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Synthesis and Anticancer Activity of Certain Fused Pyridazine Derivatives

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ABSTRACT

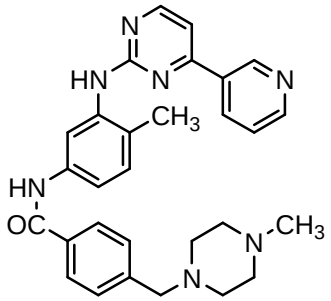
*Novel series of pyridazine containing compounds were synthesized through the reaction of N-carbamimidoyl-4-[(6 chloropyridazin-3-yl) amino]benzenesulphonamide **1** with either 2-aminophenol, acetophenone phenylhydrazone derivatives, anthranilic acid derivatives, thiosemicarbazide or substituted benzoyl hydrazide to afford the corresponding derivatives **2, 3a-c, 4a-d, 5** and **6a-c** respectively. The structure of the newly synthesized compounds was characterized by spectroscopic means and elemental analysis. All the synthesized compounds were screened for their anticancer activity in vitro on colon cancer cell line (HCT-116) and breast cancer cell line (MCF-7). Compound **4a** showed potent anticancer activity against colon cancer cell line (HCT-116) with IC_{50} 11.90 μM in comparison to imatinib (Gleevec ®).*

Keywords: Pyridazine, Colon cancer, Breast cancer, Cytotoxic activity.

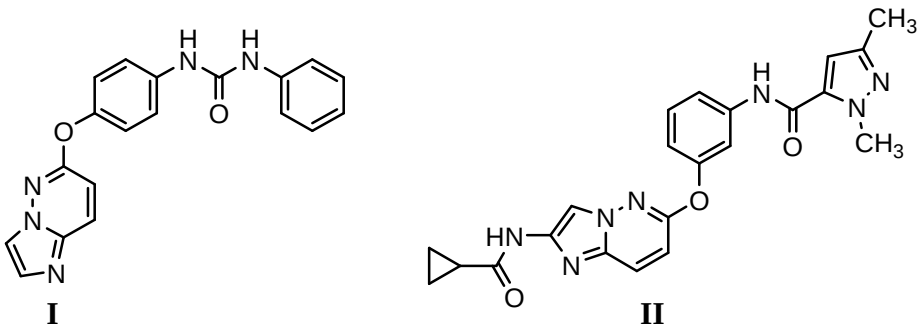
INTRODUCTION

Cancer is one of the most tremendous threats to the well being of mankind [1]. Surgery and radiation are used to treat cancer that is considered locally, where as drug therapy is essential to kill cancer cells that have spread to distant sites in the body [2]. But, cancer chemotherapy is limited by a lack of specificity resulting in damage to not only cancer cells but also normal cells [3]. Hence, the optimum goal is to find a treatment modality that specifically kills malignant cells and causes little or no side effects to normal cells. Therefore, targeted therapy was developed to target key elements that play a role in tumor development and tumor growth [4]. Targeted therapy in oncology consists of drugs that specifically interfere with abnormal signalling pathways that are dysregulated in cancer cells. Thus tyrosine kinase inhibitors (TKI) have become more widespread in use as targeted therapy for a variety of malignancies and offer excellent target for selective inhibition. These agents potentially provide low toxicity in comparison with conventional cytotoxic chemotherapy [5]. The receptor tyrosine kinase family includes receptors for many growth factors such as epidermal growth factor (EGF), platelet- derived growth factor (PDGF) and vascular endothelial growth factor (VEGFR) [6]. Neoplastic cells secrete VEGFs by themselves to stimulate new vessel formation, thus providing oxygen and nutrients to the tumor and also allowing access to the circulation to facilitate metastasis [7]. The process in which new blood vessels are formed from existing vessels is called angiogenesis. [8]. Angiogenesis plays an important role in the growth and metastasis of solid tumors. [9]. Antiangiogenic therapy is a novel field of cancer therapy, compared with traditional chemotherapies [1]. The first TKI to be used clinically is imatinib (Gleevec®) (Fig. 1). It was approved for the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors [10]. Imatinib displays good inhibitory activity for the kinase domain of the VEGFR [11].

From another point of view, some imidazopyridazine derivatives **I** and **II** were reported to have an inhibitory activity against VEGFR (Fig. 2) [12, 9].



Imatinib (Gleevec®)
Fig. 1. An example of VEGFR kinase inhibitor



I **II**

Fig. 2. Structures of a reported imidazopyridazine derivatives I and II showing potent inhibitory activity against VEGFR

The promising anticancer activity observed by pyridazine derivatives encouraged us to synthesize new derivatives.

MATERIALS AND METHODS

Chemistry

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using Varian Mercury-300 (300 MHz or 400 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Micro Analytical Center, Cairo University, Egypt and Micro Analytical Unit, Faculty of Pharmacy, Cairo University, Egypt. Coupling patterns are described as follows: s, singlet, d, doublet, t, triplet, m, multiplet. J describes a coupling constant Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Micro Analytical Center, Cairo University, Egypt and Micro Analytical Center, Al-Azhar University, Egypt. Elemental analyses were carried out at the Micro Analytical Center, Al-Azhar University, Egypt. Progress of the reactions was monitored using TLC sheets pre-coated with UV fluorescent silica gel Merck 60F 254 using Toluene/ethanol (4.5: 0.5) and were visualized using UV lamp. All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

N-Carbamimidoyl-4-[(6 chloropyridazin-3-yl) amino]benzenesulphonamid **(1)** was prepared as reported [13].

