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Synthesis, Characterization and Antitumor Activity of Some Novel Pyrimidine Sulfonamide Derivatives

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ABSTRACT

The key intermediate in this study is synthesis of pyrimidine-5-sulfonamides 2a,b. Further modification of starting material by treatment with ethanolamine, anthranilic acid and 3-bromopropanoic acid yielded the condensed pyrimidine derivatives incorporating sulfonamide moiety such as: imidazopyrimidine 3a,b, pyrimidoquinazoline 4a,b and pyrimidothiazine derivatives 5a,b, respectively, in good yields. S-Methylpyrimidine derivatives 6a,b were obtained and let to react either with morpholine and with phosphorous oxychloride to give 7a,b or 8a,b respectively. Compounds 9a,b or 10a,b were obtained via reaction of 8a,b and thiourea or hydrazine hydrate respectively. The condensed tetrazolopyrimidine derivatives 11a,b obtained by cyclocondensation of 10a,b with sodium nitrite and hydrochloric acid. Structures of newly synthesized compounds elucidated by: elemental analysis, IR, NMR and mass spectral data. The antitumor activities evaluated in vitro against four cell lines.

Keywords: Pyrimidine-5-sulfonamides derivatives; Fused pyrimidine systems; *In vitro* antitumor screening; Antitumor activities

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INTRODUCTION

Pyrimidine and fused pyrimidine systems are widely accepted as important moieties in many bioactive promising applications such as antiviral [1,2], antibacterial [3], anti-AIDS [4], anticancer [5], competitive inhibitors [6], antimicrobial [7], anti-plasmodia [8], anti-inflammatory [9], and antiproliferative activity [10]. In addition, in a series of recent publications, sulfonamide derivatives have anticancer activity [11-15]; for example, the anticancer sulfonamide agent E-7010 I [16] interacts with various cellular targets such as disruption of microtubules assembly and carbonic anhydrase inhibition [17,18]. Furthermore, the potency of KD5170 II and PXD101 (Belinostat) III (Figure 1) was observed toward tumor due to presence of NH group of sulfonamide which attached with an aryl group [19,20]. The researchers are in an urgent need to searching for and developing newly synthesizing potential anticancer agents, which should be safer, cheaper and with minimal side effects [21]. All of these classes of compounds target to overcome cancer diseases, which is a group of lethal disease in the world [22,23]. Based on the previous survey and in continuation of our work, we aim to synthesize new thiopyrimidine and its fused sulfonamide derivatives and investigate its antitumor activities against a panel of four (liver; HepG2; breast; MCF7; colon; HCT116; and lung A549). Human solid tumor cell lines and study their structure activity relationship [23-25].

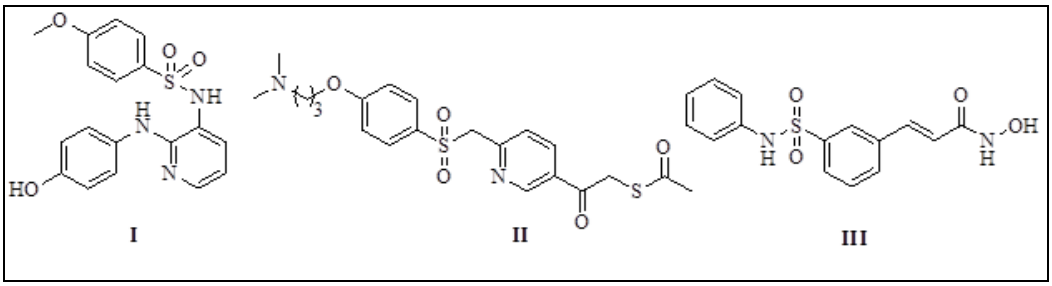


Figure 1: Anticancer drugs with sulfonamide structure

EXPERIMENTAL SECTION

All melting points were determined on a Gallenkamp melting point apparatus. The IR spectra were recorded on a Perkin Elmer 317 Grating IR spectrophotometer, using KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 MHz and 75 MHz, respectively, using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Elemental analyses were carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt. Giza, Egypt. TLC: Merck 0.2 mm silica gel 60 F154 anal aluminium plates. 4-Oxo-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-sulfonyl chloride **1**, was prepared according to the reported method [23].

General Procedure for the Synthesis of Compounds (2a,b)

A mixture of pyrimidine sulfonyl chloride **1** (1 mmol) and a proper amine (1 mmol) namely p-anisidine or 2-nitroaniline in absolute ethanol (10 mL) and pyridine (2 mmol) was heated under reflux for 12 h. The reaction mixture was poured into crushed ice in small portions while stirring. A colored solid mass separated out, which was filtered, washed with water and crystallized from DMF/water (10 mL, 4:1, v/v).

