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Anticancer Activities of Some New Synthesized Thiazolo[3,2-a]Pyrido[4,3-d]Pyrimidine Derivatives

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Abstract: Problem statement: This study describes the synthesis and anticancer activities of a new series of thiazolo[3,2-a]pyrimidines derivatives (2-7) using 3,5-bisarylmethylene-1-methyl-4-piperidone and 4-aryl-8-arylmethelene-6-methylpyrido[4,3-d]pyrimidine-2(1H)thiones as a starting materials. **Approach:** The antitumor activities of the newly synthesized compounds 4-7 were evaluated utilizing 60 different human tumor cell lines, representing leukemia, melanoma, lung, colon, brain, ovary, breast and prostate as well as kidney. **Results:** Some of the tested compounds exhibited better in vitro antitumor activities at low concentration (log10 GI_{50} = -4.7) against the used human tumor cell lines. **Conclusion:** From the obtained results, we can conclude that pyrimidine moieties fused to N-methylpipredine ring are essential for antitumor activities. In the present work, we can suggest that the anticancer activity is due to the presence of nitrogen heterocyclic rings and the presence of sulfur atom generally enhancing the activity.

Key words: Synthesis, reactions, pyrimidinethione, thiazolopyrimidine, anticancer activity, starting materials, nitrogen heterocyclic, pyrimidines derivatives, biological activity

INTRODUCTION

Cancer poses a serious human health problem despite much progress in understanding its biology and pharmacology. Consequently, the design of new lead structures employed as antitumor agents is one of the most urgent research areas in contemporary medicinal chemistry. During our ongoing studies aimed at the discovery of new heterocycles endowed with antitumour activity, we have reported on the synthesis and antitumor activities of a series of heterocyclic compounds (Hammam *et al*., 2003; 2005; Amr *et al*., 2006; Velusamy and Palaniappan, 2011; Abd El-Salam *et al*., 2010). Pyrimidine has gained considerable attention because of its diversity in biological activity and widespread applications in pharmaceuticals fields (Katritzky and Rees, 1996; Francis *et al*., 2011). For instance, as Tie-2 kinase inhibitors (Matloobi and Kappe, 2007; Chengguo *et al*., 2009), HIV-1 inhibitor (Gadhachanda *et al*., 2007; Naeem *et al*., 2009), antimalarial (Ngoy *et al*., 2011; Khan *et al*., 2009), adenosine A_1 receptor antagonist (Chang *et al*., 2004), anticancer (Capdeville *et al*., 2002), analgesic (Rezvani and Shariati, 2010), cardiovascular (Atwal, 1988 and Hasanuzzaman *et al*., 2010) and antiallergic (Ozeki *et al*., 1989; Dahmardeh, 2011) activities. On the other hand, the importance of the pyridine ring in the chemistry of biological system has been greatly realized because of their presence as substructure in many natural products of therapeutic importance, involved in oxidation-reduction process. The potent biological activity of various vitamins and drugs (Joule and Mills, 2000; Henry, 2004; Li *et al*., 1999; Vacher *et al*., 1999; Nasratun *et al*., 2009) is primarily contributed by the presence of pyridine ring in their molecular make-up. Furthermore, the pyridine ring is found in the skeleton of many compounds with potent antibacterial, antifungal and anticancer properties (Millet *et al*., 2004; Mallea *et al*., 2003; Abou-Ghalia and Amr, 2004; Amr *et al*., 2009; Jill *et al*., 2011). In view of these reports and in continuation of our previous work in heterocyclic chemistry, we herein synthesized some new derivatives containing heterocyclic ring fused with *N*-methylpiperdion and/or pyrido [4,3-d] pyrimidine

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structure for the evaluation of their anticancer activities. In view of a beforementioned biological activities and as a part of our interest in the sereach for novel anticancer agents, we report herein the synthesis of several thiazolo [3, 2-a] pyrido [4, 3-d] pyrimidine derivatives and evaluate of their anticancer activities.

MATERIALS AND METHODS

Chemistry: All melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analyses were performed on Elementar, Vario El, Microanalytical Unit, National Research Centre, Cairo, Egypt and were found win ± 0.4% of the theoretical values. Infrared (IR) spectra were recorded on Carlzeise Spectrophotometer model 'UR 10' spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6 or $CDCl_3$) and the chemical shift are given in δ (parts per million) downfield from Tetramethylsilane (TMS) as an internal standard. The Mass Spectra (MS) were measured using a Finnegan SSQ 7000 mass spectrometer. The anticancer screening occurred in United States National Institute of Health (NIH)/National Cancer Institute (NCI). The starting material, 3,5-bisarylmethylene-1-methyl-4-piperidon (1) was synthesized according to the reported procedures (Lyle *et al*., 1973; Mcelvain and Rorig, 1948; Abdel-Latif and Lamiaa, 2010).

Synthesis of thiopyrimidene derivatives (2a-e): To a solution of 1a-e (0.01 mile) in 25 ml absolute ethanol, 0.5 g potassium hydroxide and thiourea (0.76 g, 0.01 moles) were added**.** The reaction mixture was refluxed for 3 hrs. Left to cooland poured gradually onto cold water. The solid formed was filtered off, washed with water and crystallized from the proper solvent to give pyrimidin-2-(1H) thiones 2a-e, respectively.

8-Benzylidene-3,4,5,6,7,8-hexahydro-6-methyl-4-phenylpyrido[4,3-d]pyrimidine-2(1H)-thione (2a): Yield 89%, mp 190-193°C; IR (KBr) cm^{-1}: 3194, 3502; ^{1}H NMR (DMSO-d_6): 2.20 (s, 3H, CH_3), 2.85 (m, 2H, H-5), 3.10-3.19 (dd, 2H, J = 7.0 Hz, J = 5.0 Hz, H-7), 4.95 (s, 1H, H-4), 7.15-7.40 (m, 11H, Ar-H + C=CH), 9.18, 9.48 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR: 44.48 (CH_3), 53.91 (CH, pyrimidine), 125.63 (CH, methylene), 54.35, 54.39 (2CH_2-pyridine), 137.92 (C-pyridine), 138.26, 143.30, 180.6 (3C-pyrimidine), 126.65, 126.92, 128.13, 128.62, 128.74, 129.49, 136.19, 143.29 (12C, Ar-C); MS (EI): m/z 347 [M^+] (80), 346 (M^+-H^+) (100), 214 (346- C_6H_5CH=NH) (52), 254 (M^+-C_6H_5 -H^+) (19). Anal. Calcd. for $C_{21}H_{21}N_3S$: C, 72.58; H, 6.1; N, 12.1; S, 9.22. Found: C, 72.56; H, 6.12; N, 12.07; S, 9.25.

8-(4-Flourobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-flourophenyl)pyrido[4,3-d]pyrimidine-2(1H)-thione (2b): Yield 91%; mp 155-157°C; IR (KBr) cm^{-1}: 3204, 3344 (NH); ^{1}H NMR (DMSO-d_6): 2.20 (s, 3H, CH_3), 2.41-2.60 (m, 2H, H-5), 3.10-3.20 (dd, 2H, J = 7.0 Hz, J = 5.0 Hz, H-7), 4.96 (s, 1H, H-4), 7.11-7.50 (m, 9H, Ar-H+C = CH), 9.21, 9.51 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR: 44.47 (CH_3), 53.91 (CH, pyrimidine), 50.93, 52.71 (2CH_2-pyridine), 124.19 (CH, methylene), 136.81 (C-pyridine), 126.29, 126.47, 128.14, 128.61, 128.75, 128.89, 135.43, 143.33 (12C, Ar-C), 137.28, 140.41, 180.56 (3C-pyrimidine); MS (EI): m/z 383 [M^+] (100), 339 (M^+-C=S) (11), 259 (383-F-C_6H_4CH=NH) (71); Anal. Calcd. For $C_{21}H_{19}N_3SF_2$: C, 65.77; H, 4.99; N, 10.96; S, 8.36. Found: C, 65.75; H, 5.01; N, 10.94; S, 8.38.

