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Heterocyclization of Poly functionalized Pyrimidine: Novel Synthesis and Antiproliferative Evaluation of Azino and Azolo Pyrimidines

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Abstract

Mercaptopyrimidin **1** was subjected to an acidic medium reaction with urea and aromatic aldehydes, yielding pyrimidopyrimidine **5a,b** via unsaturated system **3**, followed by 1,4 additions and subsequent H_2S elimination. Using thiourea and/or guanidine instead of urea in the aforementioned process yielded pyrimidines of type (**5 c-f**). Condensation of benzaldehyde and its derivatives with compound **1** is followed by H-shift and oxidative cyclization, resulting in polycyclic compounds. **7**. To make ozoloazines **9a-b**, compound 7 undergoes a substitution reaction with urea and thiourea, followed by air oxidation. The cyclocondensation of hydrazides derivatives yielded triazolopyrimidines (**12 a, b**). *In vitro*, all novel pyrimidine derivatives were tested for antiproliferative activity against two cell lines: human gastric carcinoma (BGC-823) and human lung cancer (A-549). The results were compared to the NIH/3T3 murine fibroblast cell line. Some of these compounds, in particular **5a–f, 6,8,10a-b** and **12a-b**, were discovered to be the most effective derivatives against all cancer cell lines, with no impact on normal cells. Using the structure–activity connection as a guide, compound **10b** has a greater activity against A-549 cells (IC_{50}= 14.15 0.33 g/ml), as it contains thiadiazolidine imine nucleus which attached to thio pyran ring.

***Key words*:** Azino pyrimidines; azolo pyrimidines; pyrimidopyrimidine; antitumor agents..

1. INTRODUCTION

Pyrimidine compounds have a wide range of application in medicine due to their pronounced biological activity. The heterocyclic pyrimidine nucleus, which is a key base component of deoxyribonucleic acid's genetic material, has shown a variety of biological functions. The purpose of this review is to look at the work that has been done on the therapeutic potentials of pyrimidine scaffolds, which are useful for medical applications in the next generation. Many of these compounds provided to be active analgesic [1]. This core pyrimidine ring is a key component of all diazines, having a strong presence in DNA and RNA. Several pyrimidine-based derivatives have been developed as antitumor agents [2, 3], and antiviral agents [4 – 9]. Moreover, several pyrimidine derivatives have long been recognized as potent bactericidal and fungicidal agents [10, 11]. Newly synthesized azino Pyrimidines compounds were synthesized using the environmentally friendly reagents [12, 13]. The synthesis of several new azolo pyrimidines is reported [14 – 16]. In the light of the above findings and due to the broad biological activities of Pyrimidines also, in continuation of our previous works [17, 18] concerned with the synthesis of a variety of heterocyclic systems for biological evaluationwe envisioned our approach toward the synthesis of new series from azolo pyrimidines and

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azino pyrimidines, The results were obtained through reactions with various nucleophiles and in vitro applications of pyrimidine derivatives in order to aid in the creation of more potent and effective antiproliferative drugs, and they are reported here.

2. EXPERIMENTAL

2.1 Chemistry

2.1.1 General

Measuring of melting points, was done by using an Electro thermal IA 9100 apparatus with open capillary tube and are uncorrected. All experiments were carried out using drying solvents. The IR spectrum (KBr disc, υ, cm-1)) was recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. Also ^{1}H NMR and ^{13}C NMR (30, 75 MHz, δ, ppm, J) spectrum were measured on a JEOL-JNM-LA spectrometer using DMSO as a solvent. Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer was used to collect ESIMS data. Analytical data were obtained from the Microanalysis Center at Cairo. Finally, mass spectra were recorded on a MS-S988 instrument operating at 70 eV.

2.1.2 General procedure for the synthesis of compounds 5 a-f

A mixture of 2-Amino-6-thioxo-5, 6-dihydropyrimidin-4(3*H*)-one 1 (0.01 mol) which dissolve in DMF on warming with aromatic aldehydes 2a-b and/or pseudoamide-type (urea, thiourea, guanidine) 4a-c and few drops of diluted HCl was heated under reflux for 6 h. The reaction mixture was cooled at room temperature and poured into ice. The product that obtained was filtered off, washed with water and crystallized from ethanol and DMF (10:1) to obtain compound 5a-f.

