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Synthesis and cytotoxic evaluation of some Pyrimidine derivatives

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ABSTRACT:

***Background*:** It had been found that some 5-substituted-2-thiouracils are biologically active as anticancer and as antimicrobial agents. *Aim:* As a part of ongoing studies in developing new pyrimidine derivatives, a series of some new thio pyrimidine derivatives were synthesized and evaluated for their cytotoxic activities. *Material and method:* The synthesis of targeted compounds occured *via* chlorosulphonation of 2-thiouracil to give a very rective sulphonyl chloride (2) which in turn was reacted with all isomers of anisidine yielding sulphonamide derivatives (3a-c) which were chlorinated with $POCl_3/PCl_5$ giving chloropyrimidines (4a-c), which were reacted with *p*-aminoacetophenone to give derivatives (5a-c). In another pathway, chloropyrimidines (4a-c) were reacted with hydrazine giving hydrazine derivatives (6a-c). Furthermore, chloropyrimidines (4a-c) were cyclo condensed with glycine yielding immidazopyrimidines (7a-c). In addition, hydrazinopyrimidines (6a-c) were cyclo condensed with Ac_2O, trimethylformate and diethyloxalate giving derivatives (8a-c), (9a-c) and (10a-c), respectively. Finally, they were reacted with *p*-nitrobenzaldehyde yielding derivatives (11a-c). The cytotoxic activities of the prepared compounds were screened, and some of the synthesized compounds showed significant cytotoxic activities, either pyrimidines or condensed pyrimidines. Cell viability was determined using the trypan blue exclusion method. Cells were stained with trypan blue and counted with a hemocytometer. *Results and discussion:* 2-thiouracil-5-sulphonamides have a cytotoxic activity comparable to 2-thiouracil itself. *Conclusion*:Substitution of 2-thiouracil at 5th position may retain its cytotoxic activity.

KEYWORDS: Pyrimidines-5-sulphonamides with cytotoxic activity.

1. INTRODUCTION

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use as antiallergic[1,2] antiinflamatory[3,4], anxiolytic[5], tranquilizer[6], anticonvulsant[7,8], antimalarial[9], antiviral[10-12], and antitumor agents[13-15]. There are a large number of pyrimidine-based anti metabolites structurally related to the endogenous substrates, thus they can inhibit nucleic acid synthesis. The structural modification may be based on pyrimidine ring or the pendant sugar group. One of the early metabolites was 5-fluorouracil[16], a pyrimidine derivative. Also 5-substituted-2-thiouracils especially, 2-thiouracil-5-sulphonamides[10,15] exhibited some useful antineoplastic activites. The cytotoxic activities of compounds possessing the guanine nucleus like azathioprine[17], mercaptopurine[18], thioguanine[19], tegafur[20], …..etc were discovered and evaluated. These drugs prevent the utilization of normal cellular metabolites[21] . There are many more in recent times, like mopidamol[22], nimustine[23], raltitrexed[24], uramustine[25], trimetrixate[26]and gemcitabine[27], all are pyrimidine metabolites showed excellent antitumor activity against murine solid tumors. From 1957 to 2015, different research laboratories investigated the anticancer activity of some 5- and 6-substituted-2-thiouracils and reported that the tested compounds found to inhibit DNA synthesis[28-33]. Based on these findings the present work aims to synthesize a new group of pyrimidine compounds incorporated with different heterocycles to investigate whether the resulting compounds have better biological activities as anticancer agents.

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2. MATERIAL

2. 1. Chemicals

2-thiouracil, chlorosulphonic acid, isomers of anisidine, pyridine, absolute ethanol, dