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Synthesis and Anticancer Activity of Some Novel Fused Pyridine Ring System

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New series of pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidines (**7a**,**b**) and thieno[2,3-*b*:4,5-*b*'] dipyridine (**11a**-**c**) were synthesized from 4-aryl-6-(4-chlorophenyl)-2-thioxo-1,2-dihydro pyridine-3-carbonitriles **4a**,**b** via application of Thorpe-Zielger reaction. The novel target compounds were evaluated *in vitro* for their anticancer activity against human breast adenocarcinoma MCF-7 and colon carcinoma cell line (HCT 116). Most of the tested compounds exploited potent to moderate growth inhibitory activity, in particular compound **11d**, which exhibited superior potency to the reference drug Doxorubicin (IC_{50} = 5.95, 6.09 and 8.48, 8.15 μM, respectively). The structures of the compounds obtained were determined by spectroscopic data.

Key words: Synthesis, Pyridothienopyrimidine, Thienodipyridine, Anticancer activity

INTRODUCTION

Cancer treatment has been a major endeavor of research and development in academia and pharmaceutical industry for the last many years as it is one of the leading causes of death (Choo et al., 2002; Abdel-Aziz, 2007; Hassan et al., 2011; Taher et al., 2012). The great incidence of cancer worldwide encourages the search for newer, safer and more efficient anticancer agents, aiming at preventing or curing this illness. Although chemotherapy is the mainstay for cancer treatment, the use of available chemotherapeutics is often limited due to undesirable side effects. In addition, there is a growing incidence of drug resistance to cancer chemotherapeutic agents, which represents a serious medical problem (Li et al., 1995; Rojo et al., 2008). As a result of these challenges, significant interest has developed in the search for new anticancer agents.

The pyridine nucleus is prevalent in numerous natural products and is extremely important in the chemistry of biological systems (Bringmann et al., 2004). Derivatives of pyridine were found to have various biological activities. Among these derivatives, fused analogues are often of greater biological interest than the corresponding monocyclic compounds (Litvinov et al., 2007). Furthermore, it should be emphasized that the combination of pyridine with other hetero cycles is a well-known approach for drug-like molecules build-up. This may allow achieving new pharmacological profiles, action-strengthening or toxicity-lowering.

On the other hand, compounds containing a fused pyrimidine ring have attracted attention in the past few years. This is due by their wide range of biological activities, particularly in cancer and virus research (Baba et al., 1987; Gangjee et al., 1995). Pyrido[2,3-*d*] pyrimidines were reported to exhibit antitumor activity which may be attributed to inhibition of cyclin dependent kinase (Toogood et al., 2005; VanderWel et al., 2005), check point kinase (Palmer et al., 2005) or mammalian target of rapamycin (Malagu et al., 2009). In addition, thienopyrimidine derivatives showed promising biological (Devani et al., 1976) and anticancer activity (Dai et al., 2005; Rheault et al., 2009). Several reports showed that a single molecule containing more than one pharmacophore, each with different mechanism of action, could be beneficial for the treatment of cancer. Accordingly, the combination of these two classes to give the tricyclic pyridothienopyrimidines and the subsequent influence on the biological activities have become a subject of interest (Dave et al., 1989; Quintela

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et al., 1998; Amr et al., 2003). However, none of these publication discussed the effect of thieno[2,3-*b*:4,5-*b*']dipyridine as antitumor agents.

Motivated by the above-mentioned findings, the present study involves the synthesis and pharmacological evaluation of some substituted pyridothienopyrimidines and isosteric analogues thienodipyridines as anticancer agents.

MATERIALS AND METHODS

Chemistry

General remarks: Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp). Microanalysis was carried out at Micro-analytical Unit, Faculty of Science, Cairo University, Analyses indicated were within 0.4% of the theoretical values. Infrared Spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu), and expressed in wave number (cm^{-1}), using potassium bromide discs. The NMR spectra were recorded on a Varian Gemini 200 MHz and Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethylsulfoxide (DMSO-d_6). Chemical shifts were quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck), the developing solvents were benzene/acetone (4:1) and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber). Compounds **2a**,**b** (Bickel, 1946), **3** (Howard, 1956), **5** (Huffman and Schaefer, 1963) and **9** (Mowry, 1945; Gewald, 1965) were obtained according to the reported procedures.

4-Aryl-6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitriles 4a,b

Two methods were adopted for the synthesis of the title compounds.

Method A: A mixture of *p*-chloroacetophenone **1** (1.55 g, 0.01 mol), the appropriate aromatic aldehydes (0.01 mol), cyanothioacetamide **3** (1 g, 0.01 mol) and ammonium acetate (6.16 g, 0.08 mol) in n-butanol (30 mL) was heated under reflux for 9 h. The reaction mixture was cooled to room temperature. The separated solid was filtered, dried and crystallized from suitable solvent.