2.1.2.1 7-Amino-4-phenyl-3, 4-dihydropyrimido [4, 5-*d*] pyrimidine-2, 5*(1H, 6H)*-dione (5a)

Creamy white powder; Yield 90%; m.p. 270°C; IR (KBr): ύ/cm = 3,405 (NH), 3,323 (Ar-CH), 1680, 1657 (2 C=O) and 1,626 (C═N). ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO]-d_6): δ (ppm): 5.20 (s, 1H, C<u>H</u>NH), 5.36 (s, 1H, NH), 7.21–7.34 (m, 5H, Ar-H) 7.95 (s, 1H, 1NH), 11.84 (s, 2H, 2NH) and 12.06 (s, 1H, N<u>H</u>NH$_2$); ^{13}C-NMR (75MHz, DMSO-d_6) δ (ppm): 174.63, 173.23, 159.33,153.90,143.78, 128.92, 128.32, 128.10, 126.93, 126.82, 52.12, and 90.70; ESI MS *m/z* (rel. int.): 257.0113 $[M]^+$ ($C_{12}H_{11}N_5O_2$); Anal. Data for $C_{12}H_{11}N_5O_2$ (257.01). Calcd. C, 56.02; H, 4.27; N, 27.20%. Found: C, 56.07; H, 4.21; N, 27.12%.

2.1.2.2 7-Amino-4-(3-chlorophenyl)-3, 4-dihydropyrimido [4, 5-*d*] pyrimidine-2, 5(1*H*, 6H)-dione (5b)

Faint yellow crystals; Yield 78%; m.p. 280°C; IR (KBr): ύ/cm = 3,317 (NH), 3,052 (Ar-CH), 1650 (2 C=O) and 1,625 (C═N). ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO] -d_6): δ (ppm): 5.31 (s, 1H, C<u>H</u>NH), 6.58 (s, 1H, NH), 7.17–7.32 (m, 4H, Ar-H) 7.96 (s, 1H, 1NH), 11.89 (s, 2H, 2NH) and 12.03 (s, 1H, N<u>H</u>NH$_2$); Anal. Data for $C_{12}H_{10}ClN_5O_2$ (291.11). Calcd. C, 49.42; H, 3.27; Cl, 12.15; N, 24.20%. Found: C, 49.39 H, 3.21; Cl, 12.11; N, 24.12%.

2.1.2.3 2-Amino-5-phenyl-7-thioxo-5, 6, 7, 8-tetrahydropyrimido [4, 5-*d*] pyrimidin-4(3*H*)-one (5c)

White powder; Yield 85%; m.p. 290°C; IR (KBr): ύ/cm = 3,406 (NH), 3,352 (Ar-CH), 1662 (C=O), 1,604 (C═N) and 1,214 (C═S); ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO]-d_6): δ (ppm): 2.51 (s, 1H, NH), 5.31 (s, 1H, C<u>H</u>NH), 7.07–7.24 (m, 5H, Ar-H), 7.95 (s, 1H, 1NH), 11.83 (s, 2H, 2NH) and 12.06 (s, 1H, N<u>H</u>); ESI MS *m/z* (rel. int.): 273.0112 $[M]^+$ ($C_{12}H_{11}N_5OS$); Anal. Data for $C_{12}H_{11}N_5OS$ (273.01). Calcd.: C, 52.72; H, 4.07; N, 25.60; S, 11.96 %. Found: C, 52.62; H, 4.03; N, 25.56; S, 11.91 %.

2.1.2.4 4-(7-Amino-5-oxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimido[4,5-*d*]pyrimidin-4-yl)benzoyl chloride(5d)

Creamy white powder; Yield 80%; m.p. 280°C; IR (KBr): ύ/cm = 3,406 (NH), 3,116 (Ar-CH), 1662 (C=O), 1,651 (C═N) and 1219 (C=S); ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO]-d_6): δ (ppm): 3.33 (s, 1H, C<u>H</u>NH), 5.32 (s, 1H, NH),7.09–7.27 (m, 4H, Ar-H), 7.96 (s, 1H, 1NH), 11.85 (s, 2H, 2NH) and 12.08 (s, 1H, N<u>H</u>); ^{13}C-NMR (75MHz, DMSO-d_6) δ (ppm): 175.45, 173.28, 163.45,162.78,159.84, 153.95, 151.53, 137.56, 90.39, and 59.70; Anal. Data for $C_{12}H_{10}ClN_5OS$ (307.11). Calcd.: C, 46.82; H, 3.27; Cl, 11.55; N, 22.73%. Found: C, 46.79 H, 3.18; Cl, 11.51; N, 21.55%.

2.1.2.5 2-Amino-7-imino-5-phenyl-5, 6, 7, 8-tetrahydropyrimido [4, 5-*d*] pyrimidin-4(3*H*)-one (5e)

Pale yellow powder; Yield 75%; m.p. 240°C; IR (KBr): ύ/cm = 3,405 (NH), 3,323 (Ar-CH), 3174(=NH), 1680(C=O) and 1,606 (C═N). ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO] -d_6): δ (ppm): 2.50(s, 1H, =NH), 5.33 (s, 1H, NH), 3.33 (s, 1H, CHNH), 7.07–7.24 (m, 5H, Ar-H), 7.96 (s, 1H, 1NH), 11.82 (s, 2H, 2NH) and 12.05 (s, 1H, NH); ESI MS *m/z* (rel. int.): 256.0160 $[M]^+$ ($C_{12}H_{12}N_6O$); Anal. Data for $C_{12}H_{12}N_6O$ (256.01). Calcd.: C, 56.22; H, 4.67; N, 32.70%. Found: C, 56.17; H, 4.61; N, 32.66%.

