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RESEARCH ARTICLE

Anticancer activities of some newly synthesized pyrazole and pyrimidine derivatives

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Abstract A series of pyrazolopyridine and pyridopyrimidine derivatives **2–6** were newly synthesized using 3,5-bisarylmethylene-1-methylpiperidone as the starting material. The anticancer activities of the synthesized compounds were evaluated using 59 different human tumor cell lines, representing cancers of CNS, ovary, renal, breast, colon, lung, leukemia, and melanoma, prostate as well as kidney. Some of the tested compounds, especially those with a fluorine substituent at the para-position in the phenyl ring and those with a pyridopyrimidine-2-thione with a free –NH or –SH, exhibited greater in vitro antitumor activities at low concentrations (log 10 [GI_{50}] = −4.6) against the human tumor cell lines. Additionally, some of the compounds had moderate inhibitory effects on the growth of the cancer cell lines. The detailed synthesis, spectroscopic data and antitumor properties of the synthesized compounds are reported.

Keywords *N*-methylpiperidone · Pyrimidine derivatives · Human tumor · Anticancer activities

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Introduction

Electron-rich nitrogen heterocycles play important roles in diverse biological activities. For example, pyrimidines are an important class of compounds and have widespread applications from pharmaceuticals to industrial materials (Selvam et al. 2012) with activities such as Tie-2 kinase inhibitors (Matloobi and Kappe 2007), HIV-1 inhibitors (Gadhachanda et al. 2007), antimalarials (Agarwal et al. 2005), adenosine A1 receptor antagonism (Chang et al. 2004), anticancer agents (Capdeville et al. 2002), analgesics (Zaki et al. 2006), cardiovascular agents (Atwal 1988) and antiallergic agents (Ozeki et al. 1989). On the other hand, the importance of the pyridine ring in the chemistry of biological systems has attracted much attention because of their presence in the substructures of many natural products of therapeutic importance. The potent biological activity of various vitamins and drugs (Joule et al. 1974; Henry 2004; Vacher et al. 1999) is primarily a result of the presence of the pyridine ring in their molecular structure. 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). Pyrazole derivatives exhibit pharmacological activities; they can act as hypotensives, antibacterials, anti-inflammatory agents and antitumor agents (Hardy 1984; Orth 1968; Elnagdi et al. 1987; Elnagdi et al. 1990). 2-Pyrazoline derivatives have antimicrobial (Turan-Zitouni et al. 2005), anti-

inflammatory (Nasr and Said 2003), and antihypertensive (Turan-Zitouni et al. 2000) activities. In particular, condensed pyrazoles are known for their various biological activities; e.g., the pyrazolo[3,4-b]pyridine system has interesting biological and pharmacological properties such as adrenocorticotropic hormone–releasing factor [corticotropin-releasing factor (CRF)] antagonist activity; CRF antagonists are believed to be effective in the treatment of a wide variety of stress-related illnesses, such as depression, Alzheimer's disease, gastrointestinal diseases, anorexia nervosa, hemorrhage stress, drug and alcohol withdrawal symptoms, drug addiction and infertility (Chen 1995). On the other hand, many pyridopyrimidine derivatives possess anticancer and kinase inhibiting activities (McKennon et al. 2004; Newlander and Parrich 2010). Pyrazolo[3,4-b]pyridines are generally prepared by reaction of 5-aminopyrazole and substituted α,β-unsaturated nitriles in organic solvents (Quiroga et al. 1999, 2001) but most of them suffer from drawbacks such as lower yields, and use of organic solvents. In view of these facts, and in continuation of our interest in the synthesis of new candidates with potential activity (Mohamed et al. 2011; Amr et al. 2003, 2006 ; Abd El-Salam et al. 2010; Abdel-Hafez et al. 2009; Rashad et al. 2010; El-Sayed et al. 2010a, b), we herein report the synthesis and anticancer activity of new pyrazolopyridine and pyridopyrimidine derivatives. In addition, the effect of substituents on the aromatic ring on activity was investigated. The results on activity show the effects of substitution at C-2 in the synthesized pyridopyrimidine ring system with oxo-, thioxo- and imino groups.

Materials and methods

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analyses were done using an Elementar, Vario El, Microanalytical Unit, National Research Centre, Cairo, Egypt and were found to be within ±0.4 % of theoretical values. Infrared (IR) spectra were recorded on a Carlzeise Spectrophotometer model 'UR 10' spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer (DMSO-d_6 or $CDCl_3$) and the chemical shifts are reported as δ (parts per million) downfield from tetramethylsilane as an internal standard. Splitting patterns were designated as follows; s: singlet; d: doublet; t: triplet: m: multiplet. Mass spectra (MS) were measured using a Finnigan SSQ 7000 mass spectrometer. Follow-up of the reactions and checking the purity of the compounds was done by TLC on silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were detected by exposure to a UV lamp at λ_{245} nm for a few seconds. The starting material, 3,5-bisarylmethylene-1-methyl-4-piperidone (**1**), was synthesized according to reported procedures (Lyles et al. 1974; Mcelvain and Kurt 1948).

3-Aryl-7-arylmethylene-3,3a,4,5,6,7-hexahydro-5-methyl-2-phenyl-2*H*-pyrazolo[4,3-c]pyridine (**2**)

To a solution of compound **1** (10 mmol) in acetic acid (10 mL), phenylhydrazine (1.1 mL, 10 mmol) was added. The mixture was stirred at room temperature for 7 h, poured into 25 mL of cold water and mixed with dilute 50 % hydrochloric acid (2 mL). The solid formed was filtered and crystallized from 75 % aqueous ethanol. The crystals included the following reaction products (**2a–e**).

*7-Benzylidine-3,3a,4,5,6,7-hexahydro-5-methyl-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**2a**)*

3.4 g (89 % yield), m.p 183–184 °C; IR (KBr, cm^{-1}): 1630 (C=N); ^{1}H NMR (DMSO-d_6): δ 7.40–6.61 (m, 16H, Ar–H + C=C*H*), 4.1 (d, 1H, J = 6.1 Hz, H-3), 3.11–3.20 (dd, 2H, J = 7.0 Hz, J = 6.5 Hz, H-6), 2.31–2.61 (m, 3H, H-3a + H-4,4′), 2.31 (s, 3H, N–CH_3); ^{13}C NMR: δ = 155.80 (C=N), 143.81, 141.11, 138.10 (Ar–C, 3C), 132.51 (C=*C*H), 129.70, 128.90,128.70 (Ar–C, 6*C*H-*m*), 128.51, 126.11, 122.47, 118.51 (Ar–C, C-7 and 6CH-*o*), 118.10, 117.12, 116.20 (Ar–C, 3CH-*p*), 57.51 (C-4), 53.70 (C-3 + C-6), 48.41 (C-4a), 43.81 (CH_3); MS, *m/z* (%): 379 [M^+] (100), 350 (M^+–CH_3–N) (75.5), 336 (M^+–CH_3–N=CH_2) (83.5), 302 (M^+–C_6H_5) (81.5); anal. calcd. for $C_{26}H_{25}N_3$ (379.49); C, 82.28; H, 6.64; N, 11.07. Found: C, 82.30; H, 6.62; N, 11.06.