N-(4-Methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (2a):

Yellowish crystals; Yield (241 mg, 77%); mp 263-265°C; IR (KBr): ν_{max} 3320-3140 (3NH), 1690 (CO), 1340 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.90 (s, 3H, OCH_3), 7.21, 7.53 (dd, 4H, Ar-H), 8.10 (s, 1H, pyrimidine H), 8.20 (s, 1H, NH, D_2O-exchangeable), 11.01, 11.50 (2s, 2H, 2NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 177.1, 159.6, 157.4, 154.1, 132.2, 124.0, 123.9, 117.5, 117.1, 117.0, 54.4 ppm. MS: m/z 313 [M^+]; Anal. Calcd for $C_{11}H_{11}N_3O_4S_2$: C, 42.16; H, 3.54; N, 13.41. Found: C, 42.25; H, 3.46; N, 13.27.

N-(2-Nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide (2b):

Yellow solid; Yield (246 mg, 75%); mp 285-287°C; IR (KBr): ν_{max} 3325-3170 (3NH), 1687 (CO), 1348 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.40-7.83 (m, 4H, Ar-H), 8.16 (s, 1H, pyrimidine H), 8.22 (s, 1H, NH, D_2O-exchangeable), 11.10, 11.81 (2s, 2H, 2NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.2, 159.8, 153.7, 138.2, 134.9, 134.1, 127.2, 124.8, 119.2, 117.3 ppm. MS: m/z 328 [M^+]; Anal. Calcd for $C_{10}H_8N_4O_5S_2$: C, 36.58; H, 2.46; N, 17.06. Found: C, 36.47; H, 2.37; N, 17.14.

General Procedure for the Synthesis of Compounds (3a, b)

A mixture of **2a** (1 mmol) or **2b** (1 mmol), ethanolamine (3 mL) in isopropyl alcohol (10 mL) was reflux for 10 h. After cooling, the reaction mixture was poured gradually with stirring on the crushed ice (about 50 g), the solid formed was filtered off, washed with water, dried and crystallized from butanol.

N-(4-Methoxyphenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-sulfonamide (3a):

White solid; Yield (235 mg, 73%); mp 256-258°C; IR (KBr): ν_{max} 3250, 3175 (2NH), 1695 (CO), 1343 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.90-3.14 (m, 2H, CH_2), 3.10-3.36 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 7.20, 7.55 (dd, 4H, Ar-H), 8.12 (s, 1H, pyrimidine H), 8.55 (s, 1H, NH, D_2O-exchangeable), 10.50 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 322 [M^+]; Anal. Calcd for $C_{13}H_{14}N_4O_4S$: C, 48.44; H, 4.38; N, 17.38. Found: C, 48.31; H, 4.30; N, 17.30.

N-(2-Nitrophenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine-6-sulfonamide (3b):

White solid; Yield (226 mg, 67%); mp 287-289°C; IR (KBr): ν_{max} 3270, 3170 (2NH), 1690 (CO), 1346 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.93-3.23 (m, 2H, CH_2), 3.20-3.44 (m, 2H, CH_2), 7.30-7.65 (m, 4H, Ar-H), 8.21 (s, 1H, pyrimidine H), 8.34 (s, 1H, NH, D_2O-exchangeable), 10.77 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 337 [M^+]; Anal. Calcd for $C_{12}H_{11}N_5O_5S$: C, 42.73; H, 3.29; N, 20.76. Found: C, 42.61; H, 3.20; N, 20.84.

General Procedure for the Synthesis of Compounds (4a, b)

A mixture of **2a** (1 mmol) or **2b** (1 mmol) and anthranilic acid (1 mmol) in sodium ethoxide (Na, 2 mmol in 10 mL absolute ethanol) was heated under reflux for 8 h. After cooling, the reaction mixture was poured into acidic ice water and neutralized with HCl (pH ~ 7). The solid was filtered off, washed with water, dried and crystallized from DMF.

N-(4-Methoxyphenyl)-4,6-dioxo-6,11-dihydro-4H-pyrimido[2,1-b]quinazoline-3-sulfonamide (4a):

White solid; Yield (243 mg, 61%); mp 302-304°C; IR (KBr): ν_{max} 3200, 3100 (2NH), 1718, 1695 (2CO), 1601 (C=N), 1340 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.10 (s, 3H, OCH_3), 7.11-7.51 (m, 8H, Ar-H), 8.20 (s, 1H, pyrimidine H), 8.55 (s, 1H, NH, D_2O-exchangeable), 10.50 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 398 [M^+]; Anal. Calcd for $C_{18}H_{14}N_4O_5S$: C, 54.27; H, 3.54; N, 14.06. Found: C, 54.34; H, 3.47; N, 14.14.