2.1.2.6 4-(7-Amino-2-imino-5-oxo-1,2,3,4,5,6-hexahydropyrimido[4,5-*d*]pyrimidin-4-yl)benzoyl chloride(5f)

Pale yellow crystals; Yield 70%; m.p. 202°C; IR (KBr): ύ/cm = 3,408 (NH), 3,350 (Ar-CH), 1674 (C=O) and 1,601 (C═N). ^{1}H-NMR (300MHz, dimethyl sulfoxide [DMSO]-d_6): δ (ppm): 2.51(s, 1H, =NH), 3.39 (s, 1H, CHNH), 5.31 (s, 1H, NH), 7.17–7.32 (m, 4H, Ar-H) 7.95 (s, 1H, 1NH), 11.90 (s, 2H, 2NH) and 12.04 (s, 1H, NHNH$_2$); ^{13}C-NMR (75MHz, DMSO-d_6) δ (ppm): 173.15, 163.02, 162.77,153.57,137.42, 132.80,129.94, 129.42, 128.02, 127.11and 90.24; Anal. Data for $C_{12}H_{11}ClN_6O$ (290.10). Calcd.: C, 49.55; H, 3.82; Cl, 12.15; N, 28.90%. Found: C, 49.59 H, 3.25; Cl, 12.16; N, 28.12%.

2.1.3 2-Imino-5-thioxo-5, 6-dihydro-2*H*-[1, 2, 4] thiadiazolo [2, 3-*a*] pyrimidin-7(3*H*)-one (6)

A mixture of 2-Amino-6-thioxo-5, 6-dihydropyrimidin-4(3*H*)-one1 (0.01) which dissolve in DMF on warming with (0.01mol) of thiourea and few drops of diluted HCL was heated under reflux for 8 h. The reaction mixture was cooled at room temperature and poured into ice .The product that obtained was filtered off, washed with water and crystallized from ethanol and DMF (10:1) to obtain compound 6. Yellow powder, Yield (78%); mp >360 °C; IR: ν_{max}/cm^{-1}: 3405 (NH) , 1716 (C=O) and 1238(C=S). ^{1}H NMR (DMSO-d_6, ppm) δ: 3.33 (s, 2H, 1 CH_2) 11.77 (s, 1H, =NH) and 13.36 (br.s, H, NH); ^{13}C-NMR (75MHz, DMSO-d_6) δ (ppm): 177.33, 173.16, 109.60 and 89.58; *Anal*. Calcd for $C_5H_4N_4OS_2$ (199.87): C, 29.99; H, 2.00; N, 27.96; S, 32.03. Found: C, 29.98; H, 2.00; N, 27.91; S, 31.93.

2.1.4 2-Amino-3*H*-thiochromeno [2, 3-*d*] pyrimidin-4*(5H)*-one (8)

A mixture of 2-Amino-6-thioxo-5, 6-dihydropyrimidin-4(3*H*)-one1 (0.01) which dissolve in DMF on warming with (0.01 mol), of benzaldehyde and few drops of tri ethyl amine was heated under reflux for 4 h. The reaction mixture was cooled at room temperature and poured into ice. The product that obtained was filtered off, washed with water and crystallized from ethanol and compound 8 was formed. Yellow powder, Yield (85%); mp 250 °C; IR: ν_{max}/cm^{-1}: 3390 (NH) and 1660 (CO); ^{1}H NMR (DMSO-d_6, ppm) δ: 3.36 (m, 2H, CH_2-thiopyran), 7.07–7.30 (m, 4H, Ar-H), 11.92 (s, 1H, NH) and 11.97 (s, 2H, NH_2). ESI MS *m/z* (rel. int.): 231.1700 $[M]^+$ ($C_{11}H_9N_3OS$); *Anal.* Calcd for $C_{11}H_9N_3OS$ (231.17): C, 57.12; H, 3.91; N, 18.16; S, 13.84. Found: C, 57.11; H, 3.98; N, 18.3; S, 13.81.

2.1.5 General procedure for the synthesis of compounds 10 a-b

A mixture of 2-Amino-3*H*-thiochromeno [2, 3-*d*] pyrimidin-4(5*H*)-one 8 (0.01 mol) which dissolve in ethanol on warming with (0.01) and/or pseudoamide-type (urea, thiourea), and (0.01) of ethoxide was heated under reflux for 6 h. The reaction mixture was cooled at room temperature and poured into ice. The product that obtained was filtered off, washed with water and crystallized from ethanol and compound (10a, b) was formed.