General procedure for the preparation of 2

An equimolar amount of **1** (0.326 g, 0.001 mol) and 2-aminophenol (0.111 g, 001 mol) in 5 mL DMF was heated under reflux for 24 h. The reaction mixture was cooled, poured on ice/cold water. The formed solid was filtered, dried and recrystallized from ethanol.

N-Carbamimidoyl-4-(pyridazino[1,6-*a*]benzimidazo-2-ylamino)benzenesulphonamide (2)

Yield: 0.10 g (26.31%); mp: 230-232 ^{o}C; IR (KBr, cm^{-1}): 3417, 3325, 3197 (NH_2, 3 N-H), 1315 and 1134 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.64 (s, 2H, NH_2, D_2O exchangeable), 6.69-8.25 (m, 8H, Ar-H), 7.72 (d, 1H, pyridazine-H, *J*=8.88 Hz), 7.82 (d, 1H, pyridazine-H, *J*=8.88 Hz), 9.13 (s, 1H, NH, D_2O exchangeable), 9.28 (s, 1H, NH, D_2O exchangeable), 9.39 (s, 1H, NH, D_2O exchangeable); MS: m/z 382.00 $[M+1]^+$ (23.50%), 381.00 (M^+., 24.79%); Anal. Calcd for $C_{17}H_{15}N_7O_2S$ (381.41) (%): C, 53.53; H, 3.96; N, 25.71. Found (%): C, 53.71; H, 3.98; N, 25.94.

General procedure for the preparation of (3a-c)
A mixture of **1** (0.326 g, 0.001 mol) and the appropriate acetophenone phenylhydrazone derivative (0.001 mol) was heated at 160-180 ^{o}C for 7 h. The residue was boiled with different polar and non polar solvents and the solid was collected.

***N*-Carbamimidoyl-4-[(3-methyl-2,3-diphenyl-2,3-dihydro[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)amino] benzene sulphonamide (3a)**
Yield: 0.23 g (46.93%); mp: 270-272 ^{o}C; IR (KBr, cm^{-1}): 3348, 3336, 3169 (NH_2, 3 N-H), 2937-2856 (CH aliphatic), 1327 and 1172 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.75 (s, 3H, CH_3), 7.00 (s, 2H, NH_2, D_2O exchangeable),7.01-7.80 (m, 10H, Ar-H), 7.12 (d, 2H, Ar-H, *J*=7.20 Hz), 7.22 (d, 2H, Ar-H, *J*=7.20 Hz), 7.34 (d, 1H, pyridazine-H, *J*=8.10 Hz), 7.54 (d, 1H, pyridazine-H, *J*=8.10 Hz), 8.20 (s, 1H, NH, D_2O exchangeable), 9.00 (s, 1H, NH, D_2O exchangeable), 10.00 (s, 1H, NH, D_2O exchangeable); MS: m/z 502.00 $[M+2]^+$ (43.48%), 500.00 (M^+., 35.40%) . Anal. Calcd for $C_{25}H_{24}N_8O_2S$ (500.58) (%): C, 59.98; H, 4.83; N, 22.38. Found (%): C, 60.09; H, 4.87; N, 22.52.

***N*-Carbamimidoyl-4-[(3-methyl-2-phenyl-3-(4-chlorophenyl)-2,3-dihydro[1,2,4]triazolo [4,3-*b*]pyridazin-6-yl) amino]benzenesulphonamide (3b)**
Yield: 0.37 g (69.81%); mp: 228-230 ^{o}C; IR (KBr, cm^{-1}): 3350, 3255, 3180 (NH_2, 3 N-H), 2962-2800 (CH aliphatic), 1325 and 1153 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 6.98-7.85 (m, 13H, Ar-H), 7.00 (s, 2H, NH_2, D_2O exchangeable),7.02 (d, 1H, pyridazine-H, *J*=9.00Hz), 7.36 (s, 1H, NH, D_2O exchangeable), 7.51 (d, 1H, pyridazine-H, *J*=9.00Hz), 9.60 (s, 1H, NH, D_2O exchangeable), 10.00 (s, 1H, NH, D_2Oexchangeable); MS: m/z 536 $[M+2]^+$ (76.47%), 534 (M^+ ., 50.98%), 233 (53.92%). Anal. Calcd for $C_{25}H_{23}ClN_8O_2S$ (535.02) (%): C, 56.12; H, 4.33; N, 20.94. Found (%): C, 56.21; H, 4.39; N, 21.09.

***N*-Carbamimidoyl-4-[(3-methyl-2-phenyl-3-(4-bromophenyl)-2,3-dihydro[1,2,4]triazolo [4,3-*b*]pyridazin-6-yl) amino]benzenesulphonamide (3c)**
Yield: 0.41 g (71.92%); mp: 236-238 ^{o}C; IR (KBr, cm^{-1}): 3367, 3356-3331 (NH_2, 3 N-H), 2910-2839 (CH aliphatic), 1327 and 1176 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 7.14 (s, 2H, NH_2, D_2O exchangeable), 7.22-7.77 (m, 9H, Ar-H), 7.54 (d, 2H, Ar-H, *J*=6.90 Hz), 7.59 (d, 2H, Ar-H, *J*=6.90 Hz),.7.61 (d, 1H, pyridazine-H, *J*=8.10 Hz), 7.66 (d, 1H, pyridazine-H, *J*=8.10 Hz), 7.80 (s, 1H, NH, D_2O exchangeable), 7.91 (s, 1H, NH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable); MS: m/z 580.00 $[M+2]^+$ (47.90%), 578.00 (M^+., 62.18%). Anal. Calcd for $C_{25}H_{23}BrN_8O_2S$ (579.47) (%): C, 51.82; H, 4.00; N, 19.34. Found (%): C, 51.97; H, 4.03; N, 19.51.