N-(2-Nitrophenyl)-4,6-dioxo-6,11-dihydro-4H-pyrimido[2,1-b]quinazoline-3-sulfonamide (4b):

White solid; Yield (289 mg, 70%); mp 290-292°C; IR (KBr): ν_{max} 3220-3110 (2NH), 1720, 1690 (2CO), 1603 (C=N), 1349 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.20-7.76 (m, 8H, Ar-H), 8.10 (s, 1H, pyrimidine H), 8.54 (s, 1H, NH, D_2O-exchangeable), 10.73 (s, 1H, 1NH, D_2O-exchangeable) ppm. MS: m/z 413 [M^+]; Anal. Calcd for $C_{17}H_{11}N_5O_6S$: C, 49.40; H, 2.68; N, 16.94. Found: C, 49.28; H, 2.59; N, 16.82.

General Procedure for the Synthesis of Compounds (5a, b)

A mixture of 2a (1 mmol) or 2b (1 mmol), β-bromopropanic acid (1 mmol), anhydrous sodium acetate (0.3 g) and acetic anhydride (1 mL) in glacial acetic acid (10 mL) was reflux for 2 h. After cooling, the reaction mixture was poured gradually with stirring on the crushed ice (about 30 gm) after the completion of the reaction monitored by TLC. The solid formed was filtered off, washed with water, dried and crystallized from DMF/water (10 mL, 4:1, v/v).

N-(4-Methoxyphenyl)-4,6-dioxo-2,3,4,6-tetrahydropyrimido[2,1-b][1,3]thiazine-7-sulfonamide (5a):

White solid; Yield (268 mg, 73%); mp 220-222°C; IR (KBr): ν_{max} 3222 (NH), 1720, 1690 (2CO), 1602 (C=N), 1340 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.89 (t, 2H, CH_2), 3.20 (t, 2H, CH_2), 4.13 (s, 3H, OCH_3), 7.20, 7.54 (dd, 4H, Ar-H), 8.20 (s, 1H, pyrimidine H), 10.52 (br, 1H, NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 165.2, 157.5, 156.4, 154.2, 154.0, 125.2, 124.3, 119.6, 118.2, 117.2, 55.4, 35.6, 26.7 ppm. MS: m/z 367 [M^+]; Anal. Calcd for $C_{14}H_{13}N_3O_5S_2$: C, 45.77; H, 3.57; N, 11.44. Found: C, 45.65; H, 3.50; N, 11.56.

N-(2-Nitrophenyl)-4,6-dioxo-2,3,4,6-tetrahydropyrimido[2,1-b][1,3]thiazine-7-sulfonamide (5b):

White solid; Yield (264 mg, 69%); mp 233-222°C; IR (KBr): ν_{max} 3220 (NH), 1715, 1690 (2CO), 1602 (C=N), 1347 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.74 (t, 2H, CH_2), 3.14 (t, 2H, CH_2), 7.30-7.63 (m, 4H, Ar-H), 8.12 (s, 1H, pyrimidine H), 10.70 (br, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 382 [M^+]; Anal. Calcd for $C_{13}H_{10}N_4O_6S_2$: C, 40.83; H, 2.64; N, 14.65. Found: C, 40.76; H, 2.73; N, 14.57.

General Procedure for the Synthesis of Compounds (6a, b)

To a solution of **2a** (1 mmol) or **2b** (1 mmol), in sodium ethoxide (Na, 4 mmol in 10 mL absolute ethanol), methyl iodide (1 mmol) was added and refluxed for 3 h. The reaction mixture was cooled and poured on the crushed ice (about 30 g), the solid formed was filtered off, washed with water, dried and crystallized from butanol.

N-(4-Methoxyphenyl)-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-sulfonamide (6a):

Pal yellow crystals; Yield (186 mg, 57%); mp 268-270°C; IR (KBr): ν_{max} 3220, 3150 (2 NH), 1685 (CO), 1342 (S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.70 (s, 3H, CH_3), 4.1 (s, 3H, OCH_3), 7.21, 7.55 (dd, 4H, Ar-H), 8.32 (s, 1H, pyrimidine H), 10.20 (s, 1H, NH, D_2O-exchangeable), 10.51 (s, 1H, NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.3, 166.5, 157.0, 154.6, 132.4, 126.1, 125.5, 121.7, 117.3, 117.0, 55.4, 17.3 ppm. MS: m/z 327 [M^+]; Anal. Calcd for $C_{12}H_{13}N_3O_4S_2$: C, 44.02; H, 4.00; N, 12.84. Found: C, 44.13; H, 4.09; N, 12.76.

2-(Methylthio)-N-(2-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-sulfonamide (6b):

Yellow crystals; Yield (212 mg, 62%); mp 257-259°C; IR (KBr): ν_{max} 3230, 3170 (2NH), 1690 (CO), 1345(S=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.85 (s, 3H, CH_3), 7.31-7.86 (m, 4H, Ar-H), 8.20 (s, 1H, pyrimidine H), 10.31 (s, 1H, NH, D_2O-exchangeable), 10.72 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 342 [M^+]; Anal. Calcd for $C_{11}H_{10}N_4O_5S_2$: C, 38.59; H, 2.94; N, 16.37. Found: C, 38.71; H, 2.88; N, 16.46.