2.1.5.1 Imino-2,3-dihydro-[1,2,4]oxadiazolo[2,3-*a*]thiochromeno[2,3-*d*]pyrimidin-11*(10H)*-one(10a)

Yellow powder, Yield (73%); mp 260 °C; IR: ν_{max}/cm^{-1}: 3155 (NH) and 1612 (CO); ^{1}H NMR (DMSO-d_6, ppm) δ: 3.35 (m, 2H, CH_2- thiopyran), 7.07–7.35(m, 4H, Ar-H) and 12.01 (s, 1H, NH). MS (EI, 70 eV) *m*/*z* (%) = M^+ 374 (51.76 %) and 291(100 %). *Anal*. Calcd for $C_{12}H_8N_4O_2S$ (272.29): C, 52.93; H, 2.97; N, 20.57; S, 11.78. Found: C, 52.87; H, 2.95; N, 19.96 S, 11.75.

2.1.5.2 Imino-2,3-dihydro-[1,2,4] thiadiazolo [2,3-*a*] thiochromeno [2,3-*d*] pyrimidin-11*(10H)*-one (10b)

Yellow powder, Yield (80%); mp 262°C; IR: ν_{max}/cm^{-1}: 3398 (NH), 1612 (CO) and 1554 (CN); ^{1}H NMR (DMSO-d_6, ppm) δ: 3.35 (m, 2H, CH_2-thiopyran), 7.07–7.30 (m, 4H, Ar-H) and 11.87 (s, 1H, NH). MS (EI, 70 eV) *m*/*z* (%) = M^+ 374

(51.76 %) and 291(100 %). *Anal.* Calcd for $C_{12}H_8N_4OS_2$ (288.02): C, 49.97; H, 2.81; N, 19.47; S, 22.26. Found: C, 49.93; H, 2.79; N, 19.41; S, 22.16.

2.1.6 General procedure for the synthesis of compounds 12 a-b

A mixture of 2-Amino-6-thioxo-5, 6-dihydropyrimidin-4(3*H*)-one1 (0.02 mol) which dissolve in DMF on warming with and/or (malonic and/or succinic) hydrazide and few drops of tri ethyl amine was heated under reflux for 7 h. The reaction mixture was cooled at room temperature and poured into ice. Formed product was filtered off, washed with water and crystallized from ethanol to give compounds (12a, b).

2.1.6.1 3, 3'-Methylenebis (7-mercapto-[1, 2, 4] triazolo [4, 3-*a*] pyrimidin-5(1*H*)-one) (12a)

Brown crystals; Yield (70%); mp >360 °C; IR: ν_{max}/cm^{-1}: 3423 (NH) and 1631(C=O). ^{1}H NMR (DMSO-d_6, ppm) δ: 3.37 (s, 2H, 1 CH_2), 6.48 (s, 2H, Ar-CH), 11.62 (s, 2H, 2NH) and 11.66 (.s, 2H, 2SH); Anal. Calcd for C11H8N8O2S2 (348.37): C, 37.92; H, 2.31; N, 32.18; S, 18.43. Found: C, 37.82; H, 2.21; N, 32.17; S, 18.41.

2.1.6.2 3,3'-(Ethane-1,2-diyl)bis(7-mercapto-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one) (12b)

Orange crystals; Yield (85%); mp 280 °C; IR: ν_{max}/cm^{-1}: 3417 (NH) , 1631(C=O) and 1238(C=S). ^{1}H NMR (DMSO-d_6, ppm) δ: 3.37 (s, 2H, 2 CH_2), 6.48 (s, 2H, Ar-CH), 11.53 (s, 2H, 2NH) and 11.62 (.s, 2H, 2SH); ^{13}C-NMR (75MHz, DMSO-d_6) δ (ppm): 174.97, 162.29, 154.88, 78.64 and 40.47; ESI MS *m/z* (rel. int.): 262.1062 $[M+1]^+$ ($C_{12}H_{10}N_8O_2S_2$); *Anal.* Calcd for $C_{12}H_{10}N_8O_2S_2$ (362.07): C, 39.77; H, 2.76; N, 30.91; S, 17.73. Found: C, 39.71; H, 2.71; N, 30.81; S, 17.71.

2.2 Antiproliferative assay

Human gastric carcinoma (BGC-823), and lung cancer (A-549) cell lines were obtained from National Cancer Institut**e**, Cairo, Egypt. Cells were grown in Minimum Essential Medium Eagle media supplemented with 10% heat-inactivated fetal bovine serum, 0.01M HEPES, and 1 mM sodium pyruvate. Cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

Cell proliferation assay.

