl cation), 223 (100%, M^+-163), 163(17%, 2,4-dichloro-5-fluorophenyl cation), 111 (33%, 4-chlorophenyl cation).

Table 4
Anticancer activity screening data of compound **4, 5** and **6**

Compound	Growth percentage [a]				
	NCI code	NCI-H (lung)	MCF-7 (breast)	SF268 (CNS)	Activity [b]
4b	NSC 716879	–5	07	27	Active
4d	NSC 716880	09	14	34	Active
4e	NSC 716878	26	11	43	Active
5g	NSC 716882	37	14	27	Active
5i	NSC 716883	24	12	17	Active
5j	NSC 716881	30	19	07	Active
6d	NSC 716885	–6	–55	–67	Active
6e	NSC 716884	–24	–54	–38	Active

Fixed concentration assay (100 μM; standard NCI protocol).
[a] Percentage cell growth reduction following 48-h incubation with test compounds (optical density, sulphorhodamine proceure) [17].
[b] 'Active' when growth percentage is <32% for any one of the three cell lines. Negative numbers indicate the cell kill.

Table 5
Sixty cell line in vitro antitumour screening data (GI_{50} in μM)

Panel cell line	**4b**	**4d**	**4e**	**5g**	**5i**	**5j**	**6d**	**6e**
Leukemia								
CCRF-CEM	30.2	21.8	28.9	–	38.8	–	3.24	4.83
HL-60[TB]	11.3	**0.003**	20.6	–	27.2	**<0.001**	15.6	12.3
K-562	15.2	32.1	56.2	38.8	35.2	38.8	5.10	13.1
MOLT-4	17.7	23.4	15.6	31.9	28.2	22.4	8.92	12.8
RPMI-8226	21.7	22.8	14.9	32.5	26.1	31.1	9.32	5.16
SR	52.4	31.9	40.6	–	–	–	3.34	–
Non-small lung								
A549/ATCC	35.3	35.6	47.3	>100	46.1	>100	16.5	30.3
EKVX	28.0	22.2	31.6	–	–	–	16.6	–
HOP-62	38.7	39.5	48.2	18.8	58.7	10.6	22.6	11.5
HOP-92	24.0	25.9	27.1	31.6	31.1	26.1	22.6	12.9
NCIH-226	56.1	59.1	88.7	24.6	20.7	22.4	46.7	15.8
NCI-H23	37.5	60.8	63.9	38.5	38.6	36.5	20.6	15.3
NCI-H322M	51.3	42.2	45.7	>100	19.3	47.9	38.3	14.3
NCI-H460	20.1	25.5	34.0	48.5	36.9	60.2	17.8	10.0
NCI-H522	31.8	31.6	34.0	52.2	49.1	84.4	25.8	18.3
Colon cancer								
COLO-205	19.1	20.9	41.0	79.9	38.7	78.6	18.7	3.94
HCC-2998	23.9	23.2	23.3	20.1	–	12.8	12.9	–
HCT-116	27.0	27.5	35.8	35.1	35.4	38.0	5.10	3.56
HCT-15	28.7	37.4	46.8	33.8	34.1	35.6	9.6	8.19
HT-29	26.2	27.9	36.5	37.3	25.7	39.8	17.1	6.63
KM-12	29.2	38.0	49.4	60.1	57.5	87.5	16.6	24.5
SW-620	30.9	36.0	70.6	51.1	35.8	33.6	5.42	4.91
CNS cancer								
SF-268	48.6	45.7	65.7	35.6	46.3	36.0	28.7	13.9
SF-295	24.3	31.9	28.2	43.1	40.7	53.2	31.2	21.3
SF-539	42.3	46.6	>100	34.6	35.3	34.7	23.8	22.4
SNB-19	61.0	57.4	76.7	31.6	28.7	35.6	20.9	15.9
SNB-75	18.2	20.8	37.6	38.4	>100	63.3	17.3	29.2
U-251	26.7	22.9	29.6	34.7	29.5	31.5	19.9	8.02
Melanoma								
LOXIMVI	37.3	37.1	48.7	38.0	34.8	57.9	17.2	16.0
MALME-3M	40.6	30.7	>100	99.1	>100	>100	19.7	17.9
M-14	32.5	40.4	54.3	55.4	28.0	>100	13.9	14.5
SKMEL-2	25.0	20.9	27.1	20.1	26.0	40.4	25.1	50.4
SKMEL-28	46.9	40.2	>100	>100	91.8	>100	22.3	24.2
SKMEL-5	11.8	10.3	11.6	36.6	28.5	46.5	10.3	13.9
UACC-257	24.6	26.3	31.6	22.6	30.0	34.6	22.6	15.4
UACC-62	28.3	35.9	57.0	25.5	16.0	25.0	15.9	14.7
Ovarian cancer								
IGROVI	31.4	27.4	41.2	30.3	34.2	33.0	16.3	15.8
OVCAR-3	33.7	30.2	33.1	–	–	–	16.8	–
OVCAR-4	2.83	38.1	35.8	37.3	33.2	41.9	15.8	16.6
OVCAR-5	43.0	4.1	>100	39.4	58.8	>100	21.7	16.3
OVCAR-8	40.3	41.2	39.9	37.8	42.0	45.8	11.9	41.6
SK-OV-3	44.1	57.0	>100	21.5	21.5	192	46.5	14.8
Renal cancer								
786-O	33.6	39.9	47.9	42.3	42.9	45.3	95.1	47.1
A498	28.0	20.8	27.8	24.8	22.6	20.5	56.0	30.7
ACHN	36.9	28.8	37.7	>100	69.2	>100	16.1	16.3
CAKI-1	29.3	34.8	42.9	80.6	>100	>100	13.8	28.7
RXP 393	18.8	23.0	31.3	39.6	39.5	31.3	16.1	13.1
SN12C	21.3	27.4	29.3	47.7	57.3	76.3	18.0	54.9
TK10	32.7	28.8	45.1	12.1	77.8	13.4	23.3	16.1
UO-31	36.5	39.2	42.2	32.9	31.7	41.5	16.8	14.7