*7-(4-Flourobenzylidine)-3-(4-flourophenyl)-3,3a,4,5,6,7-hexahydro-5-methyl-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**2b**)*

3.5 g (86 % Yield), m.p 180–183 °C; IR (KBr, cm^{-1}): 1632 (C=N); ^{1}H NMR (acetone-d_6): δ 7.42–6.66 (m, 14H, Ar–H + C=C*H*), 4.62, 3.71 (dd, 1H, H-3), 3.40–3.20 (m, 3H, H-3a + H-4,4′), 2.60 (m, 2H, H-6,6′), 2.41 (s, 3H, N–CH_3); ^{13}C NMR: δ = 155.30 (C=N), 143.61, 138.11, 133.41, 133.20, 131.51, 129.50, 129.12, 128.50, 127.06, 117.41, 113.51 (18Ar–C and C-7), 132.11 (C=*C*H), 57.52 (C-4), 53.71 (C-3 + C-6), 48.42 (C-4a), 43.81 (CH_3); MS, *m/z* (%): 415 [M^+] (100), 372 (M^+–CH_3–N=CH_2) (80.5), 320 (M^+–F–C_6H_4) (39.6); anal. calcd. for $C_{26}H_{23}N_3F_2$ (415.454); C, 75.15; H, 5.58; N, 10.11. Found: C, 75.18; H, 5.55; N, 10.12.

*7-(4-Methoxybenzylidine)-3-(4-methoxyphenyl)-3,3a,4,5,6,7-hexahydro-5-methyl-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**2c**)*

3.1 g (76 % Yield), m.p 189–191 °C; IR (KBr, cm^{-1}): 1634 (C=N); ^{1}H NMR (DMSO-d_6): δ 7.61–6.82 (m, 14H, Ar–H + C=C*H*), 4.51 (d, 1H, $J = 6.0$ Hz, H-3), 3.48 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 3.20–3.11 (dd, 2H, $J = 7$, 6.5 Hz, H-6,6′), 2.80–2.61 (m, 3H, H-3a + H-4,4′), 2.31 (s, 3H, N–CH_3); ^{13}C NMR: δ = 155.81 (C=N), 143.81, 141.10, 138.10 (Ar–C, 3C), 132.15 (C=*C*H), 129.17, 128.19, 128.70 (Ar–C, 6CH-*m*), 128.50, 126.10, 113.50 (Ar–C, 6CH-*o* and C-7), 128.01, 125.92, 117.21 (Ar–C, 2CH-*p*), 57.52 (C-4), 55.81 (2OCH_3), 53.81 (C-3 + C-6), 48.62 (C-4a), 43.92 (CH_3). MS, *m/z* (%): 439 [M^+] (15), 332 (M^+–CH_3O–C_6H_4) (71), 362 (M^+–C_6H_5) (100); anal. calcd. for $C_{28}H_{29}N_3O_2$ (439.52); C, 76.51; H, 6.65; N, 9.56. Found: C, 76.53; H, 6.63; N, 9.53.

*7-(4-Chlorobenzylidine)-3-(4-chlorophenyl)-3,3a,4,5,6,7-hexahydro-5-methyl-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**2d**)*

4.1 g (90 % Yield), m.p 253–255 °C; IR (KBr, cm^{-1}): 1635 (C=N); ^{1}H NMR (acetone-d_6): δ 7.62–6.75 (m, 14H, Ar–H + C=C*H*), 4.72 (d, 1H, $J = 5.9$ Hz, H-3), 3.30–3.21 (dd, 2H, $J = 7.0$, $J = 6.6$ Hz, H-6,6′), 3.11–3.90 (m, 3H, H-3a + H-4,4′), 2.32 (s, 3H, CH_3); ^{13}C NMR: δ = 155.42 (C=N), 143.72, 138.21, 133.61, 133.30, 131.51, 129.52, 129.11, 128.60, 127.51, 117.32, 113.50 (18Ar–C and C-7), 132.21 (C=*C*H), 57.62 (C-4), 53.90 (C-3 + C-6), 42.51 (C-3a), 48.91 (CH_3); MS, *m/z* (%): 448 [M^+] (100), 452 [M^++4] (33.6), 450 [M^++2] (66.7) due to the presence of two chlorine atoms, 403 (447–CH_3–N=CH_2, –H^+) (83.2), 336 (447–Cl–C_6H_4) (30.3); anal. calcd. for $C_{26}H_{23}N_3Cl_2$ (448.384); C, 69.64; H, 5.17; N, 9.37. Found: C, 69.66; H, 5.15; N, 9.39.

*7-(4-Bromobenzylidine)-3-(4-bromophenyl)-3,3a,4,5,6,7-hexahydro-5-methyl-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**2e**)*

4.7 g (88 % Yield), m.p 161–164 °C; IR (KBr, cm^{-1}): 1630 (C=N); ^{1}H NMR (DMSO-d_6): δ 7.52–6.73 (m, 14H, Ar–H + C=C*H*), 4.92 (d, 1H, $J = 6$ Hz, H-3), 3.41–3.31 (dd, 2H, $J = 7$, 6.5 Hz, H-6,6′), 3.23–3.12 (m, 3H, H-3a + H-4,4′), 2.30 (s, 3H, CH_3); ^{13}C NMR: δ = 155.72 (C=N), 143.80, 139.12, 134.61, 134.31, 132.21, 129.70, 129.21, 128.50, 127.41, 117.52, 113.60 (18Ar–C and C-7), 132.31 (C=*C*H), 57.71 (C-4), 53.81 (C-3 + C-6), 42.70 (C-4a), 47.81 (CH_3); MS, *m/z* (%): 537 [M^+] (13.61), 539 [M^++2] (16.77), 541 [M^++4] (8.43) due to the presence of two bromine atoms, 492 (M^+–CH_3–N=CH_2) (71.2) and 368 (M^+–CHC_6H_4–Br) (100); anal. calcd. for $C_{26}H_{23}Br_2N_3$ (537.29); C, 58.12; H, 4.31; N, 7.82. Found: C, 58.10; H, 4.33; N, 7.80.

4-Aryl-8-arylmethylene-3,4,5,6,7,8-hexahydro-6-methylpyrido[4,3-d] pyrimidine-2(1*H*)-one (**3**)

A solution of compound **1** (10 mmol) in 25 ml of sodium ethoxide (0.2 g Na in 25 mL of absolute ethanol) was mixed with urea (10 mmol). The mixture was refluxed for 3 h, left to cool, and then poured gradually, with stirring, into 50 mL cold water. The solid that formed was filtered, washed with water and crystallized from ethanol. The following reaction products **3a**–**e** were identified.