8-(2-Chlorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(2-chlorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-thione (2c): Yield 82%; mp 200-202°C; IR (KBr) cm^{-1}: 3351, 3304 (NH); ^{1}H NMR (DMSO-d_6): 2.20 (s, 3H, CH_3), 2.50-2.71 (m, 2H, H-5), 3.21-3.30 (dd, 2H, J = 7.0 Hz, J = 5.0 Hz, H-7), 5.41 (s, 1H, H-4), 7.15-7.55 (m, 9H, Ar-H + C = CH), 9.10, 9.71 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR: 44.44 (CH_3), 50.98, 52.73 (2CH_2-pyridine), 53.91 (CH, pyrimidine), 125.63 (CH, methylene), 126.31, 126.52, 128.19, 128.62, 128.74, 128.89, 135.43, 143.33 (12C, Ar-C), 134.22 (C-pyridine), 137.32, 140.42, 180.59 (3C-pyrimidine); MS (EI): m/z 416 [M^+] (50), 417 [M^++2] (35), 419 [M^++4] (8), 275 (M^+-Cl-C_6H_4CH=NH, -H_2) (100); Anal. Calcd. for $C_{21}H_{19}N_3SCl_2$: C, 60.57; H, 4.6; N, 10.1; S, 7.7. Found: C, 60.6; H, 4.57; N, 10.07; S, 7.71.

8-(4-Chlorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-chlorophenyl)-pyrido[4,3-d]pyrimidine-2(1H)-thione (2d): Yield 88%; mp 202-205°C; IR (KBr) cm^{-1}: 3176, 3248 (NH); ^{1}H NMR (DMSO-d_6): 2.32 (s, 3H, CH_3), 2.42-2.63 (m, 2H, H-5), 3.31-3.43 (dd, 2H, J = 7.0 Hz, J = 5.0 Hz, H-7), 5.02 (s, 1H, H-4), 7.10-7.60 (m, 9H, Ar-H + C=CH), 9.10, 9.60 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR: 44.48 (CH_3), 50.96, 54.74 (2CH_2-pyridine), 53.91 (CH, pyrimidine), 124.28 (CH, methylene), 126.42, 126.50, 128.15, 128.61, 128.79, 128.89, 135.43, 143.37 (12C, Ar-C), 137.36, 140.46, 180.82 (3C-pyrimidine), 136.88 (C-pyridine); MS (EI): m/z 416 [M^+] (87), 417 (M^++2) (59), 419 (M^++4) (13), 414 (M^+-H_2) (100), 275 (M^+-Cl-C_6H_4CH=NH, -H_2) (72); Anal. Calcd. for

$C_{21}H_{19}N_3SCl_2$: C, 60.57; H, 4.6; N, 10.1; S, 7.7. Found: C, 60.55; H, 4.62; N, 10.13; S, 7.68.

8-(3-Bromobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(3-bromophenyl)pyrido[4,3-d]pyrimidine-2(1H)-thione (2e): Yield 85%; mp 180-182°C; IR (KBr) cm^{-1}: 3346, 3275 (NH); ^1H NMR (DMSO-d_6): 2.23 (s, 3H, CH_3), 2.32-2.53 (m, 2H, H-5), 3.32-3.41 (dd, 2H, J = 7.0 Hz, J = 5.0 Hz, H-7), 5.31 (s, 1H, H-4),7.30-7.60 (m, 9H, Ar-H+C=CH), 9.01, 9.42 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR: 44.49 (CH_3), 50.95, 52.74 ($2CH_2$-pyridine), 53.74 (CH, pyrimidine), 124.23 (CH, methylene), 126.31, 126.48, 128.21, 128.71, 128.68, 128.87, 135.45, 143.37 (12Ar-C), 136.84 (C-pyridine), 137.29, 140.44, 180.57 (3C-pyrimidine). MS (EI): m/z 505 [M^+] (47), 507 (M^++2) (95), 509 (M^++4) (45), 349 (M^+-Br-C_6H_4) (100); Anal. Calcd. For $C_{21}H_{19}N_3SBr_2$: C, 49.91; H, 3.79; N, 3.18; S, 6.34. Found: C, 49.91; H, 3.79; N, 3.18; S, 6.34**.**

Synthesis of 5-aryl-9-arylmethylene-2,3,6,7,8,9-hexahydro-7-methy-5H-thiazolo[3,2-a]pyrido-[4,3-d]pyrimidines (3a-e): To a solution of **1a-e** (0.02 mole), in a mixture of butanol (100 mL), DMSO (40 ml)and 2-aminothiazoline (2.7g, 0.023 mole) was added. The reaction mixture was refluxed for 24 hrs, the solvent was concentrated under reduced pressureand the residue was solidified with water, the formed solid was filtered offand crystallized from the proper solvent to give thiazolo[3,2-a]pyrido[4,3-d]pyrimidine**s** 3a-e, respectively.

9-Benzylidene-2,3,6,7,8,9-hexahydro-7-methyl-5H-5-phenyl-thiazolo[3,2-a]pyrido[4,3-d]-pyrimidine (3a): Yield 81%; mp 169-171°C; IR (KBr) cm^{-1}: 1635 (C=N); ^1H NMR (DMSO-d_6): 2.10 (s, 2H-C_6), 2.71 (s, 3H, CH_3), 2.99-3.01 (m, 2H-C_2), 3.05-3.15 (m, 2H-C_3), 3.60-3.58 (m, 2H-C_8), 5.51 (s, 1H-C_5), 7.36-6.57 (m, 11H, Ar-H + CH = C); ^{13}C NMR: 24.61, 50.35 ($2CH_2$, thiazole), 44.31 (CH_3), 51.71, 53.41 ($2CH_2$-pyridine), 58.31 (CH, pyrimidine), 124.28 (CH, methylene), 126.42, 127.22, 128.12, 128.65, 128.72, 135.32, 138.32 (12C, Ar-C), 129.21, 134.82, 183.66 (3C-pyrimidine), 137.1 (C-pyridine). MS (EI) m/z (%): 373 [M^+] (79), 262 (M^+-ClC_6H_4) (100), 249 (262-CH) (50); Anal. Calcd. for $C_{23}H_{23}N_3S$: C,73.95; H, 6.20; N, 11.25; S, 8.58. Found: C,73.93; H, 6.22; N, 11.27; S, 8.56**.**

9-(4-Fluorobenzylidene)-2,3,6,7,8,9-hexahydro-7-methyl-5H-5-(4-flourophenyl)-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine (3b): Yield 82%; mp 132-134°C; IR (KBr) cm^{-1}: 1631 (C=N); ^1H NMR (DMSO-d_6): 2.10 (s, 2H-C_6), 2.72 (s, 3H, CH_3), 2.99-3.11 (m, 2H-C_2), 3.21-3.25 (m 2H-C_3), 3.61-3.68 (m, 2H-C_8), 5.52 (s, 1H-C_5), 7.28-6.56 (m, 9H, Ar-H + CH=C); ^{13}C NMR: 24.59, 50.33 ($2CH_2$, thiazole), 44.29 (CH_3), 51.69, 53.40 ($2CH_2$-pyridine), 58.30 (CH, pyrimidine), 115.11, 115.32, 128.11, 129.41, 130.41, 134.30, 162.23 (12C, Ar-C), 124.28 (CH, methylene), 136.9 (C-pyridine), 129.19, 134.80, 183.64 (3C-pyrimidine); MS (EI): m/z 409 [M^+] (100), 408 (M^+-H) (97), 314 (M^+- F-C_6H_4) (70); Anal. Calcd. for $C_{23}H_{21}N_3SF_2$: C, 67.46; H, 5.16; N, 10.26; S, 7.83. Found: C, 67.48; H, 5.14; N, 10.28; S, 7.80**.**