Method B: Sodium ethoxide (0.07 g sodium in absolute ethanol 20 mL) was added to a mixture of respective chalcone analogue **2a**,**b** (0.01 mol) and cyanothioacetamide **3** (1.1 g, 0.011 mol). The reaction mixture was heated under reflux for 7 h, left to cool to room temperature. The separated solid was filtered, dried and crystallized from suitable solvent.

6-(4-Chlorophenyl)-4-(2-nitrophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (4a)

Crystallized from DMF/H_2O. Yield: method A (72%), method B (60%); mp: 208-210°C; IR ν_{max}/cm^{-1}: 3400 (NH), 2200 (CN), 1520, 1350 (NO_2), 1090 (C=S); ^1H-NMR (DMSO-d_6) δ ppm: 6.94 (s, 1H, NH, exch. D_2O), 7.50-8.75 (m, 9H, Ar-H and H-5); MS: m/z 367 [M^+, 14.44%]; Anal. Calcd for $C_{18}H_{10}ClN_3O_2S$ (367.81): C, 58.78; H, 2.74; N, 11.42. Found: C, 58.50; H, 3.00; N, 11.42.

6-(4-Chlorophenyl)-4-(3-nitrophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (4b)

Crystallized from ethanol. Yield: method A (75%), method B (55%); mp: >300°C; IR ν_{max}/cm^{-1}: 3400 (NH), 2200 (CN), 1520, 1340 (NO_2), 1090 (C=S); ^1H-NMR (DMSO-d_6) δ ppm: 6.96 (s, 1H, NH, exch. D_2O), 6.94-8.47 (m, 9H, Ar-H and H-5); Anal. Calcd for $C_{18}H_{10}ClN_3O_2S$ (367.81): C, 58.78; H, 2.74; N, 11.42. Found: C, 58.99; H,2.79; N, 11.40.

3-Amino-2-aminocyaniminomethyl-4-aryl-6-(4-chlorophenyl)thieno[2,3-*b*]pyridines 6a,b

A solution of the appropriate pyridinethione **4a**,**b** (0.003 mol) and 10% aqueous KOH (0.16 g, 0.003 mol) in DMF (20 mL) was stirred at room temperature for 5 min. N-cyanochloroacetamidine **5** (0.36 g, 0.0031 mol) was added and the mixture was stirred for additional 5 min. Finally, additional 10% aqueous KOH (0.33 g, 0.006 mol) was added to the reaction mixture with continuous stirring for 2 h. The reaction mixture was poured onto cold water, the formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

3-Amino-2-aminocyaniminomethyl-6-(4-chlorophenyl)-4-(2-nitrophenyl)thieno[2,3-*b*] pyridine (6a)

Yield: 63% ; mp: 190-191°C; IR ν_{max}/cm^{-1}: 3420, 3300 (NH_2), 3080 (CH aromatic), 2200 (CN) 1530, 1390 (NO_2); ^1H-NMR (DMSO-d_6) δ ppm: 7.60-7.62 (br s, 2H, NH_2, exch. D_2O), 7.11-8.62 (m, 9H, Ar-H and H-5), 7.97 (s, 2H, NH_2, exch. D_2O); MS m/z: 448 [M^+, 2.55%], 446 [M-2, 3.06%], 69 [NH_2CH=NCN, 22.96%]; Anal. Calcd for $C_{21}H_{13}ClN_6O_2S$: C, 56.19; H, 2.92; N, 18.72. Found: C, 56.46; H, 2.70; N, 19.00.

3-Amino-2-aminocyaniminomethyl-6-(4-chlorophenyl)-4-(3-nitrophenyl)thieno[2,3-*b*] pyridine

(6b)
Yield: 59%; mp: 225-227°C; IR ν_{max}/cm^{-1}: 3300, 3190 (NH_2), 3050 (CH aromatic), 2200 (CN), 1520, 1320 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.63 (s, 2H, NH_2, exch. D_2O), 7-8.6 (m, 9H, Ar-H and H-5), 7.99 (s, 2H, NH_2, exch. D_2O); Anal. Calcd for $C_{21}H_{13}ClN_6O_2S$: C, 56.19; H, 2.92; N, 18.72. Found: C, 55.98; H, 3.19; N, 18.50.

2,4-Diamino-9-aryl-7-(4-chlorophenyl)pyrido [3',2':4,5]thieno[3,2-*d*]pyrimidines 7a,b

Two methods were adopted for the synthesis of the title compounds.

Method A: A mixture of thienopyridine **6a**,**b** (0.001 mol) and sodium (0.07 g, 0.003 mol) in absolute ethanol (20 mL) was heated under reflux for 6 h. The precipitated solid was filtered, washed with cold ethanol, dried and crystallized from ethanol.

