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Synthesis, reactivity and antitumor activity of some new pyrazolo[3,4-d] pyrimidine and their triazole derivatives

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ABSTRACT

*Some pyrazolo [3, 4- d] pyrimidines derivatives and their triazole derivatives were synthesized starting from p-toluene sulfonyl hydrazide **1** by reaction with different electrophilic and nucleophilic reagents. Some of the newly synthesized compounds have been evaluated for their potential cytotoxicity against breast cancer cell line (MCF7), which show high activity.*

Key words: Pyrazolo [3, 4- d] pyrimidines, Triazole, Electophilic substitution, Antitumor Activity, Breast cancer.

INTRODUCTION

Pyrazole derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. Their related fused heterocycles are of interest as potential bioactive molecules. They are known to exhibit biological activities such as antiviral [1-3], anti-inflammatory [4], antitumor [5], inhibitors for platelet aggregation [6], antibacterial and antifungal agents [7,8]. Also, the chemistry of fused pyrazolo[3, 4-d] pyrimidine derivatives has drawn great attention due to their biological and chemotherapeutic importance. They are known to exhibit pharmacological activities such as CNS depressant [9], neuroleptic [10], and tuberculostatic [11]. Pyrazolo [3, 4-d] pyrimidines were identified as a general class of adenosine receptors [12,13]. There is no much difference in the basic structures of pyrazolopyrimidines and purines.

In the literature, it was found that the replacement of 1*H* of pyrazole of pyrazolo[3, 4-d] pyrimidine ring system by some other bioactive moieties drastically alters its pharmacological

properties. Pyrazolo[3, 4 - d] pyrimidine derivatives bearing a fluorinated heterocyclic moiety, particularly 8-trifluoromethyl quinoline, find much importance in the pharmaceutical field [14]. Fluorinated heterocycles in particular, are those focused much in modern-day medicinal chemistry. Incorporation of a fluorine atom instead of a hydrogen one can alter the course of the reaction as well as biological activities. [15-17]. Furthermore introduction of a fluorine atom as the CF_3 group provides a more lipophilically and pharmacologically interesting compound compared to their non-fluorinated analogues. The trifluoromethyl substituted compounds have been reported to possess biological activities as herbicides, [18], fungicides [19], analgesic [20], and antipyretic [21]. In the present work, some of pyrazolo [3, 4- d] pyrimidines derivatives and their triazole derivatives were synthesized starting from *p*-toluene sulfonyl hydrazide to evaluate their potential cytotoxicity against breast cancer cell line (MCF7).

MATERIALS AND METHODS

Experimental

Melting points were determined with a kofler block apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 1720 FTIR spectrometer for KBr disc. NMR spectra were recorded on Bruker AC-300 (300, 75 and 282 MHz for ^{1}H NMR and ^{13}C NMR). Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard and the coupling constants J values are given in Hz. Mass spectra were recorded on GC/MS Finnegan SSQ 7000 spectrophotometer & GC Ms-QP 1000 EX mass spectrometer at 70 ev. The spectral analyses were performed in chimie et photonique Moleculaires UMR6510 CNRS, Universitie de Rennes 1, Rennes, France. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F245. Elemental analyses were performed at the Microanalytical centre at Faculty of science, Cairo University, Egypt. The anti-tumor activity of the synthesized compounds was carried out at the National Cancer Institute, Cairo, Egypt.

5-Amino-1-tosyl-1*H*-pyrazol-4-carbonitrile (2)

To a solution of *p*-toluene sulfonylhydrazide **1** (1.86 g, 0.01 mol) in ethanol (30 ml) was added ethoxymethylene malononitrile (1.22 g, 0.01 mol). The reaction mixture was heated at reflux temperature for 6 hours and the completion of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The solid that obtained was recrystallized from ethanol to produce compound **2** as a yellow solid, yield 86%, (2.25 g), m.p 130-131 °C, Analysis for $C_{11}H_{10}N_4O_2S$ [Found: C, 50.40; H,3.88; N, 21.55; Calcd, C, 50.37; H,3.84; N,21.36]. IR Cm^{-1}: 3200 NH_2, 1630 (C=N); ^{1}H NMR (DMSO-d_6) ppm: 2.40 (s, CH_3), 7.48 (d, J = 8.1, 2CH phenyl), 7.64 (s, NH_2), 7.86 (d, J = 1.8, 2CH phenyl), 7.89 (s, CH pyrazole); ^{13}C NMR (DMSO-d_6): δ 21.2 (CH_3), 72.99 (C-CN), 113.15 (CN), 127.62-145.75 (Ar-C), 146.66 (CH), 154.31(C-NH_2); M/z: 262.05 (100%).