9-(2-Chlorobenzylidene)-2,3,6,7,8,9-hexahydro-7-methyl-5H-5-(2-chlorophenyl)-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine (3c): Yield 79%; mp 143-145°C; IR (KBr) cm^{-1}: 1636 (C=N); ^1H NMR (DMSO-d_6): 2.14 (s, 2H-C_6), 2.73 (s, 3H, CH_3), 2.98-3.09 (m, 2H-C_2), 3.18-3.21 (m, 2H-C_3), 3.59-3.66 (m,2H-C_8), 5.54 (s, 1H-C_5), 6.85-7.32 (m, 9H, Ar-H + CH=C); ^{13}C NMR: 24.59, 50.33 ($2CH_2$, thiazole), 44.29 (CH_3), 51.69, 53.40 ($2CH_2$-pyridine), 58.33 (CH, pyrimidine), 124.28 (CH, methylene), 115.21, 115.32, 128.30, 129.81, 130.52, 134.32, 162.20 (12C, Ar-C), 136.91 (C-pyridine), 129.21, 134.80, 183.64 (3C-pyrimidine); MS (EI): m/z 442 [M^+] (64), 444 (M^++2) (40), 446 (M^++4) (6), 406 (M^+-Cl) (17), 362 (M^+-CH_2=NCH_3, -H^+) (18), 44 (100); Anal. Calcd. for $C_{23}H_{21}N_3SCl_2$: C, 62.43; H, 4.78; N, 9.50; S, 7.24. Found: C, 62.41; H, 4.80; N, 9.58; S, 7.21.

9-(4-Chlorobenzylidene)-2,3,6,7,8,9-hexahydro-7-methyl-5H-5-(4-chlorophenyl)-thiazolo-[3,2-a]pyrido[4,3-d]pyrimidine (3d): Yield 80%; mp 187-189°C; IR (KBr) cm^{-1}: 1638 (C=N); ^1H NMR (DMSO-d_6): 2.13 (s, 2H-C_6), 2.72 (s, 3H, CH_3), 2.97-3.08 (m, 2H-C_2), 3.16-3.19 (m 2H-C_3), 3.58-3.65 (m, 2H-C_8), 5.53 (s, 1H-C_5), 6.84-7.31 (m, 9H, Ar-H + CH=C); ^{13}C NMR: 24.63, 50.31 ($2CH_2$, thiazole), 44.24 (CH_3), 51.72, 53.41 ($2CH_2$-pyridine), 58.32 (CH, pyrimidine), 115.12, 115.35, 128.13, 129.73, 130.43, 134.32, 162.25 (12C, Ar-C), 124.27 (CH, methylene), 136.85 (C-pyridine), 129.22, 134.81, 183.50 (3C-pyrimidine); MS (EI): m/z 442 [M^+] (44), 444 (M^++2) (16), 446 (M^++4) (6), 318 (M^+-CH-C_6H_4-Cl) (57), 113 (100); Anal. Calcd. for $C_{23}H_{21}N_3SCl_2$: C, 62.43; H, 4.78; N, 9.50; S, 7.24. Found: C, 62.45; H, 4.76; N, 9.52; S, 7.22.

9-(3-Bromobenzylidene)-2,3,6,7,8,9-hexahydro-7-methyl-5H-5-(3-bromophenyl)-thiazolo-[3,2-a]pyrido[4,3-d]pyrimidine (3e): Yield 78%; mp 139-142°C; IR (KBr) cm^{-1}: 1634 (C=N); ^1H NMR (DMSO-d_6): 2.15 (s, 2H-C_6), 2.73 (s, 3H, CH_3), 2.99-3.10 (m, 2H-C_2), 3.19- 3.21 (m 2H-C_3), 3.65-3.71 (m, 2H-C_8),

5.56 (s, 1H-C_5), 6.66-7.28 (m, 9H, Ar-H + CH=C); ^{13}C NMR: 24.64, 50.32 (2CH_2, thiazole), 44.25 (CH_3), 51.70, 53.40 (2CH_2-pyridine), 58.33 (CH, pyrimidine), 115.14, 115.34, 128.11, 129.76, 130.45, 134.34, 162.27 (12C, Ar-C), 124.25 (CH, methylene), 136.86 (C-pyridine), 129.20, 134.80, 183.55 (3C-pyrimidine); MS (EI): m/z 531 [M^+] (45), 533 (M^++2) (91), 535 (M^++4) (44), 451 (M^+-Br) (100); Anal. Calcd. for $C_{23}H_{21}N_3SBr_2$: C, 51.99; H, 3.98; N, 7.91; S, 6.03. Found: C, 51.97; H, 4.01; N, 7.89; S, 6.05.

Synthesis of 5-aryl-9-arylmethylene-6,7,8,9-tetrahydro-7-methyl-2H-thiazolo[3,2-a]pyrido-[4,3-d]pyrimidin-3(5H)-ones (4a-e): To a mixture of 2a-e (0.01 mole), chloroacetic acid (1 g, 0.01 mole), 6 g of fused sodium acetate in 30 ml of glacial acetic acid and 15 mL of acetic anhydride was refluxed for 3 hrs., poured gradually onto cold water. The solid formed was filtered offand crystallized from the proper solvent to give pyrido[4,3-d]pyrimidinones 4a-e, respectively.

9-Benzylidene-6,7,8,9-tetrahydro-7-methyl-5-phenyl-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (4a): Yield 69%; m.p 214-216°C; IR (KBr) cm^{-1}: 1734 (C=O); ^{1}H NMR (DMSO-d_6): 2.21 (s, 3H, CH_3), 2.62 (s, 2H-C_6), 3.70-4.02 (m, 2H-C_8), 4.12 (s, 2H-C_{-2}), 5.81 (s, 1H-C_5), 7.33-7.52 (m, 10H, Ar-H), 7.72 (s, 1H, CH=C); ^{13}C NMR: 30.61 (CH_2, thiazole), 44.21 (CH_3), 45.31 (CH, pyrimidine), 51.41, 53.43 (2CH_2-pyridine), 126.42, 126.82, 127.32, 128.11, 128.63, 128.73, 135.32, 143.22 (12C, Ar-C), 124.23 (CH, methylene), 136.91 (C-pyridine), 129.65, 134.84, 163.66 (3C-pyrimidine), 171.11 (C=O); MS (EI): m/z 387 [M^+] (44), 386 (M^+-H^+) (100), 312 (386-SCH_2-C=O) (7); Anal. Calcd. for $C_{23}H_{21}N_3SO$: C, 71.28; H, 5.46; N, 10.84; S, 8.27. Found: C, 71.30; H, 5.43; N, 10.82; S, 8.29.

9-(4-Flourobenzylidene)-6,7,8,9-tetrahydro-7-methyl-5-(4-flourophenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (4b): Yield 76%; mp 203-205°C; IR (KBr) cm^{-1}: 1732 (C=O); ^{1}H NMR (DMSO-d_6): 2.22 (s, 3H, CH_3), 2.60 (s, 2H-C_6),3.79-4.11 (m, 2H-C_8), 4.31 (s, 2H-C_{-2}), 6.31 (s, 1H-C_5), 7.31-7.50 (m, 8H, Ar-H), 7.70 (s, 1H, CH=C); ^{13}C NMR: 30.62 (CH_2, thiazole), 44.23 (CH_3), 45.33 (CH, pyrimidine), 51.40, 53.41 (2CH_2-pyridine), 126.43, 126.83, 127.30, 128.31, 128.61, 128.75, 135.22, 143.10 (12C, Ar-C), 124.25 (CH, methylene), 129.28, 134.81, 163.70 (3C-pyrimidine), 136.91 (C-pyridine), 170.89 (C = O); MS (EI): m/z 423 [M^+] (100), 422 (M^+-H^+) (92), 328 (M^+- F-C_6H_4) (71); Anal. Calcd. for $C_{23}H_{19}N_3SOF_2$: C, 65.23; H, 4.52; N, 9.92; S, 7.57. Found: C, 65.21; H, 4.54; N, 9.90; S, 7.59.