Method B: A mixture of the appropriate pyridinethione **4a**,**b** (0.001 mol) and 10% aqueous KOH (0.056 g, 0.001mol) in absolute ethanol (20 mL) was stirred for 10 min and kept at room temperature. N-Cyanochloroacetamidine **5** (0.13 g, 0.0011 mol) was added to the mixture and stirred for 20 min. Sodium (0.07 g, 0.003 mol) in absolute ethanol (20 mL) was added and the reaction mixture was heated under reflux for 4 h. The formed precipitate was filtered, washed with ethanol, dried and crystallized from ethanol.

2,4-Diamino-7-(4-chlorophenyl)-9-(2-nitrophenyl) pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine (7a)
Yield: method A (65%), method B (52%); mp: >300°C; IR ν_{max}/cm^{-1}: 3320, 3200 (NH_2), 3050 (CH aromatic), 1520, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.6 (s, 2H, 4-NH_2, exch. D_2O), 7.6-8.62 (m, 9H, Ar-H and H-8), 8.8 (s, 2H, 2-NH_2, exch. D_2O); MS m/z: 367 [M^+, 14.44%]; Anal. Calcd for $C_{21}H_{13}ClN_6O_2S$: C, 56.19; H, 2.92; N, 18.72. Found: C, 56.43; H, 3.15; N, 18.43.

2,4-Diamino-7-(4-chlorophenyl)-9-(3-nitrophenyl) pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine (7b)
Yield: method A (74%), method B (58%); mp: >300°C; IR ν_{max}/cm^{-1}: 3400, 3200 (NH_2), 3040 (CH aromatic), 1543, 1330 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.15-8.48 (m, 9H, Ar-H and H-8), 8.33 (s, 2H, 4-NH_2, exch. D_2O), 8.48 (s, 2H, 2-NH_2, exch. D_2O); ^{13}C-NMR (DMSO-*d*6) δ ppm: 165.1, 160.3, 156.1, 153.7, 147.4, 144.2, 137.9, 135.3, 132.2, 129.3, 129.1, 124.5, 122.3; Anal. Calcd for $C_{21}H_{13}ClN_6O_2S$: C, 56.19; H, 2.92; N, 18.72. Found: C, 56.38; H 3.20; N, 18.66.

3-Amino-4-aryl-2-arylcarbonyl-6-(4-chlorophenyl)thieno[2,3-*b*]pyridines 8a-c

The appropriate pyridinethione **4a**,**b** (0.002 mol) was added to a stirred solution of sodium (0.5 g, 0.021 mol) in absolute ethanol (50 mL). A solution of the substituted phenacyl bromide (0.002 mol) in dioxane (20 mL) was added portion-wise to the reaction mixture and the mixture was stirred at room temperature for 3 h. Finally, the reaction mixture was diluted with water (50 mL), the formed precipitate was filtered, dried and crystallized from ethanol.

3-Amino-2-benzoyl-6-(4-chlorophenyl)-4-(2-nitrophenyl)thieno[2,3-*b*]pyridine (8a)
Yield: 72%; mp: 145-148°C; IR ν_{max}/cm^{-1}: 3400-3100 (NH_2), 3050 (CH aromatic), 1600 (C=O), 1520, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.28-8.89 (m, 14H, Ar-H and H-5), 10.46 (s, 2H, NH_2, exch. D_2O); Anal. Calcd for $C_{26}H_{16}ClN_3O_3S$: C, 64.26; H, 3.31; N, 8.64. Found: C, 64.46; H 3.46; N, 9.05

3-Amino-6-(4-chlorophenyl)-4-(2-nitrophenyl)-2-(3-nitrobenzoyl)thieno[2,3-*b*]pyridine (8b)
Yield: 65%; mp: 229-231°C; IR ν_{max}/cm^{-1}: 3320 (NH_2), 3050 (CH aromatic), 1600 (C=O), 1520, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.36-8.78 (m, 13 H, Ar-H and H-5), 9.49 (s, 2H, NH_2, exch. D_2O); MS m/z: 532 [M+2, 5.06], 530 [M^+, 11.28]; Anal. Calcd for $C_{26}H_{15}ClN_4O_5S$: C, 58.81; H, 2.84; N, 10.55. Found:C, 58.62; H 3.14; N, 10.16.

3-Amino-6-(4-chlorophenyl)-2-(3-nitrobenzoyl)-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine (8c)
Yield: 63%; mp: >300°C; IR ν_{max}/cm^{-1}: 3300 (NH_2), 3080 (CH aromatic), 1600 (C=O), 1530, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.60-8.80 (m, 13 H, Ar-H and H-5), 8.47 (s, 2H, NH_2, exch. D_2O); Anal. Calcd for $C_{26}H_{15}ClN_4O_5S$: C, 58.81; H, 2.84; N, 10.55. Found: C, 58.61; H 3.12; N, 10.13.