*8-Benzylidene-3,4,5,6,7,8-hexahydro-6-methyl-4-phenylpyrido[4,3-d]pyrimidine-2(1H)-one (**3a**)*

2.8 g (85 % Yield), m.p 216–219 °C; IR (KBr, cm^{-1}): 3350, 3294 and 3153 (NH), 1670 (C=O); ^{1}H NMR ($CDCl_3$): δ 9.23–9.18 (bs, 2H, 2NH, exchangeable with D_2O), 6.73–7.51 (m, 11H, Ar–H + C=C*H*), 5.02 (d, 1H, $J = 4.6$ Hz, H-4), 3.78 (s, 2H, CH_2), 3.98 (s, 2H, CH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR: δ = 162.12 (C=O), 123.08–157.06 (12Ar–C, C-4a, 8a and *C*=CH), 70.05 (C-4), 58.61 (C-7), 53.52 (C-5), 43.50 (CH_3); MS, *m/z* (%): 331 [M^+] (24); anal. calcd. for $C_{21}H_{21}N_3O$ (331.38); C, 76.11; H, 6.39; N, 12.68. Found: C, 76.50; H, 5.83; N, 12.74.

*8-(4-Fluoroenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-fluoro phenyl)pyrido[4,3-d]pyrimidin-2(1H)-one (**3b**)*

3.3 g (91 % Yield), m.p 209–212 °C; IR (KBr, cm^{-1}): 3332, 3296 and 3172 (NH), 1662 (C=O); ^{1}H NMR ($CDCl_3$): δ 9.44–9.38 (bs, 2H, 2NH, exchangeable with D_2O), 7.69–6.61 (m, 9H, Ar–H + C=C*H*), 5.15 (d, 1H, $J = 6.8$ Hz, H-4), 3.59 (s, 2H, CH_2), 3.92 (s, 2H, CH_2), 2.29 (s, 3H, CH_3); ^{13}C NMR: δ = 162.12 (C=O), 160.19–124.28 (12Ar–C, C-7a, 8a and *C*=CH), 72.05 (C-4), 58.51 (C-7), 53.60 (C-5), 40.51 (CH_3); MS, *m/z* (%): 367 [M^+] (65); anal. calcd. for $C_{21}H_{19}N_3F_2O$ (367.39); C, 68.65; H, 5.21; N, 11.44. Found: C, 69.01; H, 4.90; N, 11.53.

*8-(4-Methoxybenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-methoxyphenyl)pyrido[4,3-d]pyrimidin-2(1H)-one (**3c**)*

3.5 g (90 % Yield), m.p 135–137 °C; IR (KBr, cm^{-1}): 3338, 3268 and 3175 (NH), 1665 (C=O); ^{1}H NMR ($CDCl_3$): δ 9.60–9.53 (bs, 2H, 2NH, exchangeable with D_2O), 7.80–6.67 (m, 9H, Ar–H + C=C*H*), 5.16 (d, 1H,

$J = 6.8$ Hz, H-4), 3.61 (s, 2H, CH_2), 3.57 (s, 2H, CH_2), 3.44, 3.49 (2s, 6H, 2 OCH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR: δ = 163.30 (C=O), 1243.53–161.10 (12Ar–C, C-5a, 8a and *C*=CH), 72.51 (C-4), 58.71 (C-7), 56.90 (C-5), 56.90 ($2CH_3O$), 43.52 (CH_3); MS, *m/z* (%): 391 [M^+] (50); anal. calcd. for $C_{23}H_{25}N_3O_3$ (391.41); C, 70.57; H, 6.44; N, 10.73. Found: C, 70.90; H, 5.97; N, 10.80.

*8-(4-Chlorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-chlorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-one (**3d**)*

3.6 g (91 % Yield), m.p 162–164 °C; IR (KBr, cm^{-1}): 3352, 3282 and 3170 (NH), 1665 (C=O); ^{1}H NMR ($CDCl_3$): δ 9.31–9.26 (bs, 2H, 2NH, exchangeable with D_2O), 7.60–6.71 (m, 9H, Ar–H + C=C*H*), 5.14 (d, 1H, $J = 6.2$ Hz, H-4), 3.60 (s, 2H, CH_2), 3.55 (s, 2H, CH_2), 2.35 (s, 3H, CH_3); ^{13}C NMR: δ = 164.12 (C=O), 123.21–160.60 (12Ar–C, C-4a, 8a and *C*=CH), 72.01 (C-4), 58.62 (C-7), 53.51 (C-5), 43.52 (CH_3); MS, *m/z* (%):400 [M^+] (75), 401 [M^++1] (100), 402 [M^++2] (46), 404 [M^++4] (22); anal. calcd. for $C_{21}H_{19}N_3Cl_2O$ (400.30); C, 63.01; H, 4.78; N, 10.50. Found: C, 63.30; H, 4.39; N, 10.52.

*8-(4-Bromobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-bromophenyl)pyrido[4,3-d]pyrimidin-2(1H)-one (**3e**)*

4.3 g (89 % Yield), m.p 208–211 °C; IR (KBr, cm^{-1}): 3318, 3272 and 3190 (NH), 1672 (C=O); ^{1}H NMR ($CDCl_3$): δ 9.28–9.21 (bs, 2H, 2NH, exchangeable with D_2O), 7.66–6.96 (m, 9H, Ar–H + C=C*H*), 5.12 (d, 1H, $J = 6.2$ Hz, H-4), 3.59 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR: δ = 164.12 (C=O), 123.22–160.52 (12Ar–C, C-4a, 8a and *C*=CH), 76.22 (C-4), 58.51 (C-7), 53.51 (C-5), 43.50 (CH_3); MS, *m/z* (%): 488 [M^+−1] (65); anal. calcd. for $C_{21}H_{19}N_3OBr_2$ (489.20); C, 51.56; H, 3.91; N, 8.59. Found: C, 51.75; H, 3.59; N, 8.64.

4-Aryl-8-arylmethylene-3,4,5,6,7,8-hexahydro-6-methylpyrido-[4,3-d]pyrimidine-2(1*H*)imine (**4**)

A mixture of compound **1** (10 mmol) in 25 mL absolute ethanol and sodium hydroxide (0.5 g) was mixed with guanidine hydrochloride (0.96 g, 10 mmol) and refluxed for 3 h. It was left to cool and then decanted gradually, with stirring, into cold water. The solid formed was filtered, washed with water and crystallized from the proper solvent.

*8-Benzylidene-3,4,5,6,7,8-hexahydro-6-methyl-4-phenylpyrido[4,3-d]pyrimidine-2(1H)-imine (**4a**)*

2.8 g (84 % Yield), m.p 155–158 °C; IR (KBr) ν cm^{-1}: 3150, 3300 and 3450 (NH); ^{1}H NMR (DMSO-d_6): δ 9.15 (s, 2H, 2NH, exchangeable with D_2O), 8.42 (s, 1H, NH, exchangeable with D_2O), 7.61–7.05 (m, 11H, Ar–H + C=C*H*), 5.15 (s, 1H, H-4), 4.10 (s, 2H, CH_2), 3.91 (s, 2H, CH_2), 2.70 (s, 3H, CH_3); ^{13}C NMR: δ 163.61 (*C*=*N*H), 140.30 (C-8a), 137.26 (C-4a), 136.91 (C-8), 53.61 (C-4), 143.30, 135.41, 128.80, 128.70, 128.61, 128.12, 127.01, 126.62, 126.41 (12Ar–C), 124.62 (C=*C*H), 52.80 (C-7), 50.91 (C-5), 44.40 (CH_3); MS, *m/z* (%): 330 [M^+] (88), 329 (M^+−H^+) (100), 314 (329–CH_3) (9.9), 254 (M^+–C_6H_5, –H^+) (19.9); anal. calcd. for $C_{21}H_{22}N_4$ (330.426); C, 76.32; H, 6.71; N, 16.95. Found: C, 76.35; H, 6.70; N, 16.92.