General Procedure for the Synthesis of Compounds (7a, b)

A mixture of 6a or 6b (1 mmol) with morpholine (5 mmol) in methanol (10 mL) was reflux for 15 h. After cooling, the isolated product was filtered off, washed with water, dried and crystallized from DMF.

N-(4-Methoxyphenyl)-2-morpholino-6-oxo-1,6-dihydropyrimidine-5-sulfonamide (7a):

Brownish crystals; Yield (220 mg, 60%); mp 298-300°C; IR (KBr): ν_{max} 3250, 3120 (2NH), 1685 (CO), 1340 (S=O) 1220 (C-O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.90-3.71 (m, 8H, 4 x CH_2), 4.10 (s, 3H, OCH_3), 7.22, 7.57 (dd, 4H, Ar-H), 8.21 (s, 1H, pyrimidine H), 10.22 (s, 1H, NH, D_2O-exchangeable), 10.50 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 366 [M^+]; Anal. Calcd for $C_{15}H_{18}N_4O_5S$: C, 49.17; H, 4.95; N, 15.29. Found: C, 49.29; H, 4.85; N, 15.36.

2-Morpholino-N-(2-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-sulfonamide (7b):

Brownish crystals; Yield (213 mg, 56%); mp 278-280°C; IR (KBr): ν_{max} 3260, 3150 (2NH), 1690 (CO), 1343 (S=O) 1220 (C-O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.80-3.73 (m, 8H, 4 x CH_2), 7.31-7.60 (m, 4H, Ar-H), 8.15 (s, 1H, pyrimidine H), 10.30 (s, 1H, NH, D_2O-exchangeable), 10.52 (s, 1H, NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.3, 165.2, 155.4, 137.9, 136.4, 133.2, 127.8, 125.1,124.3, 120.0, 66.4, 66.3, 46.9, 46.7 ppm. MS: m/z 381 [M^+]; Anal. Calcd for $C_{14}H_{15}N_5O_6S$: C, 44.09; H, 3.96; N, 18.36. Found: C, 44.20; H, 3.87; N, 18.22.

General Procedure for the Synthesis of Compounds (8a, b)

A mixture of 7a or 7b (1 mmol), $POCl_3$ (5 mmol) and PCl_5 (1.5 mmol) was refluxed for 5 h. The reaction mixture was cooled and poured gradually on the crushed ice (about 50 g), the solid formed was filtered off, washed with water, dried and crystallized from DMF/water (5 mL, 4:1, v/v).

4-Chloro-N-(4-methoxyphenyl)-2-(methylthio)pyrimidine-5-sulfonamide (8a):

Brownish crystals; Yield (245 mg, 71%); mp 271-273°C; IR (KBr): ν_{max} 3320 (NH), 1344 (S=O), 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.78 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.22, 7.47 (dd, 4H, Ar-H), 8.20 (s, 1H, pyrimidine H), 10.52 (br, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 345 [M^+]; Anal. Calcd for $C_{12}H_{12}ClN_3O_3S_2$: C, 41.68; H, 3.50; N, 12.15. Found: C, 41.84; H, 3.42; N, 12.27.

4-Chloro-2-(methylthio)-N-(2-nitrophenyl)pyrimidine-5-sulfonamide (8b):

Brownish crystals; Yield (192 mg, 74%); mp 253-255°C; IR (KBr): ν_{max} 3325 (NH), 1346 (S=O), 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 7.30-7.48 (m, 4H, Ar-H), 8.16 (s, 1H, pyrimidine H), 10.40 (br, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 360 [M^+]; Anal. Calcd for $C_{11}H_9ClN_4O_4S_2$: C, 36.62; H, 2.51; N, 15.53. Found: C, 36.49; H, 2.59; N, 15.64.

General Procedure for the Synthesis of Compounds (9a, b)

To a solution of chloropyrimidine 8a or 8b (1 mmol), in absolute ethanol (10 mL), thiourea (1.5 mmol) was refluxed for 10 h. The reaction mixture was cooled and poured onto cold water; the solid formed was filtered off, washed with water, dried and crystallized from DMF.

4-Mercapto-N-(4-methoxyphenyl)-2-(methylthio)pyrimidine-5-sulfonamide (9a):

Yellow crystals; Yield (260 mg,76%); mp 242-244°C; IR (KBr): ν_{max} 3320 (NH), 2320 (SH), 1340 (S=O), 1618 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.83 (s, 3H, CH_3), 4.15 (s, 3H, OCH_3), 7.10 (s, 1H, SH), 7.32, 7.65 (dd, 4H, Ar-H), 8.26 (s, 1H, pyrimidine H), 10.40 (br, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 343 [M^+]; Anal. Calcd for $C_{12}H_{13}N_3O_3S_3$: C, 41.97; H, 3.82; N, 12.23. Found: C, 41.86; H, 3.75; N, 12.15.