The antiproliferative activity of the newly synthesized coumarins was measured using MTT assay. The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, Exponentially growing cells were washed and seeded at 16000 cells/well (in 200 µl of growth medium) in 96-well microplates (Nunc, Denmark) for 24, 48, and 72 h. The antiproliferative effect of the tested compounds was examined after 72 h exposure of the cultured cells to varying concentrations of the test compound (total plate incubation time: 96 h), using MTT assay.16 The results were expressed as IC_{50} values, defined as the compound concentration required to reduce cell proliferation by 50%. Each compound was tested at every concentration in triplicate in a single experiment, which was repeated 3 times. The antiproliferative activity of tested compounds was compared to the activity of vincristine, used as a reference drug.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The current research will concentrate on the synthesis of new series from azolo pyrimidines and azino pyrimidines via reactions with various nucleophiles, as well as in vitro applications of pyrimidine derivatives, in order to aid in the development of more potent and effective antiproliferative agents. The cyclocondensation of a ternary mixture of mercaptopyrimidine **1**, [19] which was synthesised as a starting material as reported in the literature, yielded pyrimidopyrimidine of formulas **5a-f**. with aromatic aldehydes **2a-b** and/or pseudo amide-type **4a-c** under acidic condition (Scheme 1). The reaction may be involving the formation of non-isolable unsaturated system **3** followed by, conjugated addition of nucleophilic urea nitrogen leading to pyrimidine cyclization yielded **5a-f** (Mechanism 1). It has been reported that this reaction under acidic condition none of thiazine **7** was not obtained.

Pyrimidinedione derivatives **5a, b** were synthesized by the reaction of 2-Amino-6-thioxo-5, 6-dihydropyrimidin-4(3*H*)-one **1** with aromatic aldehyde **2a-b** and urea (Scheme 1). The IR spectrum of target compound **5a** revealed the presence of absorption bands at ύ 3405 , 1680 , 1657 and 1626 ppm for NH , 2 CO and C=N function groups respectively. ^{1}H-NMR spectrum of **5d** revealed down field exchanged signals that located at δ 12.06 and

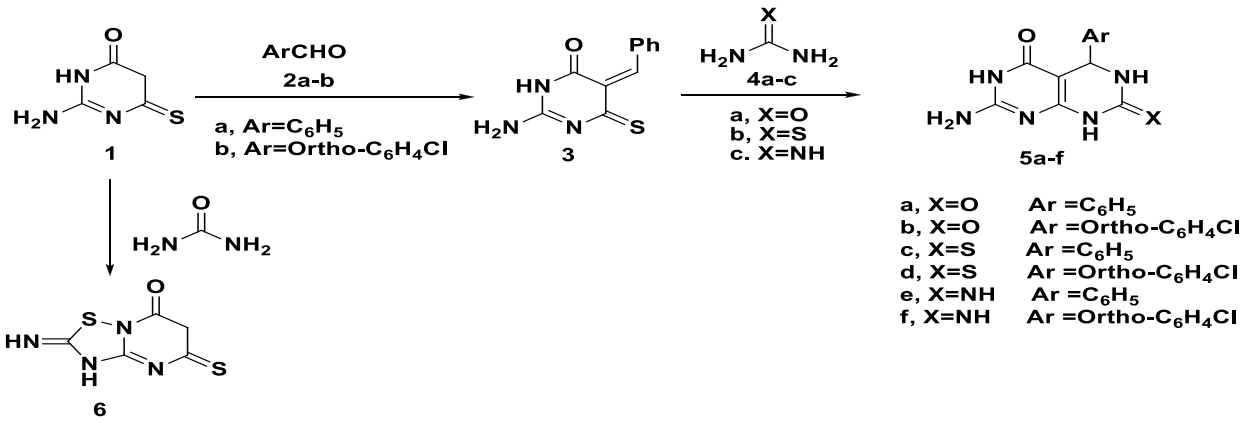
11.84 ppm for NH and NH_2 groups respectively. The other NH protons were observed at δ 7.95 and 5.36 ppm respectively. ^{13}C-NMR spectrum of **5a** showed signals attributable to two carbonyl carbons at δ 174.63 and 173.23 ppm. In the same manner, the down field exchanged signals for **5b** that located at δ 12.03 and 11.89 ppm for NH and NH_2. In addition, the other two protons of 2NH groups were observed at 7.96 and 6.58 ppm respectively. Compound **5b** showed stretching frequencies at $\acute{\upsilon}$ 3317 and 1650 (broad) for NH and carbonyl groups.

Pyrimidinethione derivatives **5c, d** were generated from the cycloaddition of a mixture of aromatic aldehyde and Thiourea (Scheme 1). NH signals of pyrimidopyrimidine **5c** displayed absorption at $\acute{\upsilon}$ 3406, 1662 (broad) and 1604 cm^{-1} due to NH, CO and C=N function respectively. In addition, IR spectrum of **5c** showed that, strong vibrational coupling is operative in the case of the nitrogen containing thiocarbonyl derivatives and three bands seem to consistently appear in the regions $\acute{\upsilon}$ 1334–1539 cm^{-1}, 1216–1435 cm^{-1}, and 940–1130 cm^{-1} due to the mixed vibrations. [20]