General procedure for the preparation of (4a-d)
A solution of **1** (0.326g, 0.001 mol) and the selected anthranilic acid derivative (0.001 mol) in 20 mL *n*-butanol was heated under reflux for 24 h. The reaction mixture was concentrated under reduced pressure then the formed solid was filtered, dried and recrystallized from the appropriate solvent.

***N*-Carbamimidoyl-4-[(10-oxo-10H-pyridazino[6,1-*b*]quinazolin-2-yl)amino] benzenesulphonamide (4a)**
Yield: 0.10 g (25.00%); solvent of crystallization ethanol, mp: 250-252 ^{o}C; IR (KBr, cm^{-1}): 3421, 3329, 3201 (NH_2, 3 N-H), 1660 (C=O), 1338 and 1138 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.76 (s, 2H, NH_2, D_2O exchangeable), 6.85-8.35 (m, 4H, Ar-H), 7.58 (d, 2H, Ar-H, *J*=7.64 Hz), 7.64 (d, 1H, pyridazine-H, *J*=8.80 Hz), 7.75 (d, 1H, pyridazine-H, *J*=8.80 Hz), 7.78 (d, 2H, Ar-H, *J*=7.64 Hz), 9.40 (s, 1H, NH, D_2O exchangeable), 9.94 (s, 1H, NH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO, 75 MHz) δ ppm: 118.23, 120.02, 121.40, 127.32, 127.42, 127.57, 128.33, 130.04, 137.00, 141.48, 143.38, 148.61, 150.23, 157.00 (Ar-C), 158.23 (C=NH), 158.38 (C=O); MS: m/z 410.10 $[M+1]^+$ (85.26%), 409.10 (M^+., 77.89%). Anal. Calcd for $C_{18}H_{15}N_7O_3S$ (409.42) (%): C, 52.80; H, 3.69; N, 23.95. Found (%): C, 52.94; H, 3.71; N, 24.08.

***N*-Carbamimidoyl-4-[(7,8-dimethoxy-10-oxo-10H-pyridazino[6,1-*b*]quinazolin-2-yl)amino]benzenesulphonamide (4b)**
Yield: 0.22 g (47.82%); solvent of crystallization benzene/ethanol, mp: 104-106 ^{o}C; IR (KBr, cm^{-1}): 3425, 3419, 3323, 3207 (NH_2, 3 N-H), 2958-2837 (CH aliphatic), 1660 (C=O), 1327 and 1132 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.99 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.58 (d, 1H, pyridazine-H, *J*=8.60 Hz), 6.97-7.68 (m, 6H, Ar-H), 7.02 (d, 1H, pyridazine-H, *J*=8.60 Hz), 7.74 (s, 1H, NH, D_2O exchangeable), 9.52 (s, 1H, NH, D_2O exchangeable); 9.36 (s, 1H, NH, D_2O exchangeable); MS: m/z 471.00 $[M+2]^+$ (57.78%), 469 (M^+., 87.78%); Anal. Calcd for $C_{20}H_{19}N_7O_5S$ (469.47) (%): C, 51.17; H, 4.08; N, 20.88. Found (%): C, 51.43; H, 4.16; N, 21.19.

***N*-Carbamimidoyl-4-[(9-iodo-10-oxo-10H-pyridazino[6,1-*b*]quinazolin-2-yl)amino]benzenesulphonamide (4c)**
Yield: 0.24 g (55.81%); solvent of crystallization ethanol, mp: 118-120 ^{o}C; IR (KBr, cm^{-1}): 3427, 3332, 3205 (NH_2, 3 N-H), 1674 (C=O), 1327 and 1134 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.62-8.60 (m, 3H, Ar-H), 6.81 (d, 1H, pyridazine-H, *J*=8.32 Hz), 7.17 (s, 2H, NH_2, D_2O exchangeable), 7.54 (d, 2H, Ar-H, *J*=7.08 Hz), 7.68 (d, 1H, pyridazine-H, *J*=8.32 Hz), 7.98 (d, 2H, Ar-H, *J*=7.08 Hz), 9.40 (s, 1H, NH, D_2O exchangeable), 9.94 (s, 1H, NH, D_2O exchangeable), 10.34 (s, 1H, NH, D_2O exchangeable); MS: m/z 537.90 $[M+2]^+$ (19.94%), 535.90 (M^+., 17.60%). Anal. Calcd for $C_{18}H_{14}IN_7O_3S$ (535.32) (%): C, 40.39; H, 2.64; N, 18.32. Found (%): C, 40.61; H, 2.61; N, 18.48.