9-(2-Chlorobenzylidene)-6,7,8,9-tetrahydro-7-methyl-5-(2-chlorophenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (4c): Yield 71%; mp 210-212°C; IR (KBr) cm^{-1}: 1736 (C=O); ^{1}H NMR (DMSO-d_6): 2.26 (s, 3H, CH_3), 2.62 (s, 2H-C_6), 3.81-4.17 (m, 2H-C_8), 4.21 (s, 2H-C_{-2}), 6.33 (s, 1H-C_5), 7.33-7.52 (m, 8H, Ar-H), 7.72 (s, 1H, CH=C); ^{13}C NMR: 30.64 (CH_2, thiazole), 44.25 (CH_3), 45.31 (CH, pyrimidine), 51.42, 53.43 (2CH_2-pyridine), 124.25 (CH, methylene), 126.41, 126.80, 127.31, 128.21, 128.59, 128.71, 135.26, 143.32 (12C, Ar-C), 136.81 (C-pyridine), 129.26, 134.86, 163.55 (3C-pyrimidine), 171.22 (C=O); MS (EI): m/z 456 [M^+] (89), 444 (M^++2) (62), 458 (M^++4) (27), 420 (M^+-Cl) (100); Anal. Calcd. for $C_{23}H_{19}N_3SOCl_2$: C, 60.50; H,4.19; N, 9.21; S, 7.02. Found: C, 60.54; H,4.17; N, 9.23; S, 7.00.

9-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-7-methyl-5-(4-chlorophenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (4d): Yield 79%; mp 232-234°C; IR (KBr) cm^{-1}: 1737 (C=O); ^{1}H NMR (DMSO-d_6): 2.28 (s, 3H, CH_3), 2.61 (s, 2H-C_6), 3.83-4.09 (m, 2H-C_8), 4.18 (s, 2H-C_{-2}), 5.90 (s, 1H-C_5), 7.31-7.54 (m, 8H, Ar-H), 7.72 (s, 1H, CH=C); ^{13}C NMR: 30.62 (CH_2, thiazole), 44.26 (CH_3), 45.33 (CH, pyrimidine), 51.41, 53.41 (2CH_2-pyridine), 124.21 (CH, methylene), 126.43, 126.81, 127.42, 128.18, 128.53, 128.73, 135.28, 143.30 (12C, Ar-C), 129.31, 134.85, 163.33 (3C-pyrimidine), 136.83 (C-pyridine), 171.00 (C=O); MS (EI): m/z 456 [M^+] (62), 444 (M^++2) (62), 458 (M^++4) (27), 420 (M^+-Cl) (39), 458 (M^++4) (7), 345 (M^+-Cl-C_6H_4) (100); Anal. Calcd. for $C_{23}H_{19}N_3SOCl_2$: C, 60.52; H, 4.19; N, 9.21; S, 7.02. Found: C, 60.50; H, 4.21; N, 9.19; S, 7.04.

9-(4-Bromobenzylidene)-6,7,8,9-tetrahydro-7-methyl-5-(4-bromophenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (4e): Yield 61%; mp 167-169°C; IR (KBr) cm^{-1}: 1735 (C=O); ^{1}H NMR (DMSO-d_6): 2.27 (s, 3H, CH_3), 2.62 (s, 2H-C_6), 3.85-4.10 (m, 2H-C_8), 4.19 (s, 2H-C_{-2}), 6.06 (s, 1H-C_5), 7.29-7.58 (m, 8H, Ar-H), 7.68 (s, 1H, CH=C); ^{13}C NMR: 30.60 (CH_2, thiazole), 44.28 (CH_3), 45.30 (CH, pyrimidine), 51.39, 53.43 (2CH_2-pyridine), 124.25 (CH, methylene), 126.41, 126.82, 127.43, 128.16, 128.51, 128.72, 135.26, 143.31 (12C, Ar-C), 136.80 (C-pyridine), 129.33, 134.82, 163.35 (3C-pyrimidine), 171.32 (C = O); MS (EI): m/z 545 [M^+] (31), 547 (M^++2) (66), 549 (M^++4) (28), 376 (M^+-BrC_6H_4=CH^+) (100); Anal. Calcd. for $C_{23}H_{19}N_3SOBr_2$: C, 50.66; H, 3.51; N, 7.70; S, 5.88. Found: C, 50.68; H, 3.53; N, 7.68; S, 5.86.

Synthesis of 5-aryl-9-arylmethylene-6,7,8,9-tetrahydro-2,7-dimethyl-2H-thiazolo[3,2-a]-pyrido[4,3-d]pyrimidin-3(5H)-ones (5a-e): A mixture of 2a-e (0.01 mole), 2-bromopropionic acid (1.54g, 0.01 mole), 6 g of fused sodium acetate in 30 ml of glacial acetic acid and 15 ml of acetic anhydride was refluxed for 2 hrs, poured onto cold water. The solid formed was filtered offand crystallized from the proper solvent to give pyrido[4,3-d]pyrimidin-3(5H)-ones 5a-e, respectively.

9-Benzylidene-6,7,8,9-tetrahydro-2,7-dimethyl-5-phenyl-2H-thiazolo[3,2-a]pyrido[4,3-d]-pyrimidine-3(5H)-one (5a): Yield 69%; mp 216-218°C; IR (KBr) cm^{-1}: 1726 (C=O); ^{1}H NMR (DMSO-d_6): 2.12 (s, 2H-C_6), 2.71 (s, 3H, CH_3, pyridine ring), 3.65-4.22 (2d, 2H-C_8), 4.51 (q, 1H-C-$_2$), 6.05 (s, 1H, CH-C_5), 1.41 (d, 3H, CH_3, thiazole ring), 7.30-7.50 (m, 11H, Ar-H + CH=C); ^{13}C NMR: 20.1 (CH_3, thiazole ring), 43.71 (CH, thiazole), 44.21 (CH_3, pyridine ring), 45.61 (CH, pyrimidine), 51.30, 53.40 (2CH_2-pyridine), 124.28 (CH, methylene), 126.41, 126.80, 127.00, 128.01, 128.64, 128.70, 135.22, 143.32 (12C, Ar-C), 136.93 (C-pyridine), 129.12, 134.81, 163.16 (3C-pyrimidine), 176.21 (*C*=O); MS (EI): m/z 401 [M^+] (50), 400 (M^+-H^+) (100), 312 (M^+-C_6H_5-CH +H^+) (9); Anal. Calcd. for $C_{24}H_{23}N_3SO$: C, 71.79; H, 5.77; N, 10.46; S, 7.98. Found: C, 71.81; H, 5.75; N, 10.46; S, 7.98.