(continued on next page)

Table 5
(continued)

Panel cell line	4b	4d	4e	5g	5i	5j	6d	6e
Prostate cancer								
PC-3	19.9	21.4	28.3	99.3	69.1	>100	22.0	11.5
DU-145	39.9	49.0	62.8	70.9	63.6	65.1	21.6	17.8
Breast cancer								
MCF7	32.6	32.2	31.2	26.9	26.0	33.5	10.0	13.3
NCI/ADR-RES	25.7	51.7	64.1	44.4	45.9	50.8	34.4	30.8
MDA-MB-231/ATCC	27.3	23.8	23.6	25.5	22.6	23.6	23.0	50.7
HS 578T	79.1	70.0	>100	34.8	14.0	29.6	61.0	56.4
MDA-MB-435	12.8	34.5	43.9	30.8	32.2	54.3	13.4	16.1
MDA-N	11.1	35.6	47.4	28.1	37.9	53.4	55.0	46.4
BT-549	32.7	57.0	>100	47.2	37.8	45.8	16.8	15.1
T-47D	18.7	20.2	19.2	19.6	21.1	19.2	17.3	19.0

The other two standard parameters TGI and LC_{50} were above 100 μM except for **5j**, which is <0.001 μM.

Hence, it is concluded that there is ample scope for further study in developing these compounds as anticancer agents.

6. Experimental

Melting points were taken in open capillary tubes and are uncorrected. IR spectra in KBr pellets were recorded on JASCO FT-IR 5300 Infrared spectrophotometer.^{1}H-NMR spectra were recorded ($CDCl_3$/$CDCl_3$-DMSO-d_6 mixture) on a Brucker AC 300 F (300 MHz) NMR spectrometer using TMS as an internal standard and the mass spectra were recorded on a JEOL JMS 300 mass spectrometer operating at 70 eV.