***N*-Carbamimidoyl-4-[(10-oxo-10H-pyrido[2′,3′,4,5]pyrimido[1,2-*b*]pyridazin-2-yl)amino]benzenesulphon - amide (4d)**
Yield: 0.17 g (42.50%); solvent of crystallization ethanol, mp: 176-178 ^{o}C; IR (KBr, cm^{-1}): 3429, 3332, 3246 (NH_2, 3 N-H), 1710 (C=O), 1321 and 1136 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.59 (s, 2H, NH_2, D_2O exchangeable), 6.61-8.28 (m, 3H, Ar-H), 6.74 (d, 2H, Ar-H, *J*=7.72 Hz), 6.75 (d, 2H, Ar-H, *J*=7.72 Hz), 7.43 (d, 1H, pyridazine-H, *J*=8.60 Hz), 7.87 (d, 1H, pyridazine-H, *J*=8.60 Hz), 9.94 (s, 1H, NH, D_2O exchangeable), 10.60 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO, 75 MHz) δ ppm: 107.77, 112.33, 112.45, 112.90, 127.72, 131.30, 133.07, 135.21, 141.02, 142.44, 150.77, 151.72,158.23 (Ar-C),158.51 (C=NH), 168.33 (C=O); MS: m/z 410.22 (M^+., 0.36%). Anal. Calcd for $C_{17}H_{14}N_8O_3S$ (410.41) (%): C, 49.75; H, 3.44; N, 27.30. Found (%): C, 49.98; H, 3.42; N, 27.53.

General procedure for the preparation of 5
An equimolar amount of **1** (0.326 g, 0.001 mol) and thiosemicarbazide (0.091 g, 0.001 mol) in 20 mL ethanol was heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure then the formed solid was filtered, dried and recrystallized from ethanol.

***N*-Carbamimidoyl-4-[(3-amino[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)amino] benzenesulphonamide (5)**
Yield: 0.29 g (85.29%); mp: 170-172 ^{o}C; IR (KBr, cm^{-1}): 3442, 3392, 3340, 3307, 3255, 3224 (2 NH_2, 3 N-H), 1244 and 1136 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.95-8.28 (m, 4H, Ar-H), 6.80 (d, 1H, pyridazine-H, *J*=8.60 Hz), 7.15 (s, 2H, NH_2, D_2O exchangeable), 7.54 (d, 1H, pyridazine-H, *J*=8.60 Hz), 8.94 (s, 1H, NH, D_2O exchangeable), 9.26 (s, 1H, NH, D_2O exchangeable), 9.48 (s, 1H, NH, D_2O exchangeable), 9.82 (s, 2H, NH_2, D_2O exchangeable); ^{13}CNMR (DMSO, 75 MHz) δ ppm: 117.60, 124.11, 126.65, 127.42, 128.25, 133.99, 135.85, 145.43, 150.86, (Ar-C), 157.23 (C=NH); MS: m/z 347 (M^+., 0.09%)Anal. Calcd for $C_{12}H_{13}N_9O_2S$ (347.36) (%): C, 41.49; H, 3.77; N, 36.29. Found (%): C, 41.63; H, 3.81; N, 36.57.

General procedure for the preparation of (6a-c)
An equimolar amount of **1** (0.326 g, 0.001 mol) and substituted benzoyl hydrazide (0.001 mol) in 20 mL ethanol **(6a** and **6b)** and in *n*-butanol **(6c)** was heated under reflux for 35 h. The reaction mixture was concentrated under reduced pressure then the formed solid was filtered, dried and recrystallized from aqueous ethanol.

***N*-Carbamimidoyl-4-[(3-phenyl[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)amino] benzenesulphonamide (6a)**
Yield: 0.12 g (30.00%); mp: 160-162 ^{o}C; IR (KBr, cm^{-1}): 3452, 3433, 3371, 3331, 3217, (NH_2, 3 N-H), 1244 and 1130 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.68 (s, 2H, NH_2, D_2O exchangeable), 6.53 (d, 1H, pyridazine-H, *J*=8.52 Hz), 6.59 (s, 1H, NH, D_2O exchangeable), 7.08-8.56 (m, 5H, Ar-H), 7.37 (d, 1H, pyridazine-H, *J*=8.52 Hz), 7.44 (d, 2H, Ar-H, *J*=7.56 Hz), 7.81 (d, 2H, Ar-H, *J*=7.56 Hz), 9.29 (s, 1H, NH, D_2O exchangeable), 10.62 (s, 1H, NH, D_2O exchangeable), ^{13}CNMR (DMSO, 75 MHz) δ ppm: 112.80, 116.96, 127.70, 127.96, 128.78, 128.97, 129.06, 129.99, 131.27, 132.41, 148.03, 151.83, (Ar-C), 158.28 (C=NH), 166.74 (Ar-C); MS: m/z 410.15 $[M+2]^+$ (3.18%), 408.10 (M^+., 9.25%). Anal. Calcd for $C_{18}H_{16}N_8O_2S$ (408.44) (%): C, 52.93; H, 3.95; N, 27.43. Found (%): C, 53.17; H, 3.99; N, 27.71.

***N*-Carbamimidoyl-4-[(3(4-methylphenyl)[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)amino]benzenesulphonamide(6b)**
Yield: 0.21 g (50.00%); mp: 148-150 ^{o}C; IR (KBr, cm^{-1}): 3431, 3392, 3346, 3219 (NH_2, 3 N-H), 2993-2854 (CH aliphatic), 1236 and 1134 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 6.56 (d, 1H, pyridazine-H, *J*=8.56 Hz), 6.63 (s, 2H, NH_2, D_2O exchangeable), 6.96-8.54 (m, 8H, Ar-H), 7.39 (d, 1H, pyridazine-H, *J*=8.56 Hz), 9.96 (s, 1H, NH, D_2O exchangeable), 10.53 (s, 1H, NH, D_2O exchangeable), 11.44 (s, 1H, NH, D_2O exchangeable), ^{13}CNMR (DMSO, 75 MHz) δ ppm: 21.49 (CH_3), 113.20, 116.96, 123.01, 127.60, 127.78, 127.97, 128.16, 129.48, 129.97, 130.02, 142.42, 147.97, (Ar-C), 158.11 (C=NH), 166.62 (Ar-C); MS: m/z 422.63 (M^+., 1.30%). Anal. Calcd for $C_{19}H_{18}N_8O_2S$ (422.46) (%): C, 54.02; H, 4.29; N, 26.52. Found (%): C, 54.17; H, 4.35; N, 26.78.