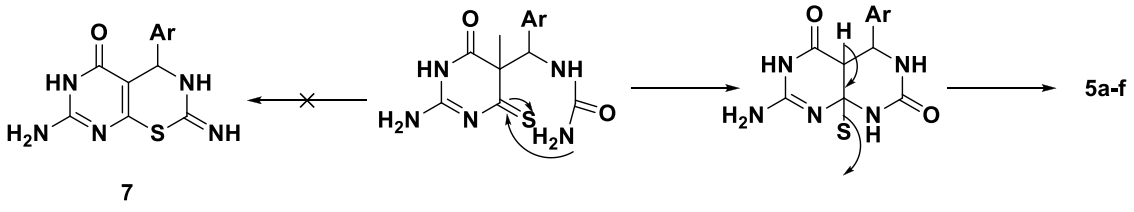
In the same manner, IR spectrum of **5d** showed stretching frequencies around 3406 and 1662 cm^{-1} corresponding to NH and CO function groups respectively. In addition, NH exchangeable signals appeared around δ 12.08, 11.85 (broad), 7.96 and 5.32 ppm. Where, the Carbonyl Carbon Signal was located at δ 173.28 ppm.

In the same manner, amino pyrimidopyrimidine **5e, f** was obtained from the reaction of aldehydes **2a, b** and guanidine with starting Compound **1** (Scheme 1). IR spectrum of Compound **5e** displayed frequencies for NH and CO function groups, in addition to its showed exchangeable signals for NH protons around δ 12.05, 11.82, 7.96 and 5.35 ppm. In the same manner, ^{13}C-NMR spectrum of **5f** showed that, carbonyl carbon at δ 173.15 ppm.

Thiourea undergoes nucleophilic substitution with the releasing of NH_3 followed by oxadizole oxidative cyclization producing fused system **6** (Scheme 1). IR spectrum of **6** revealed the presence of absorption bands at $\acute{\upsilon}$ 3405, 1716 and 1238 cm^{-1}, corresponding to NH, C=O and C=S function groups respectively. ^{1}H-NMR spectrum of **6** showed NH signal (D_2O oxide exchangeable) at δ 13.36 and 11.77 ppm. ^{13}C-NMR spectrum of **10** showed Carbon Signals corresponding to thioxo and carbonyl carbon at δ 177.33 and / or 173.16 ppm.



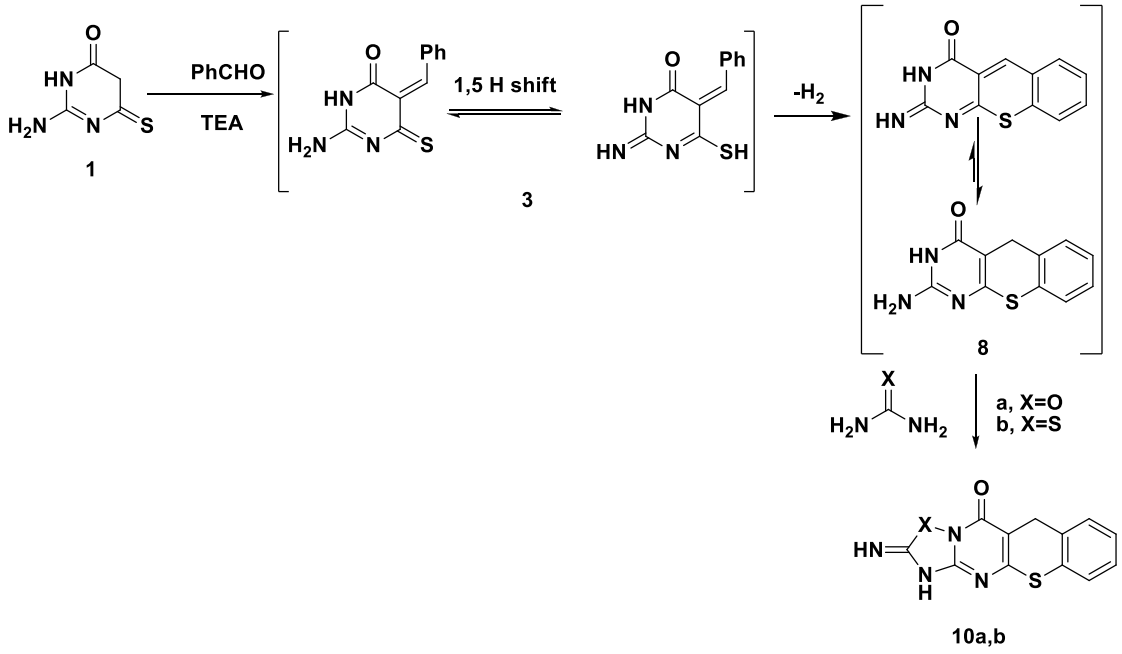
SCHEME 1. Synthesis of fused pyrimidines