4-Mercapto-2-(methylthio)-N-(2-nitrophenyl)pyrimidine-5-sulfonamide (9b):

Brownish crystals; Yield (236 mg, 66%); mp 283-285°C; IR (KBr): ν_{max} 3318 (NH), 2321 (SH), 1342 (S=O), 1620 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.85 (s, 3H, CH_3), 7.12 (s, 1H, SH), 7.23-7.57 (m, 4H, Ar-H), 8.21 (s, 1H, pyrimidine H), 10.40 (br, 1H, NH, D_2O-exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.3, 169.8, 152.6, 144.3,

139.7, 136.0, 130.1, 125.4, 124.7, 122.5, 14.8 ppm. MS: m/z 358 [M^+]; Anal. Calcd for $C_{11}H_{10}N_4O_4S_3$: C, 36.86; H, 2.81; N, 15.63. Found: C, 36.78; H, 2.75; N, 15.85.

General Procedure for the Synthesis of Compounds (10a, b)
A mixture of chloropyrimidine **8a** or **8b** (1 mmol) and hydrazine hydrate (0.5 mL) in butanol (10 mL) was refluxed for 2 h. The reaction mixture was filtered off, and crystallized from DMF/water (5 mL, 4:1, v/v).

2,4-Dihydrazinyl-N-(4-methoxyphenyl)pyrimidine-5-sulfonamide (10a):
Brown crystals; Yield (198 mg, 61%); mp 240-242°C; IR (KBr): ν_{max} 3430-3120 ($2NH_2$, 3NH), 1341 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.45 (s, 3H, OCH_3), 4.41 (br, 4H, $2NH_2$, D_2O-exchangeable), 5.50 (br, 3H, 3NH, D_2O-exchangeable), 7.12, 7.58 (dd, 4H, Ar-H), 7.98 (s, 1H, pyrimidine H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.5, 159.8, 156.7, 156.3, 130.7, 126.0, 125.5, 115.2, 114.7, 108.6, 55.4 ppm. MS: m/z 325 [M^+]; Anal. Calcd for $C_{11}H_{15}N_7O_3S$: C, 40.61; H, 4.65; N, 30.14. Found: C, 40.79; H, 4.54; N, 30.28.

2,4-Dihydrazinyl-N-(2-nitrophenyl)pyrimidine-5-sulfonamide (10b):
Brown crystals; Yield (197 mg, 58%); mp 237-239°C; IR (KBr): ν_{max} 3450-3185 ($2NH_2$, 3NH), 1344 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.83 (br, 4H, $2NH_2$, D_2O-exchangeable), 5.10 (br, 3H, 3NH, D_2O-exchangeable), 7.31-8.10 (m, 5H, Ar-H and pyrimidine H) ppm. MS: m/z 340 [M^+]; Anal. Calcd for $C_{10}H_{12}N_8O_4S$: C, 35.29; H, 3.55; N, 32.93. Found: C, 35.17; H, 3.49; N, 32.82.

General Procedure for the Synthesis of (11a, b)
A solution of sodium nitrite (0.2 mmol) in ice cold water (5 mL) was added to a stirred solution of **10a** or **10b** (1 mmol) in 20% aqueous hydrochloric acid (10 mL), the reaction mixture was stirred at room temperature for 4 h. Then the precipitate was formed, filtered off, and crystallized from DMF.

N-(4-Methoxyphenyl)ditetrazolo[1,5-a:1',5'-c]pyrimidine-6-sulfonamide (11a):
Brown crystals; Yield (187 mg, 54%); mp 266-268°C; IR (KBr): ν_{max} 3200 (NH), 1341 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.45 (s, 3H, OCH_3), 7.31, 7.67 (dd, 4H, Ar-H), 8.13 (s, 1H, pyrimidine H), 10.42 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 347 [M^+]; Anal. Calcd for $C_{11}H_9N_9O_3S$: C, 38.04; H, 2.61; N, 36.30. Found: C, 38.20; H, 2.44; N, 36.43.