2-[(6-(4-Chlorophenyl)-3-cyano-4-(2-nitrophenyl)pyridin-2-yl)sulfanyl]-1-phenylethylidene malononitrile (10)

A solution of pyridinethione **4a** (0.73 g, 0.002 mol) in absolute ethanol (15 mL) containing piperidine (0.16 g, 0.0019 mol) was heated to 50°C with stirring. 3-Bromo-2-phenyl-1,1-dicyanopropene **9** (0.5 g, 0.002 mol) was added to the reaction mixture with continuous stirring for 12 h. The reaction mixture was diluted with cold water (50 mL). The formed solid was filtered, washed with ethanol, dried and crystallized from ethanol.

Yield: 65%; mp: 145-148°C; IR ν_{max}/cm^{-1}: 3050 (CH aromatic), 2920 (CH aliphatic), 2200, 2180 (CN), 1520, 1360 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.7 (s, 2H, CH_2), 7.32-8.73 (m, 14H, Ar-H and H-5); MS m/z: 533

[M^+, 64.29%]; Anal. Calcd for $C_{29}H_{16}ClN_5O_2S$: C, 65.22; H, 3.02; N, 13.11. Found: C, 64.90; H, 3.10; N, 13.18.

2-Amino-4,9-diaryl-7-(4-chlorophenyl)thieno[2,3-*b*:4,5-*b*']dipyridine-3-carbonitriles 11a-d

Three methods were adopted for the synthesis of the title compounds.

Method A: A mixture of the suitable thienopyridine **8a-c** (0.001 mol), and malononitrile (0.2 g, 0.003 mol) in pyridine (10 mL) was heated under reflux for 12 h. After cooling, the reaction mixture was diluted with water (20 mL). The formed precipitate was filtered, dried and crystallized from ethanol.

Method B: A solution of the S-alkylated pyridinethione **10** (1.06 g, 0.002 mol) in boiling ethanol (30 mL) containing 2-3 drops piperidine was stirred with heating at 50°C for 5 h. The formed solid was filtered, washed with ethanol, dried and crystallized from ethanol.

Method C: 3-Bromo-2-phenyl-1,1-dicyanopropene **9** (12.35 g, 0.05 mol) was added to a solution of the appropriate pyridinethione **4a**,**b** (0.05 mol) and sodium (2.3 g, 0.1 mol) in absolute ethanol (50 mL). The reaction mixture was heated under reflux for 2 h. The precipitated solid was filtered, dried and crystallized from ethanol.

2-Amino-7-(4-chlorophenyl)-4-(2-nitrophenyl)-9-phenylthieno[2,3-*b*:4,5-*b*']dipyridine-3-carbonitrile (11a)

Yield: method A (83%), method B (71%), method C (90%); mp: >300°C; IR ν_{max}/cm^{-1}: 3400 (NH_2), 3050 (CH aromatic), 2200 (CN), 1520, 1385 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.46-8.31 (m, 14H, Ar-H and H-8), 8.76 (s, 2H, NH_2, exch. D_2O); MS m/z: 533 [M^+, 57.38%]; Anal. Calcd for $C_{29}H_{16}ClN_5O_2S$: C, 65.22; H, 3.02; N, 13.11 Found: C, 65.23; H, 3.02; N, 13.11.

2-Amino-7-(4-chlorophenyl)-4-(3-nitrophenyl)-9-phenylthieno[2,3-*b*:4,5-*b*']dipyridine-3-carbonitrile (11b)

Yield: method B (68%), method C (73%) ; mp: >300°C; IR ν_{max}/cm^{-1}: 3350, 3150 (NH_2), 3020 (CH aromatic), 2200 (CN), 1520, 1380 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.44-8.26 (m, 14H, Ar-H and H-8), 8.67 (s, 2H, NH_2, exch. D_2O); Anal. Calcd for $C_{29}H_{16}ClN_5O_2S$: C, 65.22; H, 3.02; N, 13.11. Found: C, 65.09; H, 3.32; N, 13.51.