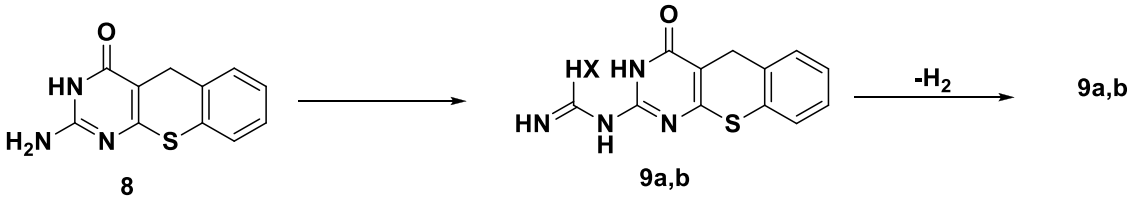
MECHANISM 1. Formation of Pyrimidopyrimidine derivaives

Base Mediated condensation of benzaldehyde and pyrimidinethione **1** resulted in polyheterocyclic **8** via the formation of non-isolable **3a**, 1,5 sigma tropic shift , followed by intra molecular oxidative Cyclization and subsequent dynamic 1,7 Sigma tropic migration equilibrium (Scheme 2) . IR spectrum of **8** showed the presence of absorption bands at $\acute{\upsilon}$ at 3390 and 1660 cm^{-1} for NH and CO function groups respectively. In addition, its ^{13}C-NMR spectrum of **8** showed that, NH Signal was observed at δ 11.92 ppm and CH_2 of thio pyran at δ 3.36 ppm.

Compound **8** was allowed to react with urea and /or thiourea gave the oxidative products **10a & 10b** through the formation of non–isolable urea **9a, b** derivative, followed by intra molecular autoxidation (Mechanism 2). IR spectrum of compound **10a** showed peaks at $\acute{\upsilon}$ 3155 and 1612 cm^{-1} for NH and CO Function groups. Also, produced signals at 12.01 ppm for NH proton and pyrimidines proton at δ 5.36 ppm. Thiourea undergoes substitution reaction with amino pyrimidine **1** with the evolution of NH_3 molecule followed by air oxidation leading to thiadiazole Cyclization (scheme 2 and Mechanism 2). Compound **10b** showed peaks at $\acute{\upsilon}$ 3398, 1612 and 1554 cm^{-1} for NH, CO, C=N groups respectively. The down field was observed at δ 11.87 ppm for NH proton.



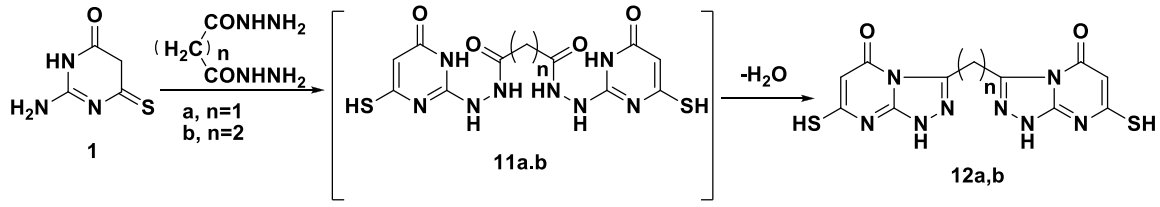
SCHEME 2. Synthesis of condensed pyrimidine derivatives



MECHANISM 2. Formation of compounds **9a-b** by intra molecular autoxidation

Malonohydrazide and glutarohydrazide undergo Cyclocondensation with pyrimidine derivative **1** providing triazole cyclization **12a,b** presumably via the non-isolable hydrazine derivative **11 a,b** that undergo intra molecular cyclodehydration via the attack of cyclic Nucleophilic iminonitrogen to Carbonyl group (Scheme 3). IR spectrum of compound **12a** showed peakes around $\acute{\upsilon}$ 3423 and 1631 cm^{-1} due to NH, Co groups respectively. The target **12a** led to SH, NH exchangeable signal around δ 11.62 and 11.52 ppm. The pyrimidine derivative **12b** displayed exchangeable signals at δ 11.62 and

11.53 ppm for SH and NH. Carbonyl Carbon signals was located at δ 174.97 ppm.



SCHEME 3. Synthesis of triazolopyrimidine **12a-b**

3.2 Antiproliferative activity

The most frequent mechanism of anticancer medication activity is to stop malignant cells from multiplying by interfering with cell division.[21] New treatments for cancer treatment, such as chemotherapy and radiotherapy, deliver the finest results, but they also have a variety of adverse effects. The cell lines of various origins – human gastric carcinoma (BGC-823) and human lung cancer (A-549) were chosen as representative examples of oncological disorders to assess the antiproliferative activity of the synthesized novel compounds. (Table 1). Furthermore, cytotoxicity was assessed on the NIH/3T3 mouse fibroblast cell line. The antiproliferative potential of compounds 5a–f, **6, 8,10a-b,** and **12a-b** was investigated in this study, with the expectation that they would have high activity against cancer cells. New compounds **5c, 5d, 6, 8,10a** and **10b** exhibited antiproliferative potential against the tested cell lines with some differences in selectivity because of the accompanying structure activity relationship's (SAR's):