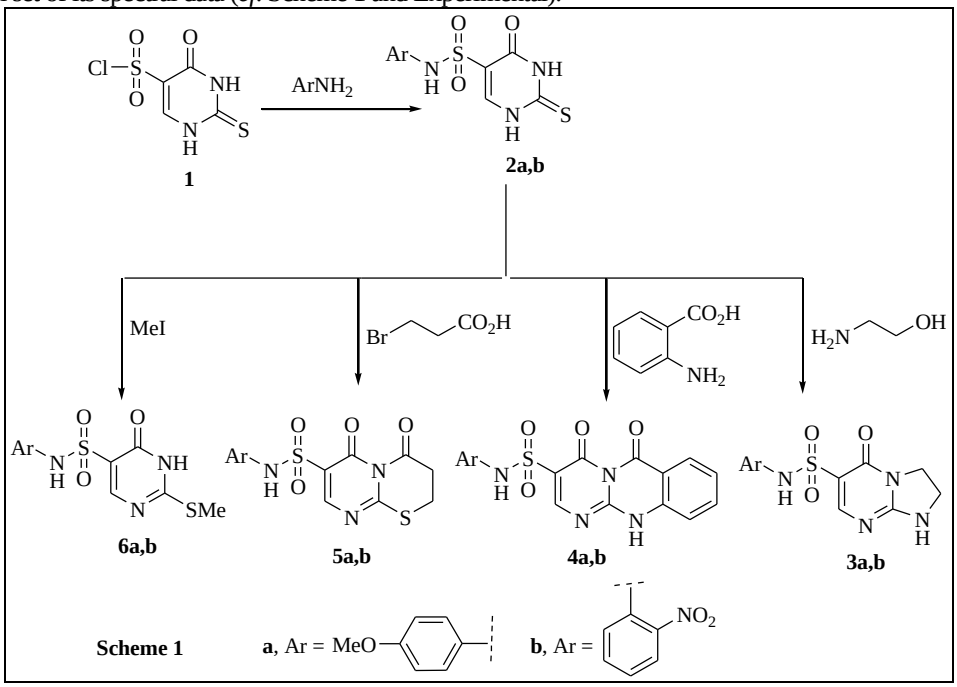
N-(2-Nitrophenyl)ditetrazolo[1,5-a:1',5'-c]pyrimidine-6-sulfonamide (11b):
Brown crystals; Yield (163 mg, 45%); mp 277-279°C; IR (KBr): ν_{max} 3250 (NH), 1346 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.15-7.83 (m, 4H, Ar-H), 8.33 (s, 1H, pyrimidine H), 10.22 (s, 1H, NH, D_2O-exchangeable) ppm. MS: m/z 362 [M^+]; Anal. Calcd for $C_{10}H_6N_{10}O_4S$: C, 33.15; H, 1.67; N, 38.66. Found: C, 33.20; H, 1.72; N, 38.60.

RESULTS AND DISCUSSION

Chemistry
4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonyl chloride [23] is used to prepare a key intermediate by reaction with *p*-anisidine and/or 2-nitroaniline to afford *N*-(substituted phenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide 2a,b in good yield as starting material for the pyrimidine sulfonamide derivatives. The ^{1}HNMR of 2a showed the appearance of new signals at δ = 3.90 ppm due to OCH_3 protons beside the aromatic protons at δ = 7.21, 7.53 ppm. When thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide 2a,b reacted with ethanolamine in isopropyl alcohol at reflux temperature, it gave the corresponding *N*-(substituted phenyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*a*]pyrimidine-6-sulfonamide 3a,b. Thus, the ^{1}HNMR for 3a showed the appearance of new signals of two methylene protons at δ = 2.90-3.14 and 3.10-3.36 ppm corresponding totetrhydroimidazole ring (*cf*. Scheme 1 and Experimental).
In addition, Condensation of 2a,b with anthranilic acid by refluxing in basic sodium ethoxide for 8h yielded pyrimido[2,1-*b*]quinazoline-3-sulfonamide derivatives 4a,b. Also, tetrahydropyrimido[2,1-*b*][1,3]thiazine-7-sulfonamide 5a,b were obtained when compounds 2a,b treated by *β*-bromopropionic acid in presence of anhydrous sodium acetate and acetic anhydride in glacial acetic acid in fairly good yield. The structure of 4a,b was confirmed on the basis of different analyses such as ^{1}HNMR of 4a that revealed presence of singlet at δ = 4.10 (s, 3H, OCH_3), singlet at δ = 8.20 (s, 1H, pyrimidine H), and two exchangeable NH signals at δ = 8.55 and 10.50 ppm. Similarly, ^{1}HNMR for 5a showed appearance of new signals of

methylene protons at δ = 2.89, 3.20 ppm, while the mass spectrum showed ion peak [M^+] at m/z = 367, which fitted the calculated molecular weight ($C_{14}H_{13}N_3O_5S_2$).

Furthermore, refluxing of compounds 1a,b with methyl iodide in the presence of sodium ethoxide, the corresponding *S*-methylpyrimidine sulfonamide derivatives 6a,b were obtained. The structure of the new compounds 6a,b is assigned on the basis of the full set of its spectral data (*cf.* Scheme 1 and Experimental).



Scheme 1: Totetrhydroimidazole ring and spectral data

In continuation of our study, we were exploring functionalization of compounds 6a,b that serve as building blocks in the synthesis of various bioactive molecules. Compounds 6a,b was refluxed for 15h with morpholine in methanol *via* nucleophilic substitution to afford the corresponding morpholinopyrimidine derivatives 7a,b which confirmed on the basis of various analytical and spectroscopic analyses. The ^{1}H NMR for 7a showed disappearance of the signals due to SCH_3-groups protons and appearance of new signals of methylene protons at δ = 2.90-3.71 ppm, while the mass spectrum showed ion peak [M^+] at *m/z* = 366 which fitted the calculated molecular weight ($C_{15}H_{18}N_4O_5S$) (*cf.* Scheme 2 and Experimental).