2-Amino-7-(4-chlorophenyl)-4-(2-nitrophenyl)-9-(3-nitrophenyl)thieno[2,3-*b*:4,5-*b*']dipyridine-3-carbonitrile (11c)

Yield: method A (79%); mp: 180-182°C; IR ν_{max}/cm^{-1} : 3350-3150 (NH_2), 3050 (CH aromatic), 2200 (CN), 1520, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.41-8.36 (m, 13H, Ar-H and H-8), 8.85 (s, 2H, NH_2, exch. D_2O); ^{13}C NMR (DMSO-*d*6) δ ppm: 168.2, 164.3, 156.1, 149.3, 146.8, 146.2, 144.6, 143.9, 141.8, 139.6, 139.1, 138.5, 135.8, 129.5, 129.2, 128.6, 125.3, 124.7, 122.0, 101.9, 88.9; Anal. Calcd for $C_{29}H_{15}ClN_6O_4S$: C, 60.15; H, 2.61; N, 14.51. Found: C, 60.08; H, 2.92; N, 14.80.

2-Amino-7-(4-chlorophenyl)-4-(3-nitrophenyl)-9-(3-nitrophenyl)thieno[2,3-*b*:4,5-*b*']dipyridine-3-carbonitrile (11d)

Yield: method A (85%); mp: 180-182°C; IR ν_{max}/cm^{-1} 3450-3320 (NH_2), 3060 (CH aromatic), 2200 (CN), 1520, 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm 7.38-8.91 (m, 13H, Ar-H and H-8), 9.57 (s, 2H, NH_2, exch. D_2O); Anal. Calcd for $C_{29}H_{15}ClN_6O_4S$: C, 60.15; H, 2.61; N, 14.51. Found: C, 60.23; H, 2.61; N, 14.53.

Biological testing

Materials and methods

The human breast adenocarcinoma cell line (MCF-7) was obtained and colon carcinoma cell line HCT-116 were obtained as a gift from NCI, MD,USA.

All solvents and chemicals were purchased from Sigma-Aldrich.

Measurement of potential cytotoxicity

The cytotoxic activity of the newly synthesized compounds was measured *in vitro* on human breast adenocarcinoma cell line (MCF-7) and Colon carcinoma cell line (HCT 116) using Sulforhodamine-B (SRB) stain assay, applying the method of Skehan et al. (1990) as follows:

Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 1, 2.5, 5 and 10 mg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37°C in an atmosphere of 5% CO_2. After 48 h, cells were fixed with trichloroacetic acid, washed with water and stained for 30 min with 0.4% (wt/vol) SRB stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell via-

Table I. IC_{50} values in μM[a] of compounds **7a**,**b** and **11a-d** against MCF7 and HCT 116

Compound no.	Cell line	
	MCF-7	HCT 116
Doxorubicin[b]	8.48±0.015	8.15±0.015
7a	19.87±0.032	9.53±0.015
7b	11.25±0.008	23.61±0.035
11a	24.34±0.047	8.29±0.021
11b	15.90±0.023	6.04±0.011
11c	33.68±0.018	22.10±0.056
11d	5.95±0.030	6.09±0.025

[a]Each experiment was independently performed three times and expressed as means±S.D. [b]Doxorubicin was used as reference drug.

bility] was calculated for each compound and results are given in Table I.