1-Tosyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ol (3)

A suspension of **2** (2.6 g, 0.01 mol) in formic acid (85%; 20ml) was heated at reflux temperature for 12 h. A solid product was obtained after cooling at room temperature, which was filtered off and recrystallized from DMF to yield **3** as a yellow powder. Yield 1.5 g (52 %), m.p 125-126°C, Analysis for $C_{12}H_{10}N_4O_3S$ [Found: C, 49.50; H, 3.48; N, 19.55; Calcd, C, 49.65; H, 3.47; N, 19.30]. IR Cm^{-1}: 3500 (OH), 1630 (C=N); ^{1}H NMR (DMSO-d_6) ppm: δ 2.29 (s, CH3), 7.48 (d, J = 8.1, 2H phenyl), 7.86 (d, J = 1.8, 2H phenyl), 7.89 (s, CH pyrazole), 8.26 (s, CH pyrimidine),

12.0 (s, OH); ^{13}C NMR (DMSO-d_6): δ 21.2 (CH_3), 105.0 (C=C), 127.62-145.75 (Ar-C), 130.0 (CH pyrazole), 147.053 (CH pyrimidine), 149.75 (C=C), 157.2 (C-OH); M/z 290.05 (100%).

1-Tosyl-1*H*- pyrazolo[3,4-d]pyrimidin-4-amine (4)

A solution of **2** (2.6 g, 0.01 mol) in formamide (10 ml) was heated at (180-190 °C) for 3 hours. The solution was then cooled to room temperature and diluted with 100 ml ice-cooled water and the precipitate obtained was filtered off and recrystallized from ethanol to give **4** as a yellow powder. Yield 2.4 g (83%), m.p 147-148 °C; Analysis for $C_{12}H_{11}N_5O_2S$ [Found: C,49.50; H,3.82; N,24.25; Calcd., C,49.82; H,3.83; N,24.21]; ^{1}H NMR (DMSO-d_6) ppm: 2.40 (s, CH_3), 7.48 (d, J = 8.1, 2CH phenyl), 7.52 (s, NH_2), 7.86 (d, J = 1.8, 2CH phenyl), 8.38 (s, CH pyrazole), 8.9 (s, CH pyrimidine); ^{13}C NMR (DMSO-d_6): δ 21.48 (CH_3), 111.8 (C=C), 127.62-145.75 (Ar-C), 136.0 (CH pyrazole), 147 (C=C), 155 (CH pyrimidine), 157 (C-NH_2); M/z 289.06 (100%).

1-Tosyl-1*H*-pyrazolo[3,4-d]pyrimidine-4,6-(5*H*,7*H*)-dithione (5)

To a solution of **2** (2.6 g, 0.01 mol) in 10% alcoholic KOH (10ml), carbon disulfide (10ml) was added. The reaction mixture was refluxed for 2 hours, cooled, poured onto cold water and neutralized with 1M HCl. The solid that obtained was filtered off, washed with water, dried and recrystallized from DMF to afford **5** as a yellow powder. Yield 1.6 g (47.3%), m.p 110-111°C; Analysis for $C_{12}H_{10}N_4O_2S_3$ [Found: C,42.55; H,2.3; N,16.50; Calcd., C,42.59; H,2.98; N,16.55]; ^{1}H NMR (DMSO-d_6) ppm: 2.48 (s, CH_3), 4.18 (s, NH), 7.48 (d, J = 8.1, 2CH phenyl), 7.86 (d, J = 1.8, 2CH phenyl), 7.93 (s, CH pyrazole), 13.83 (s, NH); M/z 338.0 (100%).