*8-(4-Fluorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-fluorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-imine (**4b**)*

3.5 g (94 % Yield), m.p 130–132 °C; IR (KBr) ν cm^{-1}: 3155, 3310 and 3452 (NH); ^{1}H NMR (DMSO-d_6): δ 9.60 (s, 2H, 2NH, exchangeable with D_2O), 8.61 (s, 1H, NH, exchangeable with D_2O), 6.85–7.04 (m, 9H, Ar–H + C=C*H*), 4.95 (s, 1H, H-4), 4.02 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 2.72 (s, 3H, CH_3); ^{13}C NMR: δ 163.27 (*C*=*N*H), 140.38 (C-8a), 137.04 (C-4a), 136.29 (C-8), 53.65 (C-4), 143.41, 135.40, 128.76, 128.49, 128.39, 128,127, 126.61, 126.20 (12Ar–C), 124.71 (C=*C*H), 52.77 (C-7), 50.99 (C-5), 44.45 (CH_3); MS, *m/z* (%): 366 [M^+] (80), 347 (M^+–F) (51), 271 (M^+–C_6H_4–F) (100), 243 (M^+–F–C_6H_4–CH=NH^+) (63); anal. calcd. for $C_{21}H_{20}N_4F_2$ (366.39); C, 68.83; H, 5.50; N, 15.29. Found: C, 68.81; H, 5.50; N, 15.30.

*8-(2-Chlorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(2-chlorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-imine (**4c**)*

3.7 g (92 % Yield), m.p 142–145 °C; IR (KBr) ν cm^{-1}: 3158, 3310 and 3455 (NH); ^{1}H NMR (DMSO-d_6): δ 9.21 (s, 2H, 2NH, exchangeable with D_2O), 8.31 (s, 1H, NH, exchangeable with D_2O), 6.82–7.12 (m, 9H, Ar–H + C=C*H*), 4.98 (s, 1H, H-4), 4.04 (s, 2H, CH_2), 3.70 (s, 2H, CH_2),2.71 (s, 3H, CH_3); ^{13}C NMR: δ 163.29 (*C*=*N*H), 141.08 (C-8a), 138.04 (C-4a), 137.59 (C-8), 54.66 (C-4), 144.40, 137.42, 128.66, 128.53, 128.43, 128,12, 126.7, 126.56 (12Ar–C), 124.91 (C=*C*H), 52.73 (C-7), 50.90 (C-5), 44.50 (CH_3); MS, *m/z* (%): 399 [M^+] (68), 401 (M^++2) (45.5), 403 (M^++4) (10.3), due to the presence of two chlorine atoms, 384 (M^+–CH_3) (10.2), 288 (M^+–Cl–C_6H_5) (36); anal. calcd. for $C_{21}H_{20}N_4Cl_2$ (399.32); C, 63.16; H, 5.04; N, 14.03. Found: C, 63.13; H, 5.07; N, 14.06.

*8-(4-Chlorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-chlorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-imine (**4d**)*

3.5 g (89 % Yield), m.p 213–215 °C; IR (KBr) ν cm^{-1}: 3150, 3312 and 3458 (NH); ^{1}H NMR (DMSO-d_6): δ 8.91

(s, 2H, 2NH, exchangeable with D_2O), 8.40 (s, 1H, NH, exchangeable with D_2O), 6.79–7.15 (m, 9H, Ar–H + C=C*H*), 5.01 (s, 1H, H-4), 4.11 (s, 2H, CH_2), 3.70 (s, 2H, CH_2), 2.70 (s, 3H, CH_3); ^{13}C NMR: δ 162.77 (*C*=*N*H), 141.45 (C-8a), 138.02 (C-4a), 136.19 (C-8), 55.25 (C-4), 144.21, 134.50, 129.61, 128.49, 127.62, 126.11, 126.31, 126.05 (12Ar–C), 124.25 (C=*C*H), 52.62 (C-7), 50.94 (C-5), 44.43 (CH_3); MS, *m*/*z* (%): 399 [M^+] (73), 401 (M^++2) (48.8), 403 (M^++4) (13), due to the presence of two chlorine atoms, 356 (M^+–CH_3–N=CH_2) (20.2), 288 (M^+–Cl–C_6H_5) (46); anal. calcd. for $C_{21}H_{20}N_4Cl_2$ (399.32); C, 63.16; H, 5.04; N, 14.03. Found: C, 63.17; H, 5.02; N, 14.06.

8-(3-Bromobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(3-bromophenyl)pyrido[4,3-d]pyrimidine-2(1H)-imine ***(4e)***

4.4 g (90 % Yield), m.p 145–148 °C; IR (KBr) ν cm^{-1}: 3156, 3309 and 3452 (NH); ^{1}H NMR (DMSO-d_6): δ 9.11 (s, 2H, 2NH, exchangeable with D_2O), 8.51 (s, 1H, NH, exchangeable with D_2O), 6.68–7.06 (m, 9H, Ar–H + C=C*H*), 4.85 (s, 1H, H-4), 4.31 (s, 2H, CH_2), 3.61(s, 2H, CH_2), 2.77 (s, 3H, CH_3); ^{13}C NMR: δ 163.31 (*C*=*N*H), 140.35 (C-8a), 137.10 (C-4a), 135.28 (C-8), 55.85 (C-4), 142.44, 135.45, 129.71, 128.78, 128.51, 128,17, 126.38, 126.07 (12Ar–C), 124.71 (C=*C*H), 52.80 (C-7), 50.95 (C-5), 43.85 (CH_3); MS, *m*/*z* (%): 488 [M^+] (56), 490 (M^++2) (100), 492 (M^++4) (54), due to the presence of two bromine atoms, 408 (M^+–Br) (5.6), 332 (M^+–Br–C_6H_5) (36); anal. calcd. for $C_{21}H_{20}N_4Br_2$ (488.23); C, 51.66; H, 4.13; N, 11.47. Found: C, 51.64; H, 4.12; N, 11.49.

4-Aryl-8-arylmethylene-3,4,5,6,7,8-hexahydro-6-methyl-pyrido[4,3-d]pyrimidine-2-(*1H*)thione (**5**)

To a solution of compound **1** (10 mmol) in 25 ml absolute ethanol, 0.5 g potassium hydroxide and thiourea (0.76 g, 10 mmol) were added. The mixture was refluxed for 3 h, left to cool, and poured gradually into cold water. The solid formed was filtered, washed with water and crystallized from the proper solvent. The following compounds were identified.