RESULTS

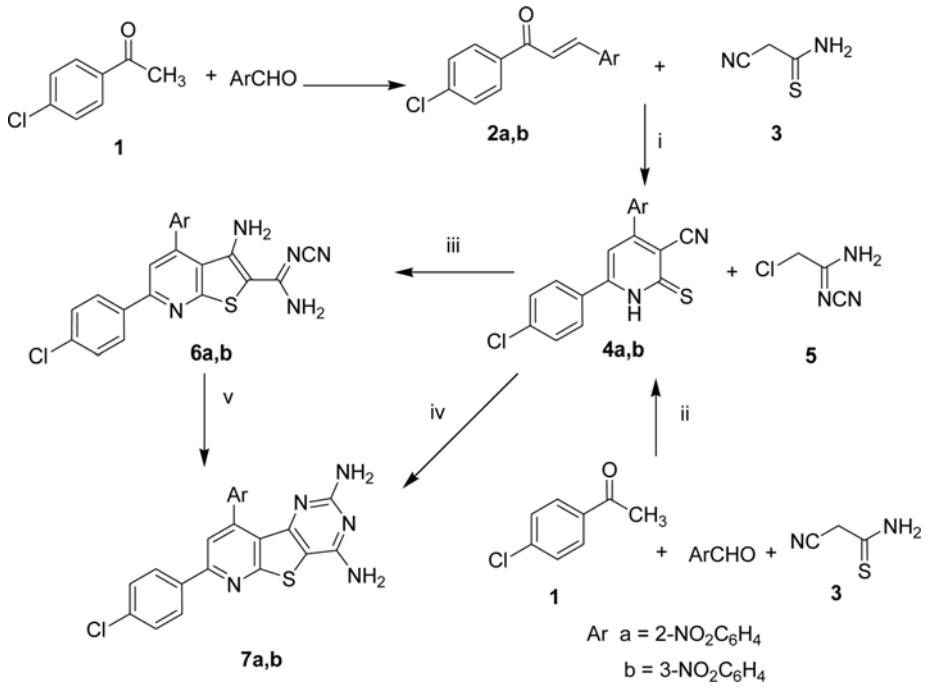
In vitro anticancer screening

The cytotoxicity of the newly synthesized compounds was evaluated *in vitro* against two cell lines, human breast adenocarcinoma cell line (MCF7) and colon carcinoma cell line (HCT 116). For comparison purposes, the cytotoxicity of doxorubicin, which is one of the most effective anticancer agents, was evaluated under the same conditions. The relationship between the surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF-7) and colon carcinoma cell line (HCT 116). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. The IC_{50} of the synthesized compounds, compared to the reference drug, are shown in Table I. From the results in Table I, it is evident that all the tested compounds displayed moderate to potent cancer cell growth inhibition. Generally, all the tested compounds tended to be more active against HCT-116 than against MCF-7 cell lines. Compounds **11b** and **11c** showed the highest potency against HCT116 while compound **11d** was the most active against MCF7 (IC_{50} = 5.95 μM). In particular the thienodipyridine derivative **11b** and **11d** were found to have improved activity against HCT116 (IC_{50} = 6.04, 6.09 μM, respectively). Although the number of tested compounds in this study is limited, some structural features that are important for explanation of their cytotoxic effects can be referred. In general, pyridothienopyrimidine derivatives **7a**,**b** were more potent against MCF-7 than thienodipyridine derivatives **11a-d** (with exception of **11d**) while in case of HCT-116 thienodipyridine derivatives **11a-d** were more effective than **7a**,**b** than pyridothienopyrimidine derivatives **7a**,**b**. Moreover, regarding the pyridothienopyrimidine derivatives **7a**,**b**, the presence of 3-nitrophenyl substituent at position 4 had a better activity against MCF7 than the 2-nitrophenyl analogue (IC_{50} = 11.25, 19.87 μM, respectively). On the other hand, regarding the influence of substitution at position 4 and position 8 on the activity of thienodipyridine derivatives **11a-d**, the presence of phenyl substituent at position 8 and 3-nitrophenyl at position 4, compound **11b**, demonstrated a higher activity to both cell lines compared to that bearing 2-nitrophenyl substituent **11a**. While regarding the replacement of phenyl group at position 8 with 3-nitrophenyl in the presence of 2-nitrophenyl substitution at position 4, **11c** led to significant decrease in activity. The best result was obtained by compound **11d** which bears 3-nitrophenyl group in both positions. On the other hand, in the presence of phenyl substituent at position 8 and 2-nitrophenyl at position 4, compound **11a** was successful inducing selectivity against HCT116 cell line. Further studies are recommended to explore the mechanism of action as well as the effect of substitution on the anticancer effect of these compounds. We hope that the synthesized compounds serve as lead chemical entities for further modification to render them clinically useful drug agents.