4-Amino-1-tosyl-1*H*-pyrazolo[3,4-d]pyrimidine-6(7*H*)-one (6a)

Five grams of compound **2** and 10 g urea were heated together at 180-190°C for 15 min. The resulting solid was dissolved in dilute sodium hydroxide and then carefully acidified with acetic acid to obtain 4 g of crude product **6a** as a yellow powder. Further purification was accomplished by reprecipitation from dilute sodium hydroxide with acetic acid. Yield 2.5 g (42.6%); m.p > 300°C, Analysis for $C_{12}H_{11}N_5O_3S$ [Found: C,47.25; H,3.5; N,22.98; Calcd., C,47.21; H,3.63; N,22.94]; ^{1}H NMR (DMSO-d_6) ppm: 2.37 (s, CH_3), 7.1 (s, NH_2), 7.4 (d, J = 7.8, 2CH phenyl), 7.7 (d, J = 1.8, 2CH phenyl), 7.72 (s, CH pyrazole), 7.9 (s, NH pyrimidine); ^{13}C NMR (DMSO-d_6): δ20.8 (CH_3), 125.5-141.75 (Ar-C), 132.55 (C=C), 148.33 (C=O), 149.9 (C=C), 155.6 (C-NH_2); M/z 305.06 (100%).

4-Amino-1-tosyl-1*H*-pyrazolo[3,4-d]pyrimidine-6(7*H*)-thione (6b)

Five grams of **2** and 10 g thiourea were heated together at 180-190°C for 45 min. The resulting solid was dissolved in dilute sodium hydroxide and then carefully acidified with acetic acid to obtain 3 g of crude product **6b** as a yellow powder. Further purification was accomplished by reprecipitation from dilute sodium hydroxide with acetic acid. Yield 1.5 g (25.5%); m.p 290-291°C; Analysis for $C_{12}H_{11}N_5O_2S_2$ [Found: C,44.56; H,3.5; N,21.75; Calcd., C,44.85; H,3.45; N,21.79]; ^{1}H NMR (DMSO-d_6) ppm: 2.37 (s, CH_3), 3.4 (s, NH pyrimidine), 7.2 (s, NH_2), 7.4 (d, J = 7.8, 2CH phenyl), 7.7 (d, J = 1.8, 2CH phenyl), 7.9 (s, CH pyrazole); M/z 321.04 (100%).

4-Imino-5-phenyl-1-tosyl-4,5-dihydro-1*H*-pyrazolo[3,4-d]-pyrimidin-6(7*H*)-one (7a)

A solution of **2** (2.6 g, 0.01 mol) and phenyl isocyanate (1.19g, 0.01 mol) in ethanol 20 ml was heated at reflux temperature for 6 hours. A solid product was obtained after cooling to room

temperature, which was filtered off and recrystallized from DMF to yield **7a** as a yellow powder; yield 1.6 g (41.9%) m.p 151-152 °C, Analysis for $C_{18}H_{15}N_5O_3S$ [Found: C,57.0; H,3.95; N,18.33; Calcd., C,56.68; H,3.96; N,18.36]; ^{1}H NMR (DMSO-d_6) ppm: 2.4 (s, CH_3), 6.0 (s, NH), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl),7.43 (m, 5H phenyl), 7.9 (s, CH pyrazole), 9.7 (s, NH); ^{13}C NMR (DMSO-d_6): δ 21.1 (CH_3), 99 (C=C), 132.9 (CH pyrazole), 127.5-130.75 (Ar-C), 128.7-139.6 (Ar-C), 146.6(C=C), 152.4 (C=O), 157 (C=NH); M/z 381.09 (100%).

4-Imino-5-phenyl-1-tosyl-4,5-dihydro-1*H*-pyrazolo[3,4-d]-pyrimidin-6(7*H*)-thione (7b)

A solution of **2** (2.6 g, 0.01 mol) and phenyl isothiocyanate (1.35g, 0.01 mol) in ethanol 20 ml was heated at reflux temperature for 8 hours. A solid product was obtained after cooling to room temperature, which was filtered off and recrystallized from ethanol to yield **7b** as a yellow powder. Yield 1.3 g (32.7%) m.p 130-131°C, Analysis for $C_{18}H_{15}N_5O_2S_2$ [Found: C,54.5; H,3.88; N,17.5; Calcd., C,54.39; H,3.8; N,17.62]; ^{1}H NMR (DMSO-d_6) ppm: 2.4 (s, CH_3), 4.5 (s, NH), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl),7.1-7. 3 (m, 5H phenyl), 7.9 (s, CH pyrazole), 11.0 (s, NH); ^{13}C NMR (DMSO-d_6): δ21.1 (CH_3), 99 (C=C), 132.9 (CH pyrazole), 127.5-130.75 (Ar-C), 128.7-139.6 (Ar-C), 146.6(C=C), 154 (C=NH), 180 (C=S); M/z 397.07 (100%).