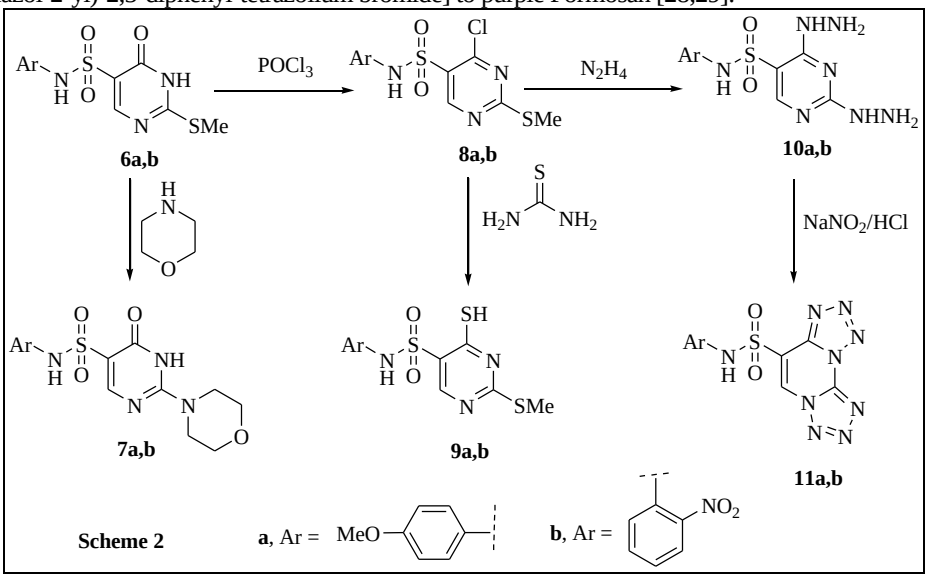
Additionally, when compounds 6a,b were refluxed in phosphoryl chloride, the corresponding 4-chloropyrimidine derivatives 8a,b were obtained. The IR of compounds 8a,b showed absence of pyrimidine carbonyl group that detected previously in the parent compound. Finally, 4-thiolpyrimidines derivatives 9a,b or 4-hydrazino pyrimidine derivatives 10a,b were obtained via treatment of the previous 4-chloropyrimidine derivatives 8a,b with thiourea and/or hydrazine hydrate respectively, in good yield. The IR spectrum of compounds 9a showed thiol group stretching at 2320 cm^{-1}. In addition, the ^{1}H NMR showed thiol group at δ = 7.10 ppm and NH group (D_2O exchangeable protons) at δ = 10.40 ppm along with the other protons in their expected location supported the suggested structure (*cf.* Scheme 2 and Experimental).

Moreover, ditetrazolo-pyrimidine-6-sulfonamides 11a,b were synthesized in low yield by treatment of 10a,b with sodium nitrite and hydrochloric acid, the structure of the products was confirmed on the basis of their elemental analyses and spectral data. The IR spectrum of compound 11a revealed an absorption band at ν3200 cm^{-1} characteristic to NH group with disappearance of the absorption band of NH_2 detected previously in the parent. Its ^{1}H NMR spectrum revealed three singlet signals at δ 3.45, 8.13 and 10.42 ppm assignable to OCH_3 group, CH of pyrimidine ring and NH protons, respectively, besides aromatic protons at δ 7.31, 7.67 ppm. (*cf.* Experimental).

In vitro Antitumor Screening

The synthesized compounds 2-20 were subjected to *in vitro* antitumor screening against human cancer cell lines using cell based disease oriented approach [26,27]. The tested compounds were used to evaluate their antitumor potency on four human tumor cell lines namely: hepatocellular carcinoma HepG2, caucasian breast adenocarcinoma MCF7, colon carcinoma

HCT116, and lung carcinoma A549. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] to purple Formosan [28,29].



Scheme 2: Disappearance of the signals

The antitumor drug doxorubicin was used as positive control. In regard to the antitumor selectivity among the used tumor cell lines, compounds 3b, 5a and 6b proved to be selective toward A549 cell line with inhibition percentage values of 60.1, 67.5 and 52.4%, respectively; while compound 11a proved to be selective toward MCF7 cell line with inhibition percentage value of 70.5%; while compound 3b and 11a proved to be selective toward HCT116 cell line with inhibition percentage value of 65.1 and 61%, respectively; while compound 5b, 6a and 7b proved to be selective toward HepG2 cell line with inhibition percentage value of 76.5, 55.7 and 78%, respectively. Compounds 6a and 11a exhibited antitumor potency comparable to the standard drug doxorubicin, where, 6a showed inhibition percentage values of 82.6% against HCT116 cell line; while compound 11a exhibited inhibition percentage values of 93.9% toward HepG2 cell line (*cf*. Table 1).