9-(4-Flourobenzylidene)-2,7-dimethyl-6,7,8,9-tetrahydro-5-(4-flourophenyl)-2H-thiazolo-[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (5b): Yield 81%; mp 206-208°C; IR (KBr) cm^{-1}: 1727 (C=O); ^{1}H NMR (DMSO-d_6): 1.43 (d, 3H, CH_3, thiazole ring), 2.10 (s, 2H-C_6), 2.72 (s, 3H, CH_3, pyridine ring), 3.63-4.20 (2d, 2H-C_8), 4.53 (q, 1H-C-$_2$), 5.81 (s, 1H-C_5), 7.32-7.60 (m, 9H, Ar-H + CH=C); ^{13}C NMR: 20.11 (CH_3, thiazole ring), 43.72 (CH, thiazole), 44.20 (CH_3, pyridine ring), 45.60 (CH, pyrimidine), 51.30, 53.41 (2CH_2-pyridine), 124.26 (CH, methylene), 126.40, 126.80, 127.05, 128.01, 128.62, 128.72, 135.18, 143.36 (12C, Ar-C), 129.21, 134.61, 163.21 (3C-pyrimidine), 136.72 (C-pyridine), 176.0 (*C*=O); MS (EI): m/z 437 [M^+] (17), 436 (M^+-H^+) (28), 341 (436-F-C_6H_4) (100); Anal. Calcd. for $C_{24}H_{21}N_3SOF_2$: C, 65.88; H, 4.83; N, 9.60; S, 7.32. Found: C, 65.90; H, 4.81; N, 9.58; S, 7.35.

9-(2-Chlorobenzylidene)-6,7,8,9-tetrahydro-2,7-dimethyl-5-(2-chlorophenyl)-2H-thiazolo-[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (5c): Yield 64%; mp 219-221°C; IR (KBr) cm^{-1}: 1723 (C=O); ^{1}H NMR (DMSO-d_6): 1.42 (d, 3H, CH_3, thiazole ring), 2.13 (s, 2H-C_6), 2.70 (s, 3H, CH_3, pyridine ring), 3.65-4.22 (2d, 2H-C_8), 4.51 (q, 1H-C-$_2$), 6.01 (s, 1H-C_5), 7.22-7.65 (m, 9H, Ar-H+ CH=C); ^{13}C NMR: 20.21 (CH_3, thiazole ring), 43.71 (CH, thiazole), 44.21 (CH_3, pyridine ring), 45.60 (CH, pyrimidine), 51.34, 53.43 (2CH_2-pyridine), 124.24 (CH, methylene), 126.43, 126.82, 127.10, 128.13, 128.52, 128.73, 135.21, 143.15 (12C, Ar-C), 136.70 (C-pyridine), 129.23, 134.64, 163.41 (3C-pyrimidine), 176.2 (*C*=O); MS (EI): m/z 470 [M^+] (91), 472 (M^++2) (60), 474 (M^++4) (31), 468 (M^+-H_2) (100), 434 (M^+-Cl) (18); Anal. Calcd. for $C_{24}H_{21}N_3SOCl_2$: C, 61.27; H, 4.50; N, 8.93; S,6.81. Found: C, 61.29; H, 4.52; N, 8.91; S,6.78.

9-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-2,7-dimethyl-5-(4-chlorophenyl)-2H-thiazolo-[3,2-a]-pyrido[4,3-d]pyrimidine-3(5H)-one (5d): Yield 62%; mp 119-121°C; IR (KBr) cm^{-1}: 1726 (C=O); ^{1}H NMR (DMSO-d_6): 1.43 (d, 3H, CH_3, thiazole ring), 2.14 (s, 2H-C_6), 2.68 (s, 3H, CH_3, pyridine ring), 3.62-4.21 (2d, 2H-C_8), 4.50 (q, 1H-C-$_2$), 5.82 (s, 1H-C_5), 7.33-7.61 (m, 9H, Ar-H+ CH=C); ^{13}C NMR: 20.23 (CH_3, thiazole ring), 43.72-(CH, thiazole), 44.23 (CH_3, pyridine ring), 45.61 (CH, pyrimidine), 51.28, 53.42 (2CH_2-pyridine), 124.21 (CH, methylene), 126.44, 126.85, 127.13, 128.15, 128.53, 128.75, 135.23, 143.16 (12C, Ar-C), 136.72 (C-pyridine), 129.25, 134.64, 163.43 (3C-pyrimidine), 176.5 (*C*=O); MS (EI): m/z 470 [M^+] (66), 472 (M^++2) (41), 474 (M^++4) (20), 469 (M^+-H^+) (100), 357 (M^+-Cl-C_6H_4) (12); Anal. Calcd. for $C_{24}H_{21}N_3SOCl_2$: C, 61.27; H, 4.50; N, 8.93; S,6.81. Found: C, 61.25; H, 4.48; N, 8.95; S,6.83.

9-(4-Bromobenzylidene)-6,7,8,9-tetrahydro-2,7-dimethyl-5-(4-bromophenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidine-3(5H)-one (5e): Yield 61%; mp 129-131°C; IR (KBr) cm^{-1}: 1725 (C=O); ^{1}H NMR (DMSO-d_6): 1.41 (d, 3H, CH_3, thiazole ring), 2.12 (s, 2H-C_6), 2.67 (s, 3H, CH_3, pyridine ring), 3.60-4.20 (2d, 2H-C_8), 4.51 (q, 1H-C-$_2$), 5.80 (s, 1H-C_5), 7.31-7.60 (m, 9H, Ar-H+ CH=C); ^{13}C NMR: 20.20 (CH_3, thiazole ring), 43.70 (CH, thiazole), 44.21 (CH_3, pyridine ring), 45.60 (CH, pyrimidine), 51.30, 53.40 (2CH_2-pyridine), 124.21 (CH, methylene), 126.39, 126.82, 127.10, 128.12, 128.51, 128.72, 135.22, 143.15 (12C, Ar-C), 136.71 (C-pyridine), 129.22, 134.62, 163.41 (3C-pyrimidine), 176.52 (*C*=O); MS (EI): m/z 559 [M^+] (29), 547 (M^++2) (60), 549 (M^++4) (27), 471 [M^+-S-CH (C=O)-CH_3] (8), 43 (100); Anal. Calcd. for $C_{24}H_{21}N_3SOBr_2$: C, 51.53; H, 3.78; N, 7.51; S, 5.73. Found: C, 51.55; H, 3.81; N, 7.49; S,5.72.

Synthesis of 5-aryl-2,9-diarylmethylene-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido-[4,3-d]pyrimidin-3(5H)-ones (6a-e): Method A. A mixture of compounds 2a, b, d (0.05 mole), chloroacetic acid (1.0g, 0.01 mole), 2g of fused sodium acetate in 20 mL of glacial acetic acid and 10 ml of acetic anhydride was refluxed for 12 min, then equimolecular amount of the appropriate aldehydes was added. The reaction mixture was refluxed for 2 h and then poured onto cold water, The solid formed was filtered off and crystallized from the proper solvent.

Method B. A mixture of 4a, b, d (0.01 mole), equimolecular amount of appropriate aldehyde and 30 ml of acetic anhydride was refluxed for 1 h, left to cool, poured onto cold water. The solid formed was filtered off and crystallized from the proper solvent to give 6a-e. The products were identified by their mp and R_f-values in comparison with authentic samples previously obtained by method A. Method A gave better yield than method B.

2,9-Dibezylidene-5-phenyl-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (6a): Yield 81% [A], 76% [B]; mp 208-210°C; IR (KBr) cm^{-1}: 1709 (C=O); ^{1}H NMR ($CDCl_3$): 2.31 (s, 3H, CH_3), 3.11 (s, 2H-C_6),3.42-3.81 (2d, 2H-C_8), 5.50 (s, 1H-C_5), 7.30-7.60 (m, 17H, Ar-H+ 2 CH=C); ^{13}C NMR: 44.22 (CH_3), 45.62 (CH, pyrimidine), 51.28, 53.42 (2CH_2-pyridine), 118 (C, thiazole), 124.30, 142.22 (2CH, methylene), 126.42, 126.81, 127.30, 128.11, 128.63, 128.73, 135.32, 143.22 (18C, Ar-C), 134.80 (C-pyridine), 129.61, 136.93, 163.20 (3C-pyrimidine), 166.61 (*C*=O); MS (EI): m/z 475 [M^+] (100), 474 (M^+-H^+) (95), 397 (M^+-C_6H_5) (59); Anal. Calcd. for $C_{30}H_{25}N_3SO$: C, 75.76; H, 5.29; N, 8.83; S,6.74. Found: C, 75.74; H, 5.31; N, 8.80; S,6.76.