Ethyl *N*-4-cyano-1-tosyl-1*H*-pyrazol-5-ylformimidate (8)

To a mixture of triethylorthoformate (0.01 mol) and acetic anhydride (20 ml), compound **2** (0.01 mol) was added and the reaction mixture was refluxed for 5h. The solvent was removed under reduced pressure and the separated solid was recrystallized from ethanol to afford **8** as a yellow powder. Yield 1.7 g (53.4%); m.p 135-136°C; Analysis for $C_{14}H_{14}N_4O_3S$ [Found: C, 52.6; H, 4.44; N, 17.5; Calcd., C, 52.82; H, 4.43; N, 17.60]; ^{1}H NMR (DMSO-d_6) ppm: 1.26 (t, CH_3), 2.4 (s, CH_3), 4.5 (q, OCH_2), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.512 (s, CH=O), 7.7 (d, *J* = 1.8, 2CH phenyl), 7.9 (s, CH=N); M/z 318.08 (100%).

4-Imino-1-tosyl-1*H*-pyrazolo[3,4-d]pyrimidin-5(4*H*)-amine (9)

To a solution of **8** (0.01 mol) in absolute ethanol (50 ml), hydrazine hydrate (0.15 mol) was added. The reaction mixture was refluxed for 4h, concentrated, cooled, and the solid product that separated out was filtered off and recrystallized from ethanol to yield **9** as a yellow powder. Yield 1.5g (49.3%), m.p 145-146°C; Analysis for $C_{12}H_{12}N_6O_2S$ [Found: C, 47.5; H, 4.0; N, 27.53; Calcd., C, 47.36; H,3.97; N, 27.62]; Cm^{-1} 3330 and 3200 (NH_2 and NH). ^{1}H NMR (DMSO-d_6) ppm: 2.0 (s, NH_2), 2.4 (s, CH_3), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl), 7.9 (s, CH pyrazole), 8.5 (s, CH pyrimidine), 11.0 (s, NH); M/z 304.07 (100%).

7-Tosyl-3,7-dihydro-2H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine-2-thione (10)

To a stirred suspension of compound **9** (3.0 g, 0.01 mol) in ethanol (20 ml), ethanolic potassium hydroxide (30 ml, 0.01 mol) and CS2 (2 ml) were added drop wise. The reaction mixture was then heated under reflux for 6h. After cooling and evaporation of the solvent, the potassium salt obtained was dissolved in water and acidified with 2N aqueous HCl. The solid product formed was filtered off and recrystallized from ethanol as yellow crystals. Yield 1.9 g (54.9%), m.p 125-126°C; Analysis for $C_{13}H_{10}N_6O_2S_2$ [Found: C, 45.0; H, 2.75; N, 24.25; Calcd., C, 45.03; H,2.91; N, 24.26]. IR; Cm^{-1} 3350 (NH), 1630 (C=N), 1190 (C=S), ^{1}H NMR (DMSO-d_6) ppm: 2.0 (s,

NH), 2.4 (s, CH_3), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl), 7.9 (s, CH pyrazole), 8.5 (s, CH pyrimidine), M/z 346.03 (100%).

General procedure for the preparation of 2-(alkylthio)-7-tosyl-7*H*-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (11a-d)

To a mixture of compound **10** (3.46 g, 0.01 mol) and sodium acetate (1.64 g, 0.02 mol) in ethanol (15 ml) was added the respective halo compound (RX, 0.01 mol), then the reaction mixture was heated under reflux for 4h. After cooling, the solid products were filtered, washed with water and recrystallized from the proper solvent.