***N*-Carbamimidoyl-4-[(3-(4-chlorophenyl)[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)amino]benzenesulphonamide(6c)**
Yield: 0.24 g (54.50%); mp: 276-278 ^{o}C; IR (KBr, cm^{-1}): 3444, 3340, 3213 (NH_2, 3 N-H), 1246 and 1134 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.67 (s, 2H, NH_2, D_2O exchangeable), 6.43-8.37 (m, 8H, Ar-H), 6.74 (s, 1H, NH, D_2O exchangeable), 7.21 (d, 1H, pyridazine-H, *J*=9.60 Hz), 8.21 (d, 1H, pyridazine-H, *J*=9.60 Hz), 9.48 (s, 1H, NH, D_2O exchangeable), 10.37 (s, 1H, NH, D_2O exchangeable), MS: m/z 444.10 $[M+2]^+$ (0.09 %), 442.10 (M^+., 0.09%). Anal. Calcd for $C_{18}H_{15}ClN_8O_2S$ (442.88) (%): C, 48.81; H, 3.41; N, 25.30. Found (%): C, 48.98; H, 3.38; N, 25.53.

ANTICANCER ACTIVITY

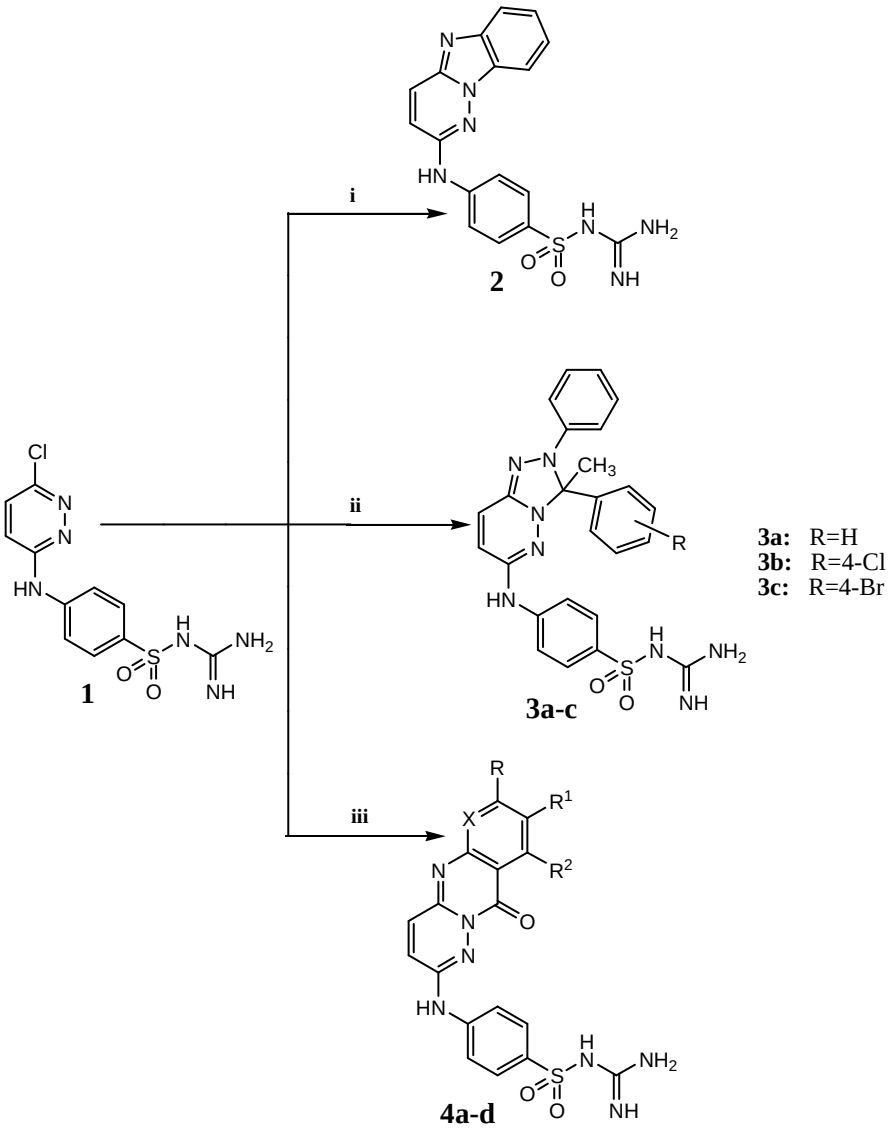
The cytotoxic activity of the tested compounds was measured *in vitro* against human colon cancer cell line (HCT-116) and breast cancer cell line (MCF-7) in comparison to imatinib applying Sulforhodamine B stain (SRB) following the method of Skehan *et al* [14]. Human tumor cell lines (MCF-7, HCT-116) used in this study were obtained frozen in liquid nitrogen (-180 ^{o}C) from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Cells were seeded in 96-well microtiter plates at a concentration of ($5x10^4$-10^5cells/well) in a fresh medium and left to attach to the plates for 24 h before treatment of the tested compounds. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. After 24 h, cells were incubated with the appropriate concentration ranges of drugs (0, 5, 12.5, 25 and 50 μg/mL), the wells were diluted to 200 μL with fresh medium and incubation was continued for 48 h at 37 °C. Control cells were treated with vehicle alone. Four wells were used for each drug concentration. After 48 h incubation, the cells were fixed with 50 μL cold 50% trichloroacetic acid for 1 h at 4 ^{o}C, washed 5 times with distilled water and then stained for 30 min at room temperature with 50 μL 0.4 % SRB dissolved in 1 % acetic acid. The wells were then washed 4 times with 1% acetic acid. The plates were air dried and the dye was solubilized with 100 μL/well of 10 mM tris base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density (O.D) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (Meter tech. Σ 960, U.S.A.). The percentage of cell survival was calculated as follows: survival fraction = O.D. (treated cells)/ O.D. (control cells). The relation between surviving fraction and compound concentration was plotted to get the survival curve for tumor cell line after the specified time. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated for each tested compound (Table 1).