8-Benzylidene-3,4,5,6,7,8-hexahydro-6-methyl-4-phenylpyrido[4,3-d]pyrimidine-2(1H)-thione ***(5a)***

3.1 g (89 % Yield), m.p 190–193 °C; IR (KBr) ν cm^{-1}: 3502, 3194 (NH); ^{1}H NMR (DMSO-d_6): δ 9.48, 9.18 (2s, 2H, 2NH, exchangeable with D_2O), 7.40–7.15 (m, 11H, Ar–H + C=C*H*), 4.95 (s, 1H, H-4), 4.09 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 2.20 (s, 3H, CH_3); ^{13}C NMR: δ 180.61 (*C*=S), 143.31 (C-8a), 138.26 (C-4a), 137.92 (C-8), 54.35 (C-4), 143.29, 136.19, 129.49, 128.74, 128.62, 128,13, 126.65 126.92 (12Ar–C), 125.63 (C=*C*H), 54.39 (C-7), 53.91 (C-5), 44.48 (CH_3); MS, *m*/*z* (%): 347 [M^+] (80.7), 346 (M^+–H^+) (100), 214 (346–C_6H_5CH=NH) (52.7), 254 (M^+–C_6H_5, –H^+) (19.9); anal. calcd. for $C_{21}H_{21}N_3S$ (347.468); 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-Fluorobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-fluorophenyl)pyrido[4,3-d]pyrimidine-2(1H)-thione ***(5b)***

4.5 g (91 % Yield), m.p 155–157 °C; (KBr) ν cm^{-1}: 3344, 3204 (NH); ^{1}H NMR (DMSO-d_6): δ 9.51, 9.21 (2s, 2H, 2NH, exchangeable with D_2O), 7.50–7.11 (m, 9H, Ar–H + C=C*H*), 4.96 (s, 1H, H-4), 4.10 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 2.20 (s, 3H, CH_3); ^{13}C NMR: δ 180.56 (*C*=S), 140.41 (C-8a), 137.28 (C-4a), 136.81 (C-8), 53.72 (C-4), 143.33, 135.43, 128.89, 128.75, 128.61, 128.14, 126.29, 126.47 (12Ar–C), 124.19 (C=*C*H), 52.71 (C-7), 50.93 (C-5), 44.47 (CH_3); MS, *m*/*z* (%): 383 [M^+] (100), 339 (M^+–C=S) (11.4), 259 (383–F–C_6H_4CH=NH) (71.4); anal. calcd. for $C_{21}H_{19}N_3SF_2$ (383.432); 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 ***(5c)***

3.4 g (82 % Yield), m.p 200–202 °C; IR (KBr) ν cm^{-1}: 3351, 3304 (NH); ^{1}H NMR (DMSO-d_6): δ 9.71, 9.10 (2s, 2H, 2NH, exchangeable with D_2O), 7.55–7.15 (m, 9H, Ar–H + C=C*H*), 5.41 (s, 1H, H-4), 4.21 (s, 2H, CH_2), 3.71(s, 2H, CH_2), 2.20 (s, 3H, CH_3); ^{13}C NMR: δ 180.59 (*C*=S), 140.42 (C-8a), 137.32 (C-4a), 136.28 (C-8), 53.71 (C-4), 143.33, 135.43, 128.89, 128.74, 128.62, 128,19, 126.31, 126.52 (12Ar–C), 124.22 (C=*C*H), 52.73 (C-7), 50.98 (C-5), 44.44 (CH_3); MS, *m*/*z* (%): 416 [M^+] (50), 418 (M^++2) (35), 420 (M^++4) (8.5), due to the presence of two chlorine atoms, 275 (M^+–Cl–C_6H_4CH=NH, –H_2) (100); anal. calcd. for $C_{21}H_{19}N_3SCl_2$ (416.36); C, 60.57; H, 4.6; N, 10.1; S, 7.7. Found: C, 60.60; 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 ***(5d)***

3.7 g (88 % Yield), m.p 202–205 °C; IR (KBr) ν cm^{-1}: 3248, 3176 (NH); ^{1}H NMR (DMSO-d_6): δ 9.60, 9.10 (2s, 2H, 2NH, exchangeable with D_2O), 7.6–7.1 (m, 9H, Ar–H + C=C*H*), 5.02 (s, 1H, H-4), 4.31 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR: δ 180.82 (*C*=S),

140.46 (C-8a), 137.36 (C-4a), 136.88 (C-8), 53.75 (C-4), 143.37, 135.43, 128.89, 128.79, 128.61, 128,15, 126.42, 126.50 (12Ar–C), 124.28 (C=*C*H), 52.74 (C-7), 50.96 (C-5), 44.58 (CH_3); MS, *m/z* (%): 416 [M^+] (87.6), 418 (M^++2) (59), 420 (M^++4) (13), due to the presence of two chlorine atoms, 414 (M^+–H_2) (100), 275 (M^+–Cl–C_6H_4CH=NH, –H_2) (72.2); anal. calcd. for $C_{21}H_{19}N_3SCl_2$ (416.36); C, 60.57; H, 4.60; N, 10.09; S, 7.70. Found: C, 60.55; H, 4.62; N, 10.13; S, 7.68.

8-(4-Bromobenzylidene)-3,4,5,6,7,8-hexahydro-6-methyl-4-(4-bromophenyl)pyrido[4,3-d]pyrimidine-2(1H)-thione ***(5e)***

4.3 g (85 % Yield), m.p 180–182 °C; IR (KBr) ν cm^{-1}: 3346, 3275 (NH); ^{1}H NMR (DMSO-d_6): δ 9.42, 9.01 (2 s, 2H, 2NH, exchangeable with D_2O), 7.61–7.30 (m, 9H, Ar–H + C=C*H*), 5.31 (s, 1H, H-4), 4.22 (s, 2H, CH_2), 3.53 (s, 2H, CH_2), 2.23 (s, 3H, CH_3); ^{13}C NMR: δ 180.57 (*C*=S), 140.44 (C-8a), 137.29 (C-4a), 136.84 (C-8), 53.74 (C-4), 143.37, 135.45, 128.87, 128.70, 128.71, 128,21, 126.31, 126.48 (12Ar–C), 124.23 (C=*C*H), 52.74 (C-7), 50.95 (C-5), 44.49 (CH_3); MS, *m/z* (%): 505 [M^+] (47), 507 (M^++2) (95), 509 (M^++4) (45), due to the presence of two bromine atoms, 349 (M^+–Br–C_6H_4,) (100); anal. calcd. for $C_{21}H_{19}N_3SBr_2$ (505.27); C, 49.91; H, 3.79; N, 8.32; S, 6.34. Found: C, 49.90; H, 3.80; N, 8.31; S, 6.35.