*6.1. General procedure for the preparation of 4-(aryl)-6-(2,4-dichloro-5-fluorophenyl)-3,4-dihydropyrimidin-2(1*H*)-thiones 4*

A mixture of 1,3-diaryl-2-propen-1-one (20 mmol) and thiourea (20 mmol) in ethanolic potassium hydroxide (2 g in 15 ml) was heated under reflux for 5 h. The volume of the reaction mixture was reduced to half of its original volume, diluted with ice cold water, then acidified with dilute acetic acid and kept over night. The solid thus obtained was filtered and washed with water and recrystallized from suitable solvents. The characterization data of these compounds are given in Table 1.

*6.2. General procedure for the preparation of 2-(5-arylfurfurylidene/5-nitrofurfurylidene)-5-(aryl)-7-(2,4-dichloro-5-fluorophenyl)-5*H*-thiazolo[2,3-*b*]pyrimidin-3-ones 5 and 6*

A mixture of thione **4** (10 mmol) monochloroacetic acid (15 mmol), anhydrous sodium acetate (2 g), glacial acetic acid (20 ml) acetic anhydride (15 ml) and 5-arylfuran-2-carboxaldehyde/5-nitro-2-furfural diacetate (10 mmol) was heated under reflux for 3 h. The reaction mixture was cooled and poured onto crushed ice with vigorous stirring. The separated solid was filtered and washed with water, recrystallized from suitable solvents to give **5** and **6**. The characterization data of the title compounds are given in Tables 2 and 3.

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References

[1] A. Doemling, Curr. Opin. Chem. Biol. 4 (2000) 318.
[2] B. Beck, M. Magnin-Larchax, E. Herdtweck, A. Doemling, Org. Lett. 3 (2001) 2875 and references cited therein.
[3] D. Lee, J.K. Sello, S.L. Schreiber, Org. Lett. 2 (2000) 709.
[4] P. Bignelli, Ber. Dtsh. Chem. Ges. 26 (1893) 447.
[5] A. Hantzsch, Justus Liebigs Ann. Chem. 1 (1882) 215.
[6] I. Brugnatelli, Ann. Chim. 8 (1818) 201.
[7] S.A. Szumski, J.J. Goodmann, U.S.Pat.2,797,187(1957), Chem. Abstr. 51 (1957) 14211.
[8] W. Sun, D. Haller, Oncology-Williston Park the Huntington, 15, 2001, pp. 49.
[9] B. Lagu, D. Tain, G. Chiu, D. Nagarathnam, J. Fang, Q. Shen, C. Forray, R.W. Ranson, S. Raymond, L. Chang, K.P. Vyas, K. Zang, C. Gluchowski, Bioorg. Med. Chem. Lett. 10 (2000) 175.
[10] N. Hans, Swiss Pat. 592103 (1977), Chem. Abstr. 88 (1978) 22886.
[11] K.J. Wilson, C.R. Utig, N. Subhasinghe, J.B. Hoffmann, N.J. Rudolph, R. Soll, C.J. Molloy, R. Bone, D. Green, Bioorg. Med. Chem. Lett. 11 (2001) 915.
[12] K.D. Berlin, M.D. Herd, Proc. Okala. Acad. Sci. 71 (1991) 29 and references cited therein.
[13] B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sorojini, N.S. Kumari, Eur. J. Med. Chem. 38 (2003) 313.
[14] R. Filler, Y. Kobayashi, Biomedicinal Aspects of Fluorine Chemistry, Elsevier, Amsterdam, 1982.
[15] B.S. Holla, B.K. Sarojini, B.S. Rao, P.M. Akberali, N.S. Kumari, V. Shetty, Il Farmaco. 56 (2001) 565.
[16] B.S. Holla, B.S. Rao, R. Gonsalves, B.K. Sarojini, K. Shridhara, Il Farmaco. 57 (2002) 693.
[17] M.R. Boyd, K.D. Paull, Drug. Dev. Res. 34 (1995) 91.