Table 1: Inhibition percentage value of the most active antitumor compounds

Code	A-549	MCF-7	HCT-116	HepG2
2a	-	14.4	-	12.6
2b	13.4	26.2	14.6	-
3a	4.1	23.9	-	3.1
3b	60.1	38.6	65.1	45.3
4a	5.6	9.3	-	-
4b	19.01	4.9	15.8	7.9
5a	67.5	33.7	38.1	18.8
5b	17.7	20.4	-	76.5
6a	13.7	8.8	82.6	55.7
6b	52.4	38.5	20.2	4.4
7a	30.6	9.6	-	11.6
7b	29.3	-	-	78
11a	46.2	70.5	61	93.9
11b	38.4	8	-	17.8

Results at 10 ppm: A-549: Lung; Mcf-7: Brest; Hct-116: Colon; HepG2: Liver

SAR (Structure Activity Relationship) showed high or moderate antitumor activity toward some of the tested compounds with inhibition percentage value ranging from 4.9 -70.5% for breast cell line (MCF-7), 3.1-93.9% for liver cell line (HepG2), 4.1-67.5% for lung cell line (A-549) and 14.6-82.6% for colon cell line (HCT-116) compared with both standard drugs.

Pyrimidine sulfonamide with fused ring contributing bridgehead nitrogen atom such as, ditetrazolo-pyrimidine, tetrahydropyrimido[2,1-*b*][1,3]thiazine**,** and tetrahydroimidazo[1,2-*a*]pyrimidine are more potent than non-fused pyrimidine sulfonamide such as, *S*-methylpyrimidine and morpholinopyrimidine; this may be due to presence of phenyl ring carrying substituent at *para*-position. Compound 11a was highly specific for the four cell lines and shown to be vital for potency

against HepG2, while compound 6a shown potent activity against HCT-116 cell line. Cytotoxicity assay results showed that compounds 6a and 11a were the most potent anticancer agents with IC_{50} between 8.88 and 19.25 μM against four different human cancer cell lines.

CONCLUSION

A new class of substituted pyrimidine sulfonamides were synthesized and their structures confirmed on the basis of elemental and spectroscopic analyses. In addition, their antitumor activity was evaluated toward a panel of four carcinoma cell lines compared with Doxorubicin as standard drug. Compounds 3b, 5a, 6a, 7b and 11a revealed moderate to high antitumor activities and SAR study showed high or moderate anti-tumor activity of some tested compounds with inhibition value ranging from 4.9-70.5% for breast cell line (MCF-7), 3.1-93.9% for liver cell line (HepG2), 4.1-67.5% for lung cell line (A-549) and 14.6-82.6% for colon cell line (HCT-116). Moreover, compounds 6a and 11a were the most potent anticancer agents with IC_{50} between 8.88 and 19.25 μM against four different human cancer cell lines compared with the standard drug.

Anticancer Evaluation

Cell culture:

A549 (Lung carcinoma), HepG2 (human Liver c arcinoma), MCF7 (Human Breast Cancer) and HCT-116 (Human Colon Cancer) cell lines were obtained from Karolinska Institute, Stockholm, Sweden. All cells were maintained in RPMI 1640 (LonzaBiowahittkar) medium, except for MCF7 cancer cells that were maintained in DMEM medium (LonzaBiowahittkar). All the media were supplemented with 1% antibiotic-antimycotic mixture (10,000 u mL^{-1} Potassium Penicillin (Biowest), 10,000 µg mL^{-1} Streptomycin Sulfate (Biowest), 25 µg mL^{-1} Amphotericin B (Biowest) and 1% L-glutamine (Biowest).

MTT cytotoxicity assay:

The cell viability was investigated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [28,29]. This reaction depends on the mitochondrial dependant reduction of yellow MTT into purple formazan. All the preceding steps were carried out in sterile laminar air flow cabinet Biosafty class II level (Baker, SG403INT, Sanford, ME, USA). All incubations were done at 37°C in 5% CO_2 incubator in humidified atmosphere (Sheldon, TC2323, Cornelius, OR, USA). Cells were seeded into 96-well microtiter plastic plates at the concentration of (104 cells per well) and allowed to adhere for 24 h. Media were aspirated and fresh medium (without serum) was added to the cells with various concentrations of the tested compounds (10, 5, 2.5 and 1.25 µg mL^{-1}) and incubated for 48 h. For the untreated cells (negative control), media were added instead of the tested compounds. A positive control Adrinamycin (Doxorubicin) [Mw = 579.99] was used as appositive control. Medium was aspirated and 40 µL MTT salt (2.5 µg mL^{-1}) (BiobasicInc) was added to each well and incubated for further 4 h. To stop the reaction and dissolve any formed formazan crystals, 200 µL of 10% sodium dodicyl sulfate (SDS) added to each well and incubated overnight at 37°C. The amount of formazan product measured at 595 nm and a reference λ 620 nm as a background using a microplate reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA).

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