RESULTS AND DISCUSSION

Chemistry

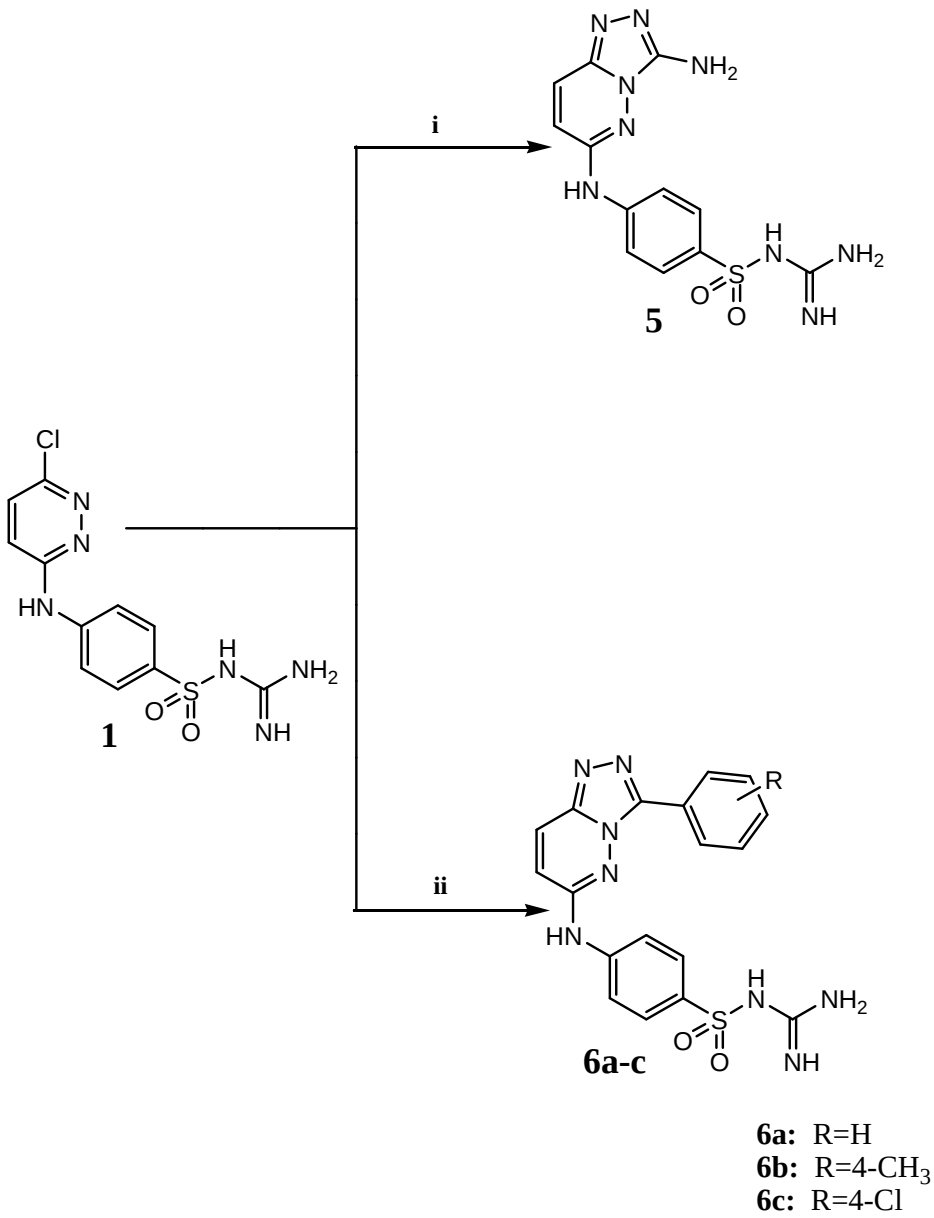
The synthetic pathway for the target compounds **2, 3a-c** and **4a-d** is outlined in Scheme 1. *N*-carbamimidoyl-4-[(6 chloropyridazin-3-yl) amino]benzenesulphonamide (**1)** was prepared from the commercially available 3,6-dichloropyridazine and sulfaguanidine according to a reported procedure [13]. On the other hand, compound **2** was obtained by reacting **1** with 2-aminophenol. Moreover, the target compounds triazolo[4,3-*b*]pyridazines **3a-c** were synthesized from **1** via the reaction with acetophenone phenylhydrazone derivatives. Furthermore, reaction of compound **1** with anthranilic acid derivatives afforded the required compounds **4a-d**. ^{1}H-NMR spectra of compounds **3a, 3b** and **3c** revealed the presence of a singlet signal at δ: 2.75 ppm, 2.27 ppm and 2.30 ppm respectively integrated for three protons due to CH_3. In addition, mass spectrum of **3c** showed the characteristic M and M+2 peaks confirming the presence of Br. The IR spectra of compounds **4a-d** demonstrated the appearance of C=O stretching at 1660, 1660, 1674 and 1710 cm^{-1} respectively. In addition, ^{1}H-NMR spectrum of compound **4b** showed the presence of two singlet signals at δ 3.99 and 4.00 ppm assigned for the three protons of the two OCH_3 groups.