2-(Methylthio)-7-tosyl-7*H*-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c] pyrimidine (11a)

Using methyl iodide, compound **11a** was obtained as white crystals from ethanol. Yield 1.9 g (52.7%), m.p 140-141°C; Analysis for $C_{14}H_{12}N_6O_2S_2$ [Found: C, 46.5, H, 3.4; N, 23.5; Calcd., C, 46.65; H, 3.36; N, 23.32].IR; Cm^{-1} 1630 (C=N), ^1H NMR (DMSO-d_6) ppm: 2.4 (s, CH_3), 2.5 (s, CH_3), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl), 8.1 (s, CH pyrazole), 9.5 (s, CH pyrimidine); M/z 360.05 (100%).

2-(Ethylthio)-7-tosyl-7*H*-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (11b)

Using ethyl iodide, compound **11b** was obtained as white crystals from ethanol. Yield 1.7 g (45.4 %), m.p 130-131°C; Analysis for $C_{15}H_{14}N_6O_2S_2$ [Found: C, 48.5, H, 3.4; N, 22.5; Calcd., C, 48.11; H, 3.77; N, 22.44].IR; Cm^{-1} 1630 (C=N), ^1H NMR (DMSO-d_6) ppm: 1.3 (t, CH_3), 2.4 (s, CH_3), 3.1 (q, CH_2), 7.4 (d, *J* = 8.1, 2CH phenyl), 7.7 (d, *J* = 1.8, 2CH phenyl), 8.2 (s, CH pyrazole), 9.5 (s, CH pyrimidine); M/z 374.06 (100%).

7-Tosyl-7*H*-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2-ylthio)-propane-1,2-diol (11c)

Using 3-bromo-propane-1,2-diol, compound **11c** was obtained as white crystals from ethyl acetate. Yield 1.6 g (38 %), m.p 135-136°C; Analysis for $C_{16}H_{16}N_6O_2S_4$ [Found: C, 45.5, H, 3.5; N, 19.5; Calcd., C, 45.7; H, 3.84; N, 19.99].IR; Cm^{-1} 3500, 3450 (2OH), 1630 (C=N), M/z 420.07 (100%).

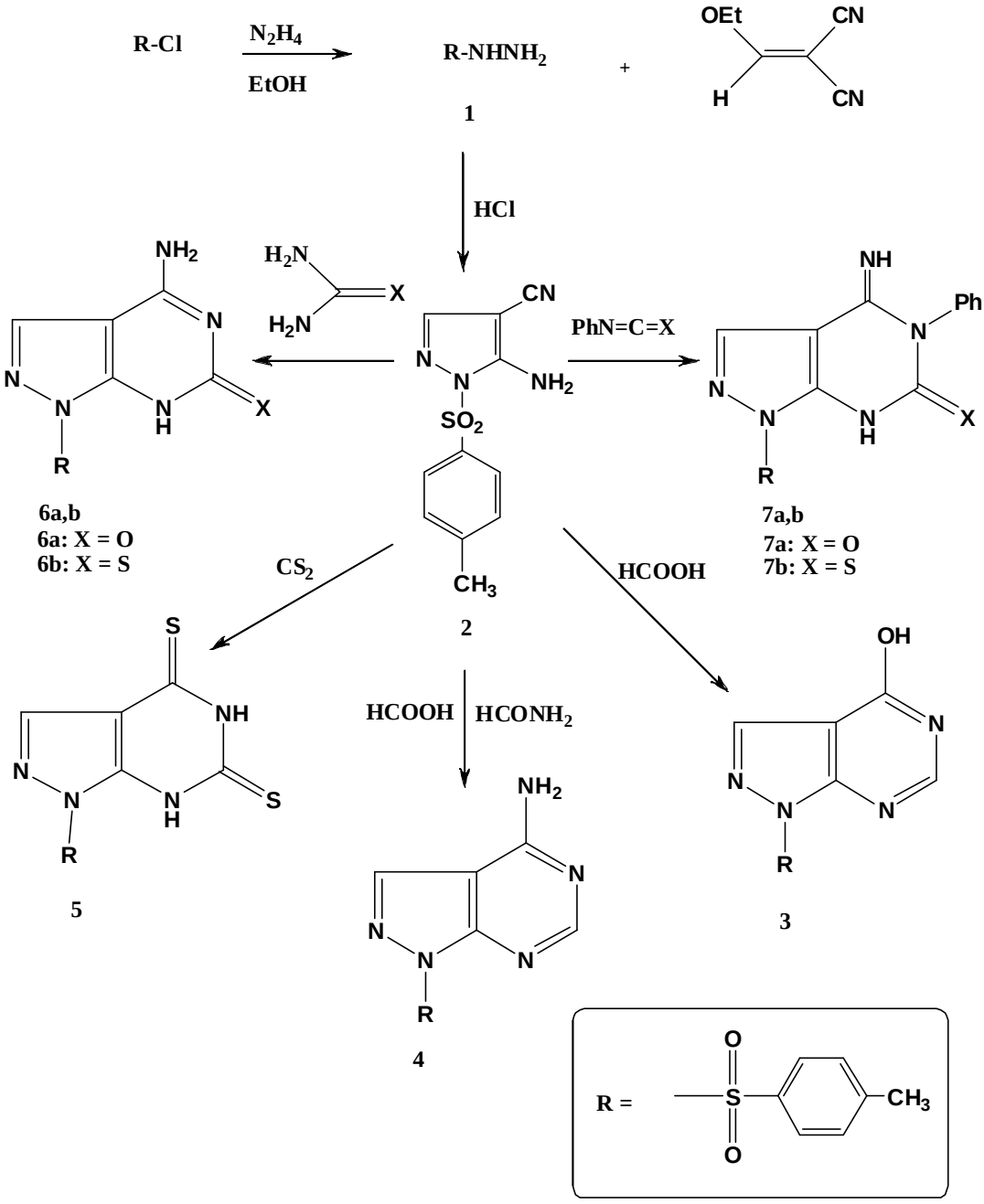
2-(Allylthio)-7-tosyl-7*H*-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (11d)