2-(4-Chlorobenzylidene)-9-bezylidene-5-phenyl-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (6b): Yield 89% [A], 66% [B]; mp 151-153°C; IR (KBr) cm^{-1}: 1719 (C=O); ^{1}H NMR ($CDCl_3$): 2.41 (s, 3H, CH_3), 2.60-3.11 (d, 2H-C_6), 3.22-3.41 (2d, 2H-C_8), 6.10 (s, 1H-C_5), 7.30-7.62 (m, 14H, Ar-H), 7.71 (s, 1 H, CH=C_9), 7.89 (s, 1 H, CH=C_2); ^{13}C NMR: 44.25 (CH_3), 45.65 (CH, pyrimidine), 51.35, 53.45 (2CH_2-pyridine), 118.20 (C, thiazole), 124.60, 142.23 (2CH, methylene), 126.45, 126.84, 127.33, 128.14, 128.66, 128.76, 135.35, 143.25 (18C, Ar-C), 134.70 (C-pyridine), 129.64, 136.90, 163.23 (3C-pyrimidine), 166.63 (*C*=O); MS (EI): m/z 510 [M^+] (39), 512 (M^++2) (63), 509 (M^+-H^+) (100), 432 (509-C_6H_5); Anal. Calcd. for $C_{30}H_{24}N_3SOCl$: C, 70.64; H, 4.74; N, 8.24; S, 6.28. Found: C, 70.62; H, 4.72; N, 8.26; S, 6.26.

2-(4-Flourodibezylidene)-9-(4-chlorobenzylidene)-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (6c): Yield 85% [A], 68% [B]; mp 182-185 °C; IR (KBr) cm^{-1}: 1721 (C=O); ^{1}H NMR ($CDCl_3$): 2.45 (s, 3H, CH_3), 2.61-3.13 (d, 2H-C_6), 3.19-3.83 (2d, 2H-C_8), 5.60 (s, 1H-C_5), 7.10-7.60 (m, 12H, Ar-H), 7.76 (s, 1 H, CH=C_9), 7.91 (s, 1 H, CH=C_2); ^{13}C NMR: 44.23 (CH_3), 45.64 (CH, pyrimidine), 51.34, 53.43 (2CH_2-pyridine), 118.10 (C, thiazole), 124.62, 142.21 (2CH, methylene), 126.40, 126.80, 127.30, 128.12, 128.64, 128.75, 135.33, 143.24 (18C, Ar-C), 134.69 (C-pyridine), 129.58, 136.91, 163.20 (3C-pyrimidine), 166.60 (*C*=O); MS (EI): m/z 562 [M^+] (90), 564 (M^++2) (59), 566 (M^++4) (33), 467 (M^+-F-C_6H_4) (100); Anal. Calcd. for $C_{30}H_{22}N_3SOCl_2F$: C, 64.05; H, 3.94; N, 7.47; S, 5.70. Found: C, 64.07; H, 3.96; N, 7.49; S, 5.67.

2-(4-Chlorodibezylidene)-9-(4-flourobenzylidene)-5-(4-flourrophenyl)-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (6d): Yield 85% [A], 75% [B]; mp 155-157°C; IR (KBr) cm^{-1}: 1723 (C=O); ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H, CH_3), 2.62-3.15 (d, 2H-C_6), 3.21-3.84 (2d, 2H-C_8), 5.62 (s, 1H-C_5), 7.11-7.61 (m, 12H, Ar-H), 7.70 (s, 1 H, CH=C_9), 7.82 (s, 1 H, CH=C_2); ^{13}C NMR: 44.20 (CH_3), 45.61 (CH, pyrimidine), 51.30, 53.41 (2CH_2-pyridine), 118.00 (C, thiazole), 124.63, 142.22 (2CH, methylene), 126.44, 126.83, 127.33, 128.13, 128.65, 128.75, 135.35, 143.25 (18C, Ar-C), 134.68 (C-pyridine), 129.59, 136.92, 163.21 (3C-pyrimidine), 166.61 (*C*=O); MS (EI): m/z 545 [M^+] (71), 547 (M^++2) (27), 544 (M^+-H^+) (100), 433 (544-Cl-C_6H_4) (85); Anal. Calcd. for $C_{30}H_{22}N_3SOClF_2$: C, 65.99; H, 4.06; N, 7.69; S, 5.87. Found: C, 65.97; H, 4.04; N, 7.68; S, 5.89.

2-(4-Methoxydibezylidene)-9-(4-chlorobenzylidene)-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-7-methylthiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (6e): Yield 72% [A], 65% [B]; mp 162-164°C; IR (KBr) cm^{-1}: 1711 (C=O); ^{1}H NMR ($CDCl_3$): 2.25 (s, 3H, CH_3), 2.90 (s, 3H, OCH_3), 3.11-3.36 (d, 2H-C_6), 3.51-3.81 (2d, 2H-C_8), 5.91 (s, 1H-C_5), 7.11-7.63 (m, 14H, Ar-H + 2 CH=C); ^{13}C NMR: 44.19 (CH_3), 45.58 (CH, pyrimidine), 51.37, 53.38 (2CH_2-pyridine), 56.01 (OCH_3), 118.20 (C, thiazole), 124.50, 142.20 (2CH, methylene), 126.40, 126.80, 127.30, 128.10, 128.60, 128.70, 135.30, 143.20 (18C, Ar-C), 134.60 (C-pyridine), 129.61, 136.90, 163.20 (3C-pyrimidine), 166.60 (*C*=O); MS (EI): m/z 574 [M^+] (86), 576 (M^++2) (57), 578 (M^++4) (31), 543 (M^+-OCH_3) (60),

467 (M^+-$OCH_3C_6H_4$) (100); Anal. Calcd. for $C_{31}H_{25}N_3SO_2Cl_2$: C, 64.80; H, 4.38; N, 7.31; S, 5.58. Found: C, 64.78; H, 4.36; N, 7.34; S, 5.59.

Synthesis of 5-Aryl-9-arylmethylene-6,7,8,9-tetrahydro-7-methyl-2-arylyldiazenyl-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-ones (7a-d): The aromatic amine, namely, aniline, p-toluidine or p-anisidine (0.01 mole) was dissolved in 3 ml HCl (70%) and cooled to 0°C, then treated with a solution of 0.7 g sodium nitrite in 2 ml water, the diazonium salt was cooled for 15 min, added gradually with stirring to cooled solution of compound 4a,d (0.01 mole) in 10 mL of pyridine, the reaction mixture was cooled for 30 min and poured onto 100 mL water. The solid formed was crystallized from the proper solvent to give compounds 7a-d.

9-Benzylidene-6,7,8,9-tetrahydro-5-phenyl-7-methyl-2-(4-tolyldiazenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (7a): Yield 79%; mp 215-217°C; IR (KBr) cm^{-1}: 1728 (C=O); ^{1}H NMR ($CDCl_3$): 2.28 (s, 3H, CH_3-N), 2.34 (s, 3H, CH_3-Ph), 2.82-3.15 (d, 2H-C_6), 3.51-3.84 (m, 2H-C_8), 4.10 (s, 1H, CH=C_2), 5.72 (s, 1H-C_5), 6.60 (s, 1H + CH=C_9), 7.21-7.61 (m, 14H, Ar-H); ^{13}C NMR: 24.32 (CH_3-Ph), 44.22 (CH_3), 45.41 (CH, pyrimidine), 53.42, 64.60 ($2CH_2$-pyridine), 64.00 (CH, thiazole), 124.23 (CH, methylene), 126.24, 126.73, 127.13, 128.13, 128.63, 128.74, 135.26, 143.22 (18C, Ar-C), 136.67 (C-pyridine), 129.18, 136.90, 163.23 (3C-pyrimidine), 170.61 (C=O); MS (EI): m/z 505 [M^+] (22), 386 (M^+-CH_3-C_6H_5-N=N) (100); Anal. Calcd. for $C_{30}H_{27}N_5OS$: C, 71.25; H, 5.38; N, 13.85; S, 6.34. Found: C, 71.23; H, 5.40; N, 13.82; S, 6.36.