(a) In compounds **(5c and 5d)** which contain thioxo bearing pyrimidopyrimidine ring.
(b) In compound **(6)** which contains both thioxo and thiadiazolo pyrimidine rings.
(c) In compound **(8)** that contains thiochromeno pyrimidine ring.
(d) In compound **(10a)** that contains oxadiazole thiochromeno pyrimidine ring.
(e) Because of the thiadiazolidine imine nucleus linked to the thio pyran ring, compound (**10b)** had a stronger antiproliferative potential than the other compounds. When compared to the results obtained, the antiproliferative effect of other drugs in vitro was substantially moderate against cell lines.

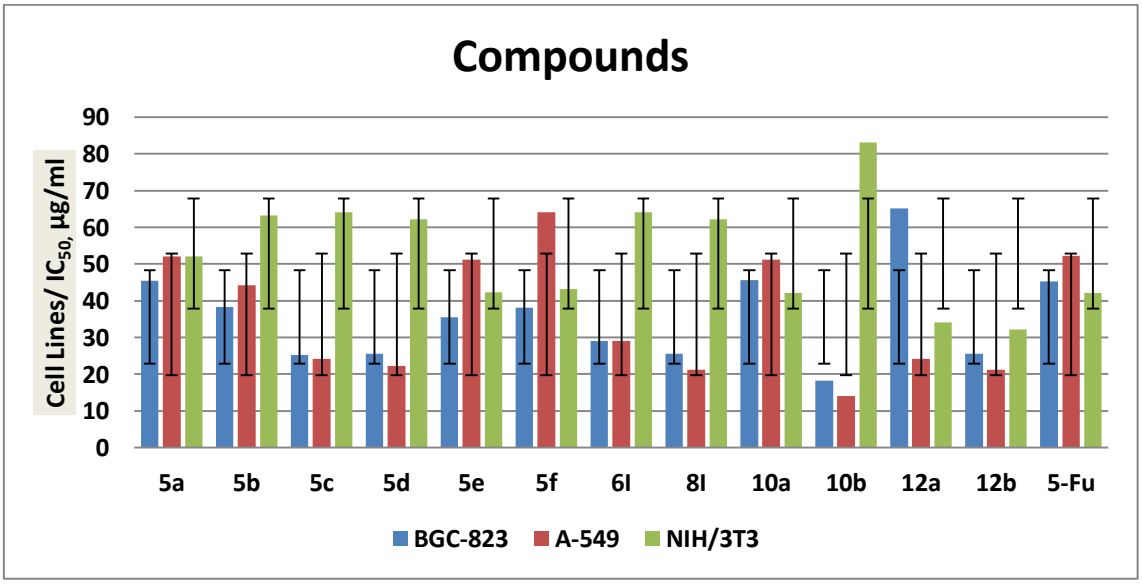


Figure 1. Antiproliferative activity of the synthesized compounds

Table 1. Antiproliferative activity of the synthesized compounds

Compounds	Cell lines / IC_{50}, µg/ml		
	BGC-823	**A-549**	**NIH/3T3**
5a	45.51 ± 0.12	52.13 ± 0.34	52.14 ± 0.12
5b	38.21 ± 0.11	44.15 ± 0.23	63.14 ± 0.13
5c	25.15± 0.11	24.21 ± 0.24	64.11 ± 0.23
5d	25.51 ± 0.43	22.25 ± 0.23	62.15 ± 0.15
5e	35.48 ± 0.12	51.15 ± 0.84	42.24 ± 0.12
5f	38.12 ± 0.22	64.15 ± 0.33	43.21 ± 0.14
6	29.11± 0.12	29.11 ± 0.24	64.11 ± 0.21
8	25.51 ± 0.44	21.15 ± 0.23	62.24 ± 0.21
10a	45.58 ± 0.42	51.15 ± 0.84	42.14 ± 0.22
10b	18.22 ± 0.22	14.15 ± 0.33	83.14 ± 0.13
12a	65.15± 0.12	24.11 ± 0.24	34.11 ± 0.21
12b	25.51 ± 0.44	21.15 ± 0.23	32.14 ± 0.25
5-Fu	45.18 ± 0.12	52.25 ± 0.81	42.14 ± 0.32

[a] 5-Fu= 5-Fluorouracil

4. CONCLUSIONS

In conclusion, we have shown that mercaptopyrimidine reacts with urea and aromatic aldehydes to generate azino and azolo Pyrimidines in both acidic and basic media. As seen by the results against various cell lines, several of these compounds have good-moderate efficacy when compared to commercial Vincristine (positive control). Using the structure–activity connection as a guide. The activity of a compound thiadiazolidine imine nucleus linked to a thio pyran ring against A-549 cells was greater.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests.

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