Using allyl bromide, compound **11d** was obtained as yellow crystals from ethanol. Yield 1.7 g (44 %), m.p 125-126°C; Analysis for $C_{16}H_{14}N_6O_2S_2$ [Found: C, 49.5, H, 3.5; N, 21.5; Calcd., C, 49.73; H, 3.65; N, 21.75].IR; Cm^{-1} 1630 (C=N), M/z 420.07 (100%).

RESULTS AND DISCUSSION

Chemistry

p-Toluene sulfonylhydrazide **1** was prepared from reaction of *p*-toluene sulfonylchloride with hydrazine hydrate in absolute ethanol according to a reported procedure [22]. When *p*-toluene sulfonylhydrazide **1** was reacted with ethoxymethylene malononitrile in absolute ethanol yielded 5-amino-1-tosyl-1*H*-pyrazol-4-carbonitrile **2** in 86% yield [23]. The ^1H NMR spectrum of **2** revealed strong singlet peak at δ 7.889 ppm corresponding to =CH group and a singlet peak at δ 7.63 ppm corresponding to NH_2 group adjacent to C-CN group of the pyrazole ring. The ^{13}C NMR of **2** revealed the presence of =CH group of pyrazole ring at δ 146.66 ppm.

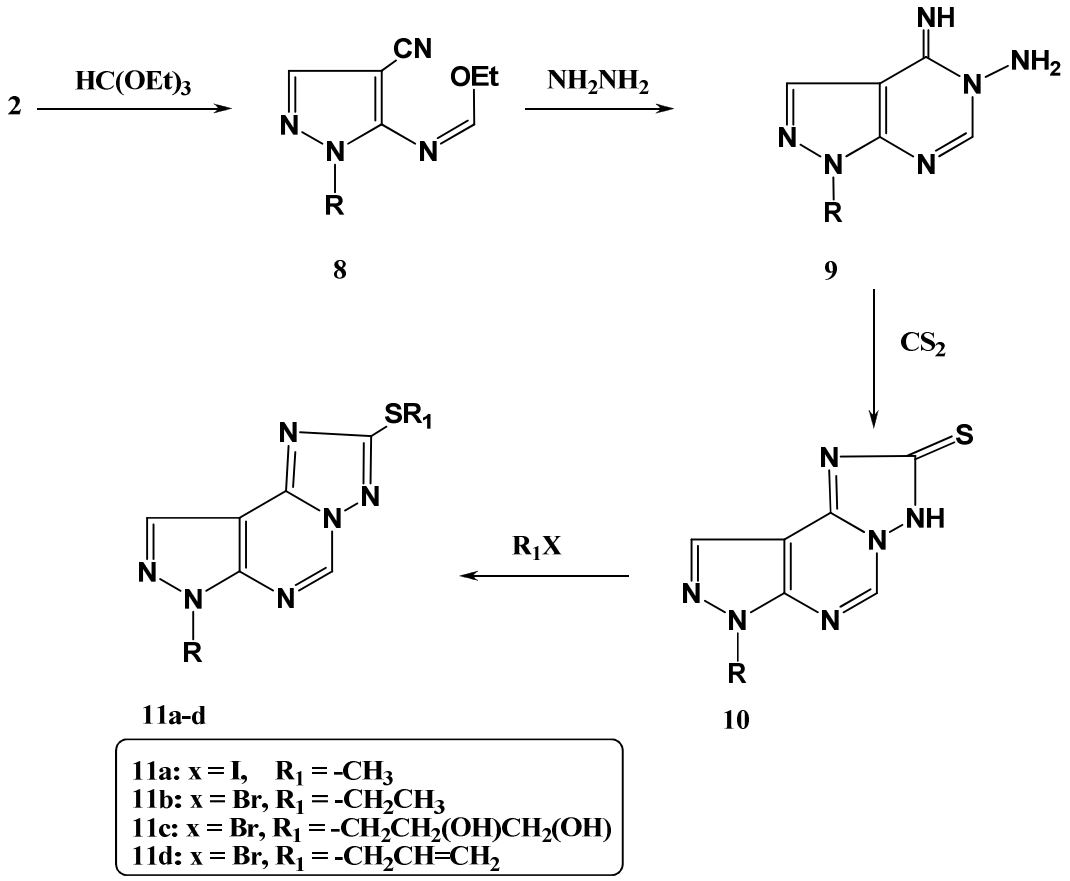


Scheme 1

When compound **2** was reacted with formic acid at reflux temperature afforded 1-tosyl-1*H*-pyrazolo [3, 4-d] pyrimidin- 4-ol **3,**[24]. The ^{1}H NMR spectrum of **3** revealed the absence of NH_2 and presence of OH singlet peak at δ 12.0 ppm. The ^{13}C NMR spectrum of **3** indicated the presence of =CH group at δ 147.053 ppm and C-OH group at δ 157.2 ppm, and when compound **2** was reacted with formamide in the presence of formic acid and dimethylformamide (DMF) afforded 1-tosyl-1*H*-pyrazolo [3, 