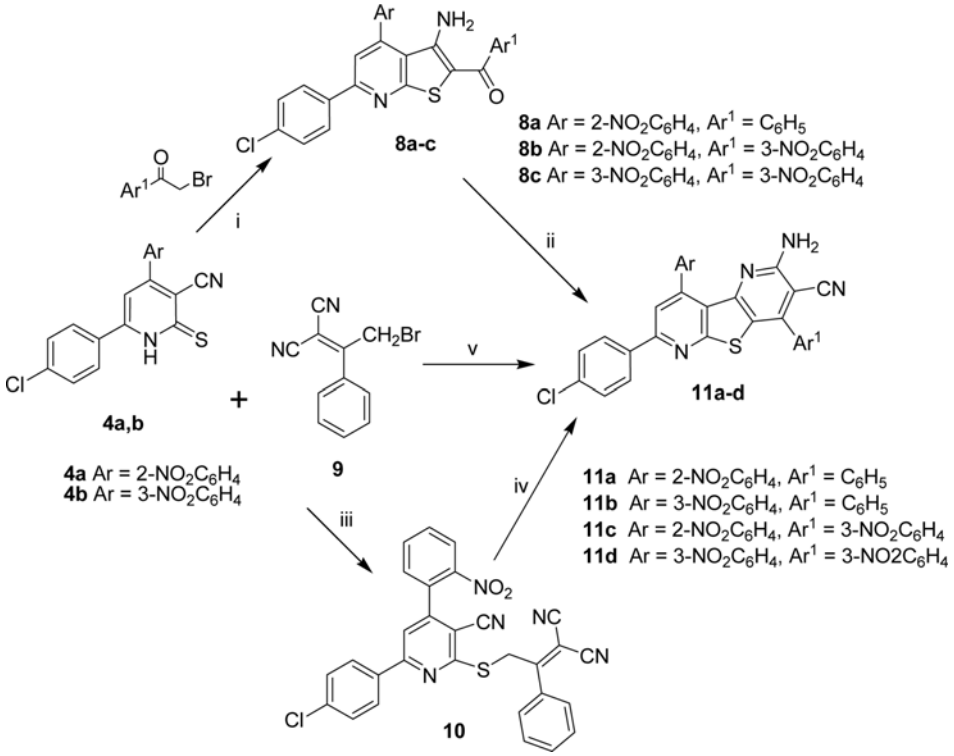
DISCUSSION

Chemistry

The synthetic approaches adopted to obtain the target compounds are outlined in Schemes 1 and 2. The starting compounds, 4-aryl-6-(4-chlorophenyl)-2-thioxo-1, 2-dihydropyridine-3-carbonitriles **4a**,**b** were prepared in a good yield using two pathways. The first pathway involved one pot reaction of *p*-chloroacetophenone **1** with the appropriate aromatic aldehydes and cyanothioacetamide **3** (Howard, 1956) in the presence of ammonium acetate, following the reaction conditions reported for the preparation of related compounds (Fathalla et al., 2002). Whereas the second pathway was adopted via refluxing a mixture of respective chalcone analogues **2a**,**b** and cyanothioacetamide **3** using sodium ethoxide in absolute ethanol, according to the previously described procedure for the preparation of analogous compounds (Vieweg et al., 1989). The derivatives **4a**,**b** were characterized by spectral studies using IR, ^{1}H-NMR and MS. IR spectra of compounds **4a**,**b** showed the characteristic C≡N stretching absorption at υ 2200 cm^{-1}, in addition to NH absorption at υ 3400 cm^{-1}. The ^{1}H-NMR spectra of **4a**,**b**



Scheme 1. Reagent and condition : (i) $NaOC_2H_5$, reflux 7 h; (ii) amm acetate, *n*-butanol, reflux 9 h; (iii) 10% KOH, DMF, stir 2 h; (iv) a-10% KOH, ethanol, stir 10 min; b-$NaOC_2H_5$ reflux 4 h; (v) $NaOC_2H_5$ rreflux 6 h



Scheme 2. Reagent and condition: (i) Dioxane, stir 3, H_2O; (ii) $CH_2(CN)_2$, pyridine, reflux 12 h; (iii) piperidine/ethanol, stir 12 h, 50°C; (iv) piperidine/ ethanol, stir 5 h; (v) C_2H_5ONa, reflux 2 h.

revealed an exchangeable proton (NH) at δ 6.94-6.96 ppm. Further structural evidence stemmed from the mass spectrum of compound **4a**, which corroborates the spectral data and the proposed structure, giving a molecular ion peak with m/z 367.

The regioselective reaction of **4a**,**b** with N-cyano-

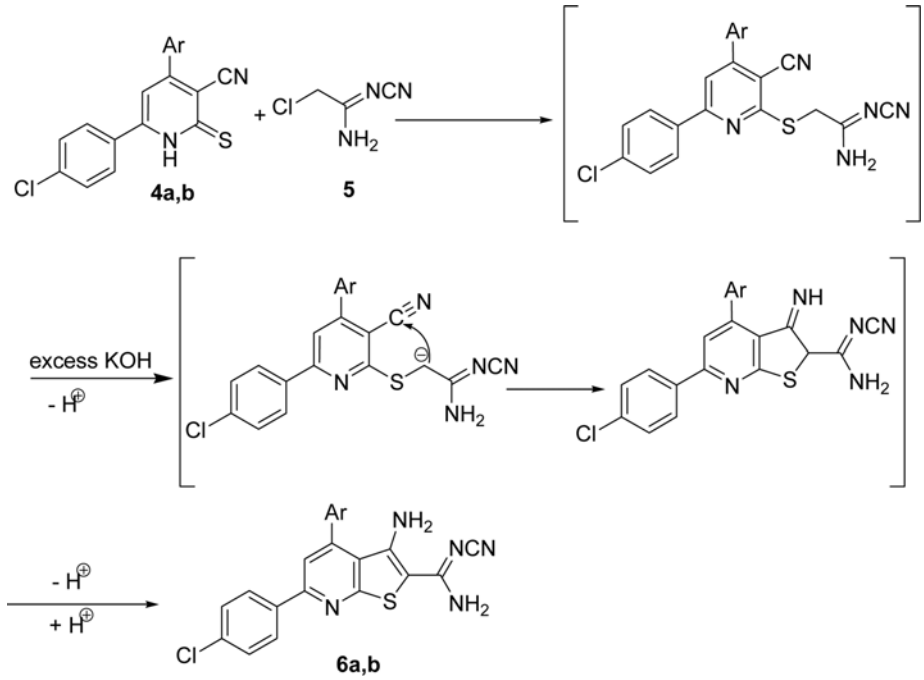


Chart 1. Formation of compounds 6a,b according to Thorpe-Zielger reaction.