4-Aryl-8-arylmethylene-2-(alkylthio)-3,4,5,6,7, 8,-hexahydro-6-methylpyrido[4,3-d]pyrimidine-2-alkylthione (**6**)

To a mixture of compound **5** (10 mmol) in 50 mL of ethyl alcohol, sodium hydroxide (0.3 g) and methyl iodide or ethyl iodide (10 mmol) was added. The mixture was refluxed for 2 h, excess ethanol was evaporated and the solid formed was collected and crystallized from ethanol.

8-Benzylidene-3,4,5,6,7,8,-hexahydro-6-methyl-2-(methylthio)-4-phenylpyrido[4,3-d]pyrimidine ***(6a)***

3.1 g (85 % Yield), m.p 119–121 °C; IR (KBr) ν cm^{-1}: 1642 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.24 (s, 1H, NH), 7.62–7.20 (m, 11H, Ar–H + C=C*H*), 4.97 (s, 1H, H-4), 4.20 (s, 2H, CH_2), 3.70 (s, 2H, CH_2), 2.45 (s, 3H, N–CH_3), 2.20 (s, 3H, SCH_3); ^{13}C NMR: δ 159.37 (*C*=*N*H), 141.26 (C-8a), 138.25 (C-4a), 135.94 (C-8), 56.77 (C-4), 143.06, 135.58, 128.28, 128.03, 128.1, 127,21, 126.21, 126.15 (12Ar–C), 123.68 (C=*C*H), 52.26 (C-7), 50.38 (C-5), 44.11 (CH_3), 26.16 (SCH_3); MS: *m/z* (%): 361 [M^+] (19), 318 (M^+–CH_3–N=CH_2) (51), 284 (M^+–C_6H_5) (100); anal. calcd. for $C_{22}H_{23}N_3S$ (361.484); C, 73.09; H, 6.41; N, 11.62; S, 8.87. Found: C, 73.11; H, 6.43; N, 11.60; S, 8.85.

8-(4-Bromobenzylidene)-3,4,5,6,7,8,-hexahydro-6-methyl-2-(methylthio)-4-(4-bromophenyl)pyrido[4,3-d]pyrimidine ***(6b)***

4.3 g (82 % Yield), m.p 163–165 °C; IR (KBr) ν cm^{-1}: 1645 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.69 (s, 1H, NH), 6.9-7.65 (m, 9H, Ar–H + C=C*H*), 4.99 (s, 1H, H-4), 3.61 (s, 2H, CH_2), 3.03 (s, 2H, CH_2), 2.47 (s, 3H, N–CH_3), 2.26 (s, 3H, SCH_3); ^{13}C NMR: δ 159.48 (*C*=*N*H), 141.72 (C-8a), 138.38 (C-4a), 136.02 (C-8), 56.79 (C-4), 144.13, 135.72, 128.52, 128.31, 128.41, 127.28, 126.21, 126.30 (12Ar–C), 123.70 (C=*C*H), 52.40 (C-7), 50.45 (C-5), 44.35 (CH_3), 26.79 (SCH_3); MS: *m/z* (%): 519 [M^+] (21), 521 [M^++2] (43), 523 [M^++4] (22), due to the presence of two bromine atoms, 472 (M^+–SCH_3) (100), 363 (M^+–C_6H_4Br) (51); anal. calcd. for $C_{22}H_{21}N_3SBr_2$ (519.276); C, 50.88; H, 4.08; N, 8.09; S, 6.17. Found: C, 51.10; H, 3.98; N, 8.10; S, 6.21.

8-(4-Bromobenzylidene)-2-(ethylthio)-3,4,5,6,7,8,-hexahydro-6-methyl-4-(4-bromophenyl)pyrido[4,3-d]pyrimidine ***(6c)***

4.3 g (81 % Yield), m.p 129–131 °C; IR (KBr) ν cm^{-1}: 1643 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.48 (s, 1H, NH), 7.72–6.91(m, 9H, Ar–H + C=C*H*), 4.84 (s, 1H, H-4), 3.72 (q, 2H, J = 6.2 Hz, CH_2CH_3), 3.63 (s, 2H, CH_2), 3.13 (s, 2H, CH_2), 2.78 (s, 3H, N–CH_3), 1.21 (*t*, 3H, J = 6.2 Hz, CH_2CH_3); ^{13}C NMR: δ 160.05 (*C*=*N*H), 141.84 (C-8a), 138.31 (C-4a), 136.05 (C-8), 57.10 (C-4), 143.16, 135.67, 128.29, 128.14, 128.02, 127.13, 126.02, 126.15 (12Ar–C), 123.70 (C=*C*H), 52.30 (C-7), 51.18 (C-5), 46.38 (CH_2), 45.22 (CH_3), 27.59 (SCH_3); MS: *m/z* (%): 533 [M^+] (18), 535 [M^++2](37), 537[M^++4] (19), due to the presence of two bromine atoms, 375 (M^+–C_6H_4Br) (100); anal. calcd. for $C_{23}H_{23}N_3SBr_2$ (533.32); C, 51.79; H, 4.35; N, 7.88; S, 6.01. Found: C, 51.97; H, 3.99; N, 7.90; S, 6.06.

8-(4-Fluorobenzylidene)-3,4,5,6,7,8,-hexahydro-6-methyl-2-(methylthio)-4-(4-fluorophenyl)pyrido[4,3-d]pyrimidine ***(6d)***

3.4 g (86 % Yield), m.p 160–162 °C; IR (KBr) ν cm^{-1}: 1649 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.52 (s, 1H, NH), 6.91–7.45 (m, 9H, Ar–H + C=C*H*), 4.96 (s, 1H, H-4), 3.61 (s, 2H, CH_2), 3.25 (s, 2H, CH_2), 2.47 (s, 3H, N–CH_3), 2.27 (s, 3H, SCH_3); ^{13}C NMR: δ 160.11 (*C*=*N*H), 141.93 (C-8a), 138.49 (C-4a), 136.28 (C-8), 58.05 (C-4), 143.81, 135.95, 128.40, 128.22, 128.90, 127.85, 126.53, 126.19 (12Ar–C), 123.99 (C=*C*H), 52.75 (C-7), 51.62 (C-5), 45.29 (CH_3), 26.38 (SCH_3); MS: *m/z* (%): 395 [M^+] (20), 300 (M^+–C_6H_4F) (100); anal. calcd. for $C_{22}H_{21}N_3SF_2$ (397.48);

C, 66.48; H, 5.33; N, 10.57; S, 8.07. Found: C, 66.83; H, 5.11; N, 10.64; S, 8.12.