4- d] pyrimidin -4-amine **4,**[24]. The ^{1}H NMR spectrum of **4** revealed the presence of NH_2 singlet peak at δ 7.522 ppm and =CH singlet peak at δ 8.38 ppm. The ^{13}C NMR spectrum of **4** indicated the presence of C-NH_2 at δ 157 ppm and =CH at δ 154

ppm. 1-Tosyl-1*H*-pyrazolo [3, 4-d] pyrimidine-4, 6-(5*H*, 7*H*)-dithione **5** was synthesized via reaction of compound **2** with CS_2 in absolute ethanol [25]. The ^1H NMR spectrum of **5** revealed the presence of two NH groups of pyrimidine moiety as singlet peaks at about δ 13.83 ppm and δ 4.18 ppm respectively **(Scheme 1).**

Refluxing of **2** with urea and / or thiourea in absolute ethanol afforded 4-amino-1-tosyl-1*H*-pyrazolo [3, 4 -d] pyrimidine-6(7*H*)-one **6a** and / or 4-amino-1-tosyl-1*H*-pyrazolo [3,4-d] pyrimidine-6(7*H*)-thione **6b** respectively [26]. The ^1H NMR spectrum of **6a** revealed the presence of three singlet peaks of NH, =CH, NH_2 at about δ 7.93,7.7 and 7.1 ppm respectively and the ^{13}C NMR spectrum of **6a** indicated the presence of C-NH_2 group at δ 155.63 ppm and -C=O group at δ 148.33 ppm and the ^1H NMR spectrum of **6b** revealed the presence of three singlet peaks of =CH, NH_2 and NH at about δ 7.991, 7.293 and 3.35 ppm respectively and reaction of **2** with phenylisocyanate and / or phenylisothiocyanate in ethanol afforded 4-imino-5-phenyl-1-tosyl-4,5-dihydro-1*H*-pyrazolo [3, 4- d] pyrimidin-6(7*H*)-one **7a** and / or 4-imino-5-phenyl-1-tosyl-4,5-dihydro-1*H*-pyrazolo [3,4-d] pyrimidin-6(7*H*)-thione **7b**.