Scheme 2 deals with the preparation of the target pyridazine derivatives **5** and **6a-c** through the reaction of **1** with thiosemicarbazide or substituted benzoyl hydrazide respectively. The IR spectrum of **5** indicated the appearance of 2 forked peaks at 3442, 3392, 3340, 3307 cm-^{1} which proved the presence of 2 NH_2 groups. Furthermore, ^{1}H-NMR spectrum of compound **6b** showed a singlet signal at δ 2.38 ppm confirming the presence of CH_3. Further evidence was obtained from the ^{13}C-NMR spectrum which demonstrated the appearance of a peak at δ 21.49 ppm due to CH_3.



4a: R=H, R^1=H, R^2=H, X=C
4b: R=OCH_3, R^1=OCH_3, R^2=H, X=C
4c: R=H, R^1=H, R^2=I, X=C
4d: R=H, R^1=H, R^2=H, X=N

Scheme 1: Reagents and conditions: i) 2-aminophenol/DMF/10h, ii) acetophenone phenylhydrazone derivatives/160-180 ^oC/ 7h, iii) anthranilic acid derivatives/ n-butanol/24h



Scheme 2: Reagents and conditions: i) thiosemicarbazide/ethanol/20 h, ii) substituted benzoyl hydrazide/ 20 h.

Anticancer activity

All the synthesized compounds **2, 3a-c, 4a-d, 5** and **6a-c** were evaluated for cytotoxic activity against colon cancer cell line (HCT-116) and breast cancer cell line (MCF-7) using Sulforhodamine B stain (SRB) colorimetric assay. For comparison purpose, the cytotoxic activity of imatinib (Fig. 1) was evaluated under the same condition. The IC_{50} values are shown in Table 1 and were represented graphically in (Fig. 3 and 4). From the analysis of Table 1, it was found that almost all the compounds exhibited significant cytotoxic activity. Interestingly, compounds **4a** and **4d** were the most potent against colon cancer cell line (HCT-116) with IC_{50} 11.90 μM and 10.43 μM. Furthermore, compounds **3a, 4b, 4c, 6a** and **6b** showed better cytotoxic activity than that produced by imatinib (Gleevec ®) with IC_{50} 34.30 μM.

On the other hand, compounds **3b, 3c** and **5** exhibited comparable cytotoxicity to imatinib. Moreover, compounds **2** and **6c** were the least active among all the tested compounds against colon cancer cell line (HCT-116).

In addition, analysis of (Table 1) revealed that compound **4a** with IC_{50} 11.10 μM was the most active among all the synthesized compounds against breast cancer cell line (MCF-7). While compounds **4d, 5, 6a** and **6c** were the least active compounds. Other compounds were found to possess moderate cytotoxic activity.

Table1: Cytotoxic activity of the tested compounds against colon cancer cell line (HCT-116) and breast cancer cell line (MCF-7) *in vitro*

Compound number	$(IC_{50})^{a,b}$ in µM against (HCT-116)	$(IC_{50})^{a,b}$ in µM against (MCF-7)
2	47.70	33.00
3a	23.80	16.90
3b	34.40	20.00
3c	40.10	24.00
4a	**11.90**	**11.10**
4b	28.90	25.70
4c	23.70	17.80
4d	**10.43**	70.73
5	31.41	ND
6a	28.43	120.09
6b	20.45	28.67
6c	74.80	50.39
Imatinib[c]	34.30	6.00

[a] IC_{50}: dose of the compound which inhibit tumor cell proliferation by 50%
[b] Values are means of three experiments
[c] Used as positive control
ND not determined

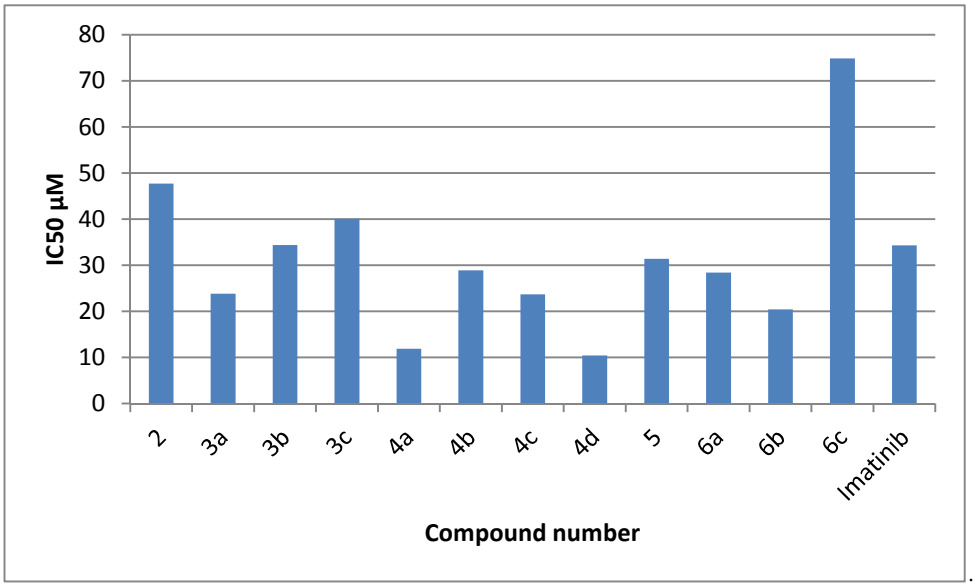


Fig. 3. Cytotoxic activity of compounds 2, 3a-c, 4a-d, 5, 6a-c and imatinib against colon cancer cell line (HCT-116)