9-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-5-(4-chlorophenyl)-7-methyl-2(2-phenyldiazenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (7b): Yield 78%; mp 211-213°C; IR (KBr) cm^{-1}: 1726 (C=O); ^{1}H NMR ($CDCl_3$): 2.49 (s, 3H, CH_3), 2.81-3.20 (d, 2H-C_6), 3.50-3.79 (m, 2H-C_8), 4.20 (s, 1 H, CH=C_2), 5.82 (s, 1H-C_5), 6.63 (s, 1H, + CH=C_9), 7.28-7.66 (m, 13H, Ar-H); ^{13}C NMR: 44.20 (CH_3), 45.40 (CH, pyrimidine), 51.40, 53.40 ($2CH_2$-pyridine), 64.50 (CH, thiazole), 124.00 (CH, methylene), 126.20, 126.70, 127.10, 128.03, 128.60, 128.70, 135.20, 143.20 (18C, Ar-C), 136.64 (C-pyridine), 129.09, 134.91, 163.00 (3C-pyrimidine), 171.61 (C=O); MS (EI): m/z 560[M^+] (92), 562 (M^++2) (60), 564 (M^++4) (35), 455 (M^+-C_6H_5-N=N) (100); Anal. Calcd. for $C_{29}H_{23}N_5OSCl_2$: C, 62.14; H, 4.13; N, 12.49; S, 5.72. Found: C, 62.11; H, 4.11; N, 12.51; S, 5.70.

9-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-5-(4-chlorophenyl)-7-methyl-2(4-tolyl-diazenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (7c): Yield 77%; mp 202-205°C; IR (KBr) cm^{-1}: 1730(C = O); ^{1}H NMR ($CDCl_3$): 2.28 (s, 3H, CH_3-N), 2.35 (s, 3H, CH_3-Ph), 2.83-3.25 (d, 2H-C_6), 3.52-3.85 (m, 2H-C_8), 4.10 (s, 1H, CH=C_2), 5.82 (s, 1H-C_5), 6.60 (s, 1H + CH=C_9), 7.23-7.81 (m, 12H, Ar-H); ^{13}C NMR: 24.31 (CH_3-Ph), 44.23 (CH_3), 45.40 (CH, pyrimidine), 51.43, 53.43 ($2CH_2$-pyridine), 64.20 (CH, thiazole), 124.10 (CH, methylene), 126.24, 126.73, 127.13, 128.14, 128.63, 128.70, 135.24, 143.21 (18C, Ar-C), 136.66 (C-pyridine), 129.05, 134.88, 163.22 (3C-pyrimidine), 171.51 (*C*=O); MS (EI): m/z 574 [M^+] (79), 576 (M^++2) (41), 578 (M^++4) (25), 483 (M^+-$CH_3C_6H_4$) (100); Anal. Calcd. for $C_{30}H_{25}N_5OSCl_2$: C, 62.71; H, 4.38; N, 12.19; S, 5.58. Found: C, 62.73; H, 4.40; N, 12.20; S, 5.56.

9-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-5-(4-chlorophenyl)-7-methyl-2(4-methoxyphenyl-diazenyl)-2H-thiazolo[3,2-a]pyrido[4,3-d]pyrimidin-3(5H)-one (7d): Yield 80%; mp 198-201°C; IR (KBr) cm^{-1}: 1710 (C = O); ^{1}H NMR (DMSO-d_6): 2.28 (s, 3H, CH_3-N), 2.83-3.15 (d, 2H-C_6), 3.86 (s, 3H, OCH_3-Ph), 3.51-3.95 (m, 2H-C_8), 4.10 (s, 1H, CH=C_2), 5.72 (s, 1H-C_5), 7.15-7.81 (m, 15H, Ar-H+ CH=C_9); ^{13}C NMR: 44.23 (CH_3), 45.43 (CH, pyrimidine), 51.42, 53.41 ($2CH_2$-pyridine), 57.10 (OCH_3-Ph), 64.62 (CH, thiazole), 124.11 (CH, methylene), 126.24, 126.73, 127.13, 128.13, 128.63, 128.74, 135.44, 143.31 (18C, Ar-C), 136.68 (C-pyridine), 128.54, 134.93, 163.24 (3C-pyrimidine), 170.55 (C=O); MS (EI): m/z 590 [M^+] (79), 592 (M^++2) (41), 594 (M^++4) (25), 479 (M^+-ClC_6H_4) (100); Anal. Calcd. for $C_{30}H_{25}N_5O_2SCl_2$: C, 61.01; H, 4.26; N, 11.86; S, 5.43. Found: C, 61.03; H, 4.24; N, 11.88; S, 5.41.

Anticancer activity: Some of the synthesized compounds were selected and screened for their anticancer activity. Each compound was tested at five different concentrations against 60 cell lines of nine types of human cancers, namely, leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer. Results are expressed as $log_{10}GI_{50}$, which the drug concentration (M) is causing a 50% reduction in the net protein increase in control cells during the drug incubation (Negaoui *et al*., 2009) Table 1. Some of the synthesized compounds showed good anticancer activity at low concentration compared with 5-fluorodeoxyuridine log_{10} GI_{50}= -4.7 as reference control.

Table 1: In vitro inhibition results of cancer cell lines of the tested derivatives (GI 50 (iM))*

	Compound									
Panel/cell line	3b	3d	4a	4b	4d	5b	5c	5d	6b	7b
Non-small cell lung cancer										
A 549/ATCC	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
EKVX	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
HOP-62	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
HOP-92	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
NCI-H226	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
NCI-H23	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
NCI-H322M	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
NCI-H460	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
NCI-H522	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3
Leukemia										
CCRF-CE	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
CCRF-CEM	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
HL-60 (TB)	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
K-562	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
MoLT-4	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
RPMI-8226	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
SR	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
CNS cancer										
SF-268	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
SF-295	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
SNB-19	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
SNB-75	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
U 251	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
Colon Cancer										
COLO 205	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
HCC-2998	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
HCT-116	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
HCT-15	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
HT29	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
KM12	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
SW-620	-4.3	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3
Breast Cancer										
MCF 7	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
NCI/ADR-RES	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
MDA-MB-231/ATCC	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
HS 578T	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
MDA-MB-435	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
BT-549	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
T – 47D	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
Ovarian Cancer										
IGROVI	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0	-4.0	-4.3	-4.3
OVCAR-3	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0	-4.0	-4.3	-4.3
OVCAR-4	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0.0	-4.3	-4.3	-4.3
OVCAR-5	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0	-4.0	-4.3	-4.3
OVCAR-8	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0	-4.0	-4.3	-4.3
SK-OV-3	-4.3	-4.0	-4.0	-4.0	-4.3	-4.3	-4.0	-4.0	-4.3	-4.3
Prostate Cancer										
PC-3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
DU-145	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3
Renal Cancer										
786-0	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
A 498	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
ACHN	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
CAKI-1	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
RXF-393	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
SN12C	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
TK-10	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
UO-31	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
Melanoma										
LOXIMVI	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
MALME-3M	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
M 14	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
SK-MEL-2	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
SK-MEL-28	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
SK-MEL-5	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
UACC-257	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3
UACC-62	-4.0	-4.3	-4.3	-4.3	-4.3	-4.3	-4.0	-4.3	-4.3	-4.3