Scheme 2

The ^1H NMR spectra of **7a** revealed the presence of two singlet peaks of free =NH and cyclic NH at about δ 9.70 and 6.0 ppm respectively and ^{13}C NMR spectrum of **7a** revealed the presence of C=NH and C=O at about δ 154.23 and 152.44 ppm respectively, while ^1H NMR of **7b** revealed the presence of two singlet peaks of free =NH and cyclic NH at about δ 11.06 and 4.5

ppm respectively and ^{13}C NMR spectrum of **7b** revealed the presence of C=NH at δ 154.23 ppm.**(Scheme 1).**

In the other hand, compound **2** was used to prepare some interesting triazolopyrazolopyrimidine derivatives using carbon and nitrogen reagents used in large scale in medicine. Thus, when compound **2** was reacted with triethylorthoformate in acetic anhydride afforded ethyl *N*-4-cyano-1-tosyl-1*H*-pyrazol-5-ylformimidate **8**. The ^{1}H NMR spectrum of **8** revealed the presence of two singlet peaks of CH=O and N=CH at about δ 7.512 and 7.9 ppm respectively. When compound **8** was reacted with hydrazine hydrate in absolute ethanol afforded 4-imino-1-tosyl-1*H*-pyrazolo[3,4-d]pyrimidin-5(4*H*)-amine **9** in good yield [24]. The ^{1}H NMR of **9** revealed the presence of two singlet peaks of =NH and NH_2 at about δ 11.0 and 2.0 ppm respectively, and when compound **9** was allowed to react with carbon disulfide in absolute ethanol afforded 7-tosyl-3, 7-dihydro-2H-pyrazolo [4, 3-e] [1, 2, 4] triazolo [1, 5-c] pyrimidine-2-thione **10**. The reaction proceeds via addition of one mole of carbon disulfide on the imine group followed by elimination of one mole of H_2S to produce the triazolopyrimidine moiety. The structure of **10** was elucidated from elemental analysis as well as the M/z 346.03 (100%) and IR spectra which revealed strong NH and C=S peaks at 3350 and 1190 Cm^{-1} respectively. When compound **10** was reacted with alkylhalides in ethanol and sodium acetate afforded 2-(alkylthio)-7-tosyl-7*H*-pyrazolo [4, 3- e] [1, 2, 4] triazolo [1, 5- c] pyrimidine **11a-d**. [27], **(Scheme 2).** The reaction proceeded via electophilic substitution reaction mechanism of the alkyl carbonium ion on the C=S group.

***In vitro* Antitumor Activity**

Measurement of Potential Cytotoxicity by SRB assay:-

Some of the newly synthesized compounds have been evaluated for their Potential Cytotoxicity testing against breast cancer **(MCF7)** using the method of skehan et al. (1990) (28). Cells were plated in 96-multiwell plate (10^4 cells / well) for 24 hrs before treatment with the compounds to allow attachment of cell to the wall of the plate.

Table 1: The IC50 (µg/mL) of some of the selected new compounds against Breast cancer cell line (MCF7)

Compound	IC_{50} µg/ml
DOX	2.97
1	25.7
2	21.3
3	18.2
4	18.8
5	22.6
6a	26.3
6b	4.09
7a	3.7
7b	17
8	16.2
9	17.8
10	22.2

Different concentration of the compound under test (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were

incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5 % CO_2. After 48 hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color Intensity was measured in an ELISA reader

The relation between survivning fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specific compound. The IC_{50} percent control of infected and uninfected response values were calculated for the various active compounds were reported in Table 1. Doxirubsin (DOX) was used as positive standard. Compounds having $IC_{50} < 5$ µg/ml are considered potentially active and exposed to further in *vivo* studies.

The results obtained in table 1 show that compounds **7a** and **6b** possess the highly significant effect against breast cancer cell line **(MCF7)** and this is might be due to the presence of such NH-C=O and NH-C=S moieties in addition to the *N*-tosylpyrazolopyrimidine moiety in the two compounds while, the other compounds show less effect against breast cancer cell line **(MCF7).**

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