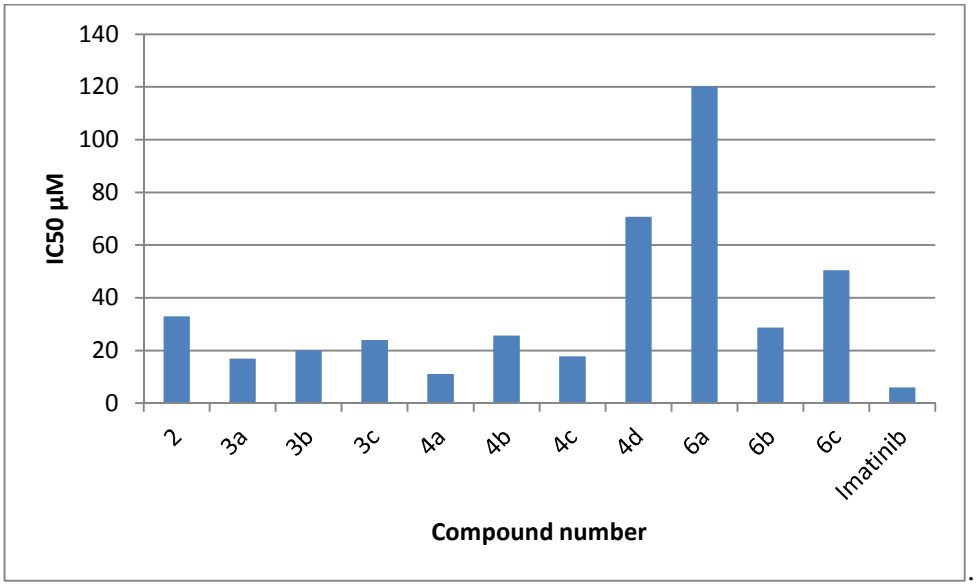


Fig. 4. Cytotoxic activity of compounds 2, 3a-c, 4a-d, 5, 6a-c and imatinib against breast cancer cell line (MCF-7)

CONCLUSION

In this study the synthesis and anticancer activity of novel pyridazine containing compounds **2, 3a-c, 4a-d, 5** and **6a-c** were done. The result revealed that compound **4a** was the most potent one with IC_{50} 11.90 μM against colon cancer cell line (HCT-116) while compounds **3a, 4b, 4c, 6a** and **6b** showed cytotoxic activity with IC_{50} lower than that of imatinib. Moreover, compounds **3b, 3c** and **5** showed cytotoxic activity comparable to the reference drug imatinib. Furthermore, compound **4a** was the most active one against breast cancer cell line (MCF-7) with IC_{50} 11.10μM.

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REFERENCES

[1] H. L. Zhao, F. Yang, X. Huang, Q. H. Zhou, *Thoracic Cancer*, **2014**, 5, 487.
[2] B. Al-Lazikani, U. Banerji, P. Workman, *Nat. Biotechnol*., **2012**, 30, 679.
[3] H. C. Wu, C. T. Huang, D. K. Chang, *J. Cancer Mol*., **2008**, 4, 37.
[4] N. Steeghs, J. W. R. Nortier, H. Gelderblom, *Ann. Surg. Oncol*., **2007**, 14, 942.
[5] M. B. Lodish, C. A. Stratakis, *Endocr. Relat. Cancer*, **2010**, 17, R233.
[6] S. R. Hubbard, *Prog. Biophys. Mol. Biol*., **1999**, 71, 343.
[7] N. Simiantonaki, M. Taxeidis, C. Jayasinghe, C. J. Kirkpatrick, *Anticancer Res*., **2007**, 27, 3245.
[8] A. Sudhakar, *J. Cancer Sci. Ther*., **2009**, 1, i.
[9] Y. Oguro, D. R. Cary, N. Miyamoto, M. Tawada, H. Iwata, H. Miki, A. Hori, S. Imamura, *Bioorg. Med. Chem*., **2013**, 21, 4714.
[10] S. Parkkila, A. Innocenti, H. Kallio, M. Hilvo, A. Scozzafava, C. T. Supuran, *Bioorg. Med. Chem. Lett*., **2009**, 19, 4102.
[11] J. Dietrich, C. Hulme, L. H. Hurley, *Bioorg. Med. Chem.*, **2010**, 18, 5738.
[12] N. Miyamoto, Y. Oguro, T. Takagi, H. Iwata, H. Miki, A. Hori, S. Imamura, *Bioorg. Med. Chem*., **2012**, 20, 7051.
[13] K. A. M. Abouzid, N. A. Khalil, E. M. Ahmed, K. O. Mohamed, *Arch. Pharm. Res.*, **2013**, 36, 41.
[14] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.*, **1990**, 82, 1107.