*8-(4-Fluorobenzylidene)-2-(ethylthio)-3,4,5,6,7,8,-hexahydro-6-methyl-4-(fluorophenyl)pyrido[4,3-d]pyrimidine (**6e**)*

3.3 g (81 % Yield), m.p 142–144 °C; IR (KBr) ν cm^{-1}: 1651 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.72 (s, 1H, NH), 6.92–7.44 (m, 9H, Ar–H + C=C*H*), 4.97 (s, 1H, H-4), 3.73 (q, 2H, $J = 6.2$ Hz, CH_2CH_3), 3.60 (s, 2H, CH_2), 3.22 (s, 2H, CH_2), 2.51 (s, 3H, N–CH_3), 1.21 (*t*, 3H, $J = 6.2$ Hz, CH_2CH_3); ^{13}C NMR: δ 159.27 (*C*=*N*H), 141.21 (C-8a), 138.10 (C-4a), 136.20 (C-8), 57.11 (C-4), 143.16, 135.66, 128.29, 128.18, 127.62, 126.84, 126.14, 125.65 (12Ar–C), 123.64 (C=*C*H), 52.14 (C-7), 51.18 (C-5), 46.29 (CH_2), 45.08 (CH_3), 27.84 (SCH_3); MS: *m/z* (%): 411 [M^+] (100), 316 (M^+–C_6H_4F) (95); anal. calcd. for $C_{23}H_{23}N_3SF_2$ (411.491); C, 67.13; H, 5.63; N, 10.21; S, 7.79. Found: C, 67.11; H, 5.62; N, 10.24; S, 7.81.

*8-(4-Chlorobenzylidene)-3,4,5,6,7,8,-hexahydro-6-methyl-2-(methylthio)-4-(4-chlorophenyl)pyrido[4,3-d]pyrimidine (**6f**)*

3.7 g (87 % Yield), m.p 132–134 °C; IR (KBr) ν cm^{-1}: 1648 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.47 (s, 1H, NH), 7.60-7.22 (m, 9H, Ar–H + C=C*H*), 4.99 (s, 1H, H-4), 3.71 (s, 2H, CH_2), 3.45 (s, 2H, CH_2), 2.55 (s, 3H, N–CH_3), 2.20 (s, 3H, SCH_3); ^{13}C NMR: δ 159.04 (*C*=*N*H), 141.39 (C-8a), 138.18 (C-4a), 135.73 (C-8), 57.02 (C-4), 142.93, 135.11, 128.04, 127.84, 127.42, 127.05, 126.02, 126.03 (12Ar–C), 123.60 (C=*C*H), 52.31 (C-7), 51.26 (C-5), 45.19 (CH_3), 27.18 (SCH_3); MS: *m/z* (%): 430 [M^+] (22),432 [M^++2] (15), 434 [M^++4] (3.8), due to the presence of two chlorine atoms, 306 (M^+–ClC_6H_4–CH) (100); anal. calcd. for $C_{22}H_{21}N_3SCl_2$ (430.39); C, 61.39; H, 4.92; N, 9.76; S, 7.45. Found: C, 61.64; H, 4.89; N, 9.83; S, 7.46.

Antitumor activity

Some of the synthesized compounds were screened for their anticancer activity. Each test compound was studied at five different concentrations against 59 different human tumor cell lines, representing cancers of lung, leukemia, brain, colon, breast, prostate, ovary, renal, melanoma as well as kidney, according to a previously reported procedure (Ally et al. 1988; Grever et al. 1992; Boy and Paull 1995). Measurements of in vitro growth in micro culture wells by cell-mediated reduction of tetrazolium showed a good correlation with measurements of cellular protein in adherent cell line cultures as well as viable cell counts in suspensions of cell line cultures. The results are expressed as log 10 GI_{50} which is the drug concentration (*M*) causing a 50 % reduction in the net protein increase in control cells during the drug incubation.

Results and discussion

3,5-Bisarylmethylene-1-methyl-4-piperidon (**1**) was synthesized according to the reported procedure (Lyles et al, 1974; Mcelvain and Kurt 1948) and used as the starting material. It was reacted with phenyl hydrazine in dioxan and/or acetic acid to afford the corresponding pyrazolo[4,3-*c*]pyridine derivative **2**. Both IR and ^{1}H NMR spectral data showed an absence of NH groups, indicating that the formation of the cyclic product **2A** is favored over the isomeric structure **2B**. The ^{13}C NMR of **2a–e** revealed signals at 155.25–155.79 ppm that were assigned to C=N. The presence of a signal corresponding to C=N in the pyrazolopyridine system in compounds **2a–e** indicated that products **2A** are favored over their isomeric structure **2B**.

Compound **1** was condensed with urea in the presence of ethanolic potassium hydroxide, guanidine hydrochloride in the presence of sodium ethoxide, and/or thiourea in the presence of ethanolic potassium hydroxide under reflux to afford the corresponding pyrimidine derivatives **3**, **4** and **5**. The structures of the pyridopyrimidine derivatives produced were confirmed by their spectral and analytical data. The IR spectra of compounds **3a–e** showed characteristic absorption bands for NH, C=O and C=N groups. The presence of signals corresponding to C-4a and C-8a in the ^{13}C NMR spectra of **3a–e** proved formation of the pyridopyrimidine ring system in these compounds. The ^{1}H NMR spectra of compounds **4** and **5** revealed the presence of NH signals, signals of methyl protons, as well as aromatic proton signals (see "Materials and methods" section). The ^{13}C NMR spectra of **5a–e** showed signals at 180.16–180.22 ppm corresponding to C=S groups.