chloroacetamidine 5 (Huffman and Schaefer, 1963) in DMF and excess KOH afforded the corresponding 3-amino-2-aminocyaniminomethyl-4-aryl-6-(4-chlorophenyl)thieno[2,3-*b*]pyridines **6a**,**b**. The regioselectivity of the reaction is a consequence of two consecutive reactions, namely nucleophilic substitution and Thorpe-Zielger reaction (Ivanov et al., 1996) (Chart 1).

^{1}H-NMR spectrum of **6a**,**b** revealed the presence of two exchangeable singlet signals corresponding to protons of the amino group at position-3 and amino group of the amidine moiety, respectively. Refluxing the substituted 3-amino-2-aminocyaniminomethyl-thieno[2,3-b] pyridines **6a**,**b** with sodium ethoxide in ethanol produced pyridothienopyrimidinederivatives **7a**,**b** (method A) (Artemov et al., 1996). The latter compounds were also obtained by condensation of **4a**,**b** with N-cyanochloroacetamidine **5** in presence of potassium hydroxide in quantitative amount followed by addition of sodium ethoxide (method B) (Artemov et al., 1994). The structures of compounds **7a**,**b** were confirmed by IR and ^{1}H-NMR spectra. The IR spectra proved useful in tracing the disappearance of the CN stretching absorption of the parent compounds **4a**,**b** and **6a**,**b**. The ^{1}H-NMR spectra of compounds **7a**,**b** displayed two singlet signals D_2O exchangeable at δ 7.60-8.33 and δ 8.48-8.80 corresponding to the two NH_2 groups at position 4 and 2.

One of the principle objectives of the present work was the synthesis of substituted thieno [2,3-*b* :4,5-*b*']dipyridines **11a-d** (Scheme 2). The formation of these condensed pyridines was synthesized by applying Litvinov's synthetic route (Artyomov et al., 1997) which was found to proceed as a result of a sequence of consecutive reactions: nucleophilic substitution at the sulfur atom, Thorpe-Ziegler closure of the thiophene ring to afford the intermediate thienopyridine and finally Thorpe-Guareschi closure of the pyridine ring (Ivanov et al., 1996). This was achieved through three different synthetic routes previously described for the preparation of analogous compounds (Ivanov et al., 1996; Artyomov et al., 1997). The first technique involved the synthesis of intermediate 3-amino-4-aryl-2-arylcarbonyl-6-(4-chlorophenyl)thieno[2,3-*b*]pyridines **8a-c** via stirring 4,6-disubstituted pyridinethiones **4a**,**b** with appropriate phenacyl bromide in the presence of sodium ethoxide. The ^{1}H-NMR spectra of **8a-c** showed a D_2O exchangeable singlet signal at δ 10.46, 9.49 and 8.47 attributed to NH_2, respectively. Besides, the mass spectrum of **8b** displayed molecular ion peaks at m/z 530 and 532 corresponding to (M^+) and (M+2) in ratio 3:1. Condensation of 2-substituted benzoylthieno [2,3-*b*] pyridines **8a-c** with malononitrile in pyridine afforded the desired substituted thieno [2,3-*b*:4,5-*b*'] dipyridines **11a**, **11c** and **11d**.

The second route involved Alkylation of 4,6-disubstituted pyridinethiones **4a**,**b** with 3-bromo -2-phenyl-1,1-dicyanopropene **9** (Mowry, 1945; Gewald, 1965) in the presence of piperidine to produce the intermediate compound **10**. ^{1}H-NMR spectrum of compound **10** displayed the appearance of a singlet signal at δ 3.7 cor-

responding to CH_2 and the disappearance of NH absorption of the precursor **4a**. Cyclization of the latter compound in the presence of piperidine furnished thieno[2,3-*b*:4,5-*b*'] dipyridines **11a**,**b.** The third route involved cyclocondensation of 2-thioxopyridine-3-carbonitriles **4a**,**b** with 3-bromo-2-phenyl-1,1-dicyanopropene **9** in the presence of sodium ethoxide yielding the corresponding thieno [2,3-*b*:4,5-*b*'] dipyridines **11a**,**b**. The ^{1}H-NMR spectra of compounds **11a**,**b** displayed the disappearance of CH_2 absorption of the precursor reagent **9** and the presence of the expected D_2O exchangeable singlet signal at δ 8.67-9.57, which was attributed to NH_2 group. The cascade reactions in this route proceeded under much milder conditions and the yield of thienodipyridine was much higher.

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