*: Data obtained from NCI's in vitro disease-oriented tumor cell screen; GI_{50}: drug molar concentration causing 50% cell growth inhibition, NA= No Activity

RESULTS

In continuation to our search for new heterocyclic chemistry based anticancer, the suggestion, synthesis, structure elucidation of some thiopyrimidine derivatives 2-7 were realized herein using 3,5-bisarylmethylene-1-methyl-4-piperidone and 4-aryl-8-arylmethelene-6-methylpyrido[4,3-d]pyrimidine-2(1H)thiones as a starting materials. Some of the synthesized compounds were selected and screened for their anticancer activity. Each compound was tested at five different concentrations against 60 cell lines of nine types of human cancers, namely, leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer. Some of the tested compounds were better exhibited *in vitro* antitumor activities at low concentration (log_{10} GI_{50} = -4.7) against the used human tumor cell lines. From the in vitro observed data it has been noticed that, some of the synthesized compounds seem to be the most active prepared derivatives against all the tested cell lines.

DISCUSSION

Chemistry: The synthetic strategy to synthesize the target products 2-7 is depicted in Fig. 1 and 2. Preparation of 3, 5-bisarylmethylene-1-methyl-4-piperidone (1a-f) was established according to the reported procedure (Lyle *et al*., 1973; Mcelvain and Rorig, 1948; Pattaraporn and Tharapong, 2009). The corresponding pyrimidine thione derivatives (2a-e) were obtained from condensation of 1a-e with thiourea in ethanolic potassium hydroxide solution under reflux. When compounds 1a-d, f reacted with 2-amino-2-thiazoline in butanol/DMSO mixture afforded compounds 3a-e in good yields. Reaction of compounds 2a-e with chloroacetic acid or with 2-bromopropionic acid in the presence of sodium acetate in acetic acid/acetic anhydride mixture gave thiazolopyrimidene derivatives 4a-e and 5a-e, respectively (Fig. 1). Compounds 4a,b,d contain an active methylene group, when these compounds reacted with aromatic aldehydes in the presence of acetic acid/acetic anhydride mixture, the corresponding arylmethylene thiazolopyrimidines derivatives 6a-e were obtained (Fig. 2). The products 6a-e could be also obtained from reaction of 2a,b,d with chloroacetic acid, followed by treatment with aromatic aldehyde in the presence of sodium acetate in refluxing acetic acid/acetic anhydride mixture. Compounds 4a,b,d were coupled with aryldiazonium salts in the presence of pyridine to give arylazo-thiazolopyrimidines derivatives 7a-d (Fig. 2).

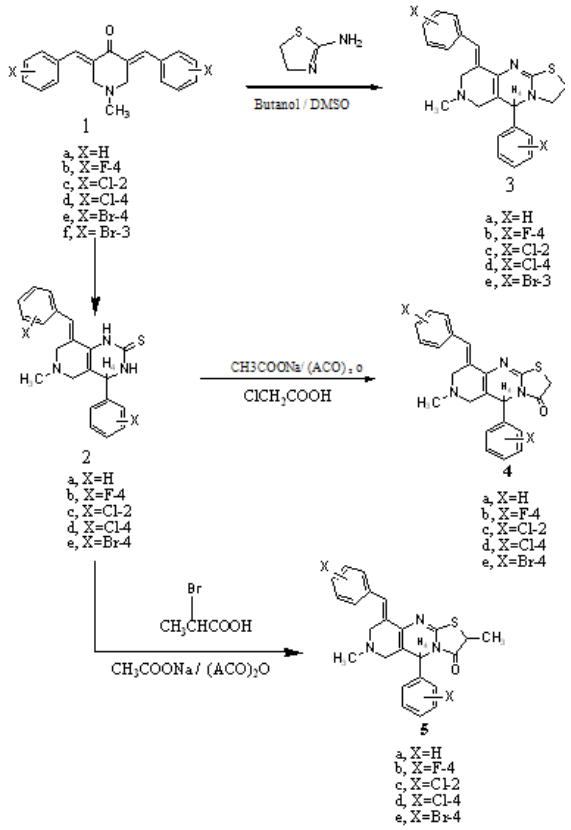

Fig. 1: Synthetic routes for compounds 1-5

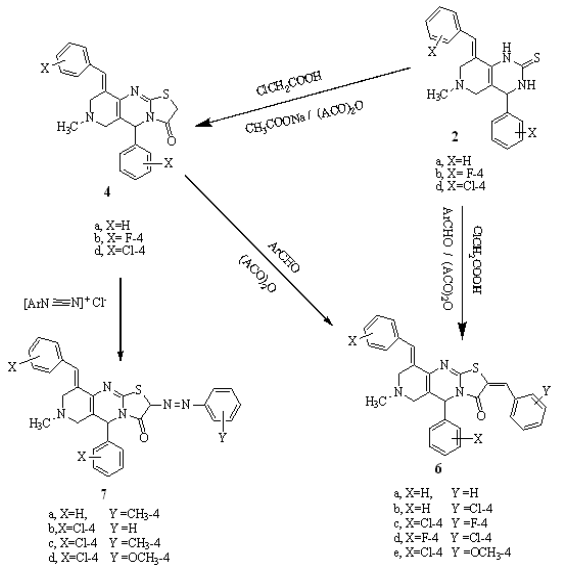

Fig. 2: Synthetic routes for compounds 6 and 7

Antitumor screening: Antitumor activity screening for the synthesized compounds utilizing 59 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate as well as kidney, was carried out according to the previously reported standard procedure (Fylaktakidou *et al*., 2004; Jung *et al*., 2005; Ngoy *et al*., 2011 and Shuangning *et al*., 2010). The obtained results (Table 1) represent concentrations of the used investigated compounds resulting in growth inhibition of 50% (GI_{50}) for the tested human tumor cell lines. From the in vitro observed data it has been noticed that, the selected compounds 3b, 3c, 3d, 4a, 4b, 4d, 5b, 5c, 5d, 6c, 6b, 6d, 7b and 7d seem to be the most active prepared derivatives against all the tested cell lines.

Structural-Activity Relationship (SAR): From the above-obtained results (Table 1), we can conclude that thiopyrimidine moieties fused to N-methylpipredine ring are essential for antitumor activities. In the present work, we can suggest that the anticancer activity is due to:

- The presence of nitrogen heterocyclic rings
- The most active compounds being 3b, 3c, 3d, 4a, 4b, 4d, 5b, 5c, 5d, 6c, 6b, 6d, 7b and 7d against all the tested cell lines.
- The presence of the nitrogen and sulfur atoms generally enhancing the activity
- The difference in activity between the compounds which is due to the indicated subsistents in the phenyl group of the molecule

CONCLUSION

In our previous works, we reported that fused pyrimidine derivatives were proved to be active anticancer agents. In the present work, a series of thiopyrimidine derivatives were synthesized using 3,5-bisarylmethylene-1-methyl-4-piperidone and 4-aryl-8-arylmethelene-6-methylpyrido[4,3-d]pyrimidine-2(1*H*)thiones as a starting materials.

The antitumor activities of the newly synthesized compounds were evaluated utilizing 60 different human tumor cell lines, representing leukemia, melanoma, lung, colon, brain, ovary, breast, prostate as well as kidney. Some of the tested compounds were better exhibited *in vitro* antitumor activities at low concentration ($\log_{10} GI_{50} = -4.7$) against the used human tumor cell lines. In the present work, we can suggest that the anticancer activity is due to the presence of nitrogen heterocyclic rings and the presence of the sulfur atom.

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