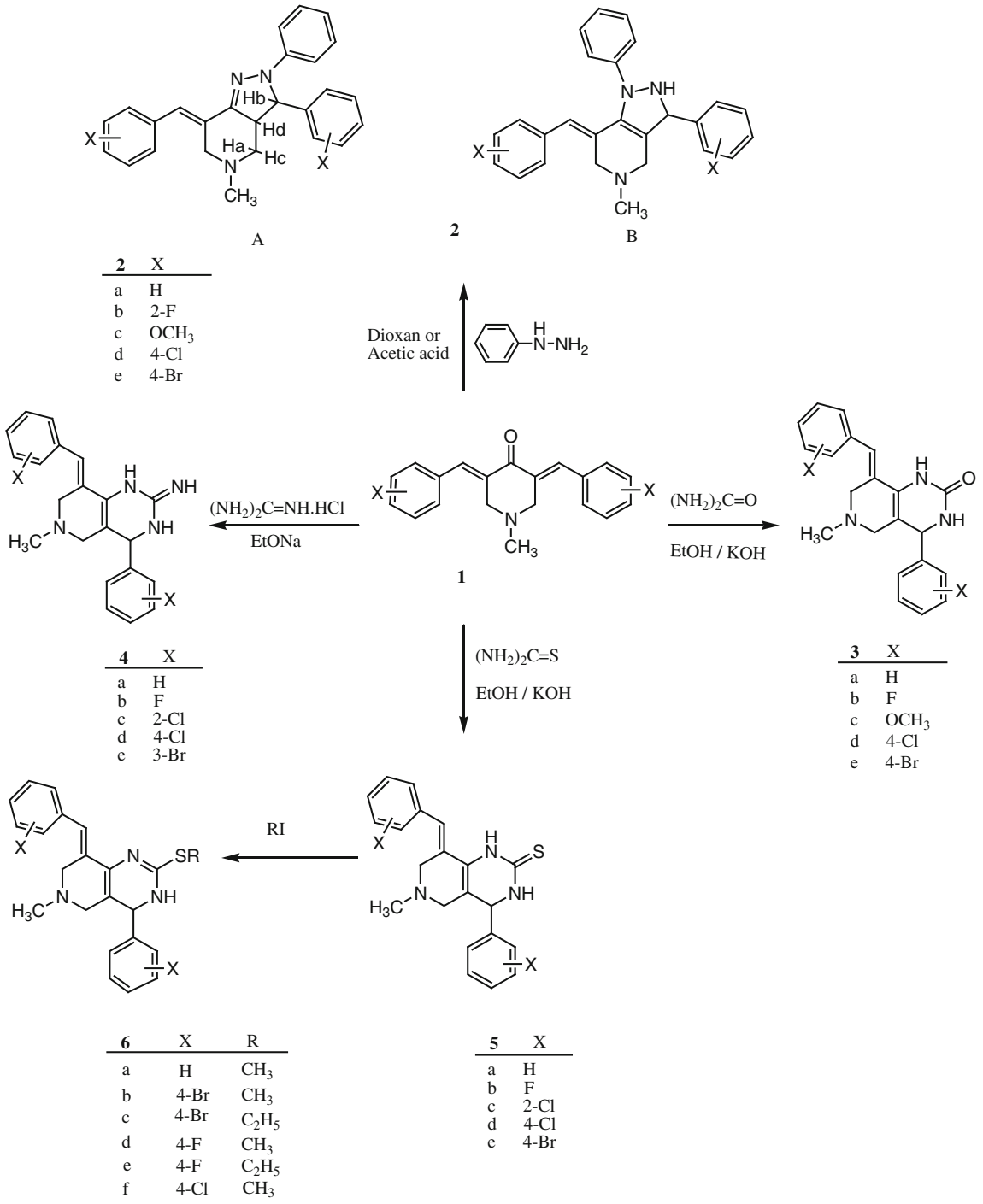
When compounds **5a–e** were allowed to react with methyl iodide and/or ethyl iodide in the presence of alcoholic sodium hydroxide, sodium ethoxide or acetic acid and sodium acetate, the corresponding alkylated products **6a–f** were afforded in 80–86 % yields. The ^{1}H NMR spectra of the resulting *S*-alkyl derivatives showed signals corresponding to either the alkyl groups as singlets or the ethyl proton signals as triplet and quartets. The spectra also showed signals at 9.24–9.72 ppm corresponding to NH groups which were assigned to position N-3. The absence of a signal corresponding to C=S in the ^{13}C NMR spectra of compounds **6a–f** indicates that alkylation had taken place at the sulfur atom, not the nitrogen atom. This is also in agreement with their ^{1}H NMR spectra which revealed a relatively low chemical shift for CH_3 or CH_2CH_3

indicating their attachment to the sulfur atom as *S*–CH_3 or *S*–CH_2CH_3 (Scheme 1).

Antitumor activity

The synthesized compounds were screened for their anticancer activity. Each compound was tested at five different concentrations against 59 different human tumor cell lines, representing cancers of lung, leukemia, brain, colon, breast, prostate, ovary, renal, melanoma as well as kidney. Some compounds showed little or no activity while other compounds such as **2b**, **2d**, **3a**, **4b**, **5b** and **5d** exhibited moderate to high activities.

The results are expressed as log 10 GI_{50}, which is the drug concentration (*M*) causing a 50 % reduction in the net



2	X
a	H
b	2-F
c	OCH_3
d	4-Cl
e	4-Br

4	X
a	H
b	F
c	2-Cl
d	4-Cl
e	3-Br

3	X
a	H
b	F
c	OCH_3
d	4-Cl
e	4-Br

6	X	R
a	H	CH_3
b	4-Br	CH_3
c	4-Br	C_2H_5
d	4-F	CH_3
e	4-F	C_2H_5
f	4-Cl	CH_3

5	X
a	H
b	F
c	2-Cl
d	4-Cl
e	4-Br

Scheme 1 Synthesis of fused *N*-methyl pyridine derivatives

Table 1 Concentrations resulting in 50 % inhibition of (log 10 GI_{50}) of the growth of in vitro human tumor cell lines

Panel/cell line	Compound					
	2b	**2d**	**3a**	**4b**	**5b**	**5d**
Non-small cell lung cancer						
A 549/ATCC	−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
HOP-62	−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
NCI-H226	−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
NCI-H322M	−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
NCI-H522	−5.3	−5.3	−5.3	−5.3	−5.3	−5.3
Leukemia						
CCRF-CEM	−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
K-562	−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
RPMI-8226	−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
CNS cancer						
SF-268	−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
SNB-19	−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
U 251	−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
HCC-2998	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
HCT-116	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
HCT-15	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
HT29	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
KM12	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
SW-620	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
Breast cancer						
MCF 7	−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
MDA-MB-231/ATCC	−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
MDA-MB-435	−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
T-47D	−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
OVCAR-3	−4.3	−4.0	−4.0	−4.0	−4.3	−4.3
OCAR-4	−4.3	−4.0	−4.0	−4.0	−4.3	−4.3
OVCAR-5	−4.3	−4.0	−4.0	−4.0	−4.3	−4.3
OVCAR-8	−4.3	−4.0	−4.0	−4.0	−4.3	−4.3
SK-OV-3	−4.3	−4.0	−4.0	−4.0	−4.3	−4.3
Prostate cancer						
PC-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
Renal cancer						
786-0	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
A 498	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
ACHN	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
CAKI-1	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
RXF-393	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
SN12C	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
TK-10	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
UO-31	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
Melanoma						
LOXIMVI	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
MALME-3 M	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
M 14	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
SK-MEL-2	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
SK-MEL-28	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
SK-MEL-5	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
UACC-257	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3
UACC-62	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3

protein increase in control cells during the drug incubation. The results are presented in Table 1.

From the in vitro data it was noticed that compounds **2b**, **2d**, **3a**, **4b**, **5b** and **5d** were the most active derivatives against all tested cell lines. An inspection of these data shows that the majority of the compounds tested exhibit activity against lung cancer at low concentration comparable with that of 5-fluorodeoxyuridine (log 10 $GI_{50} = -4.7$), which was used as the reference compound (Bhatt et al. 1994) (see Table 1). The results obtained clearly showed differences in activity between compounds with different substituents on the phenyl ring, indicating the effect of substituents on the resulting activity.

From the results outlined in Table 1 and structure activity correlations it was concluded that pyrazole and pyrimidine moieties fused to nitrogen-methylpiperidone ring systems result in increased antitumor activities. It is also suggested that the presence of fluorine and chlorine in the para-position especially in the pyrazolopyrimidine ring system results in enhancement of anticancer activity. Substitution in the *para*-position with a fluorine atom in the aromatic ring attached to pyrazolopyrimidine, 2-imino-pyridopyrimidine and pyridopyrimidine-2-thione systems is essential for enhanced activity. We also conclude that substitution on the pyridopyrimidine-2-thione ring system

with a free –NH or –SH with thione–thiol tautomerism shows higher activity than the corresponding *S*-alkyl derivatives (the activity was reduced when the free SH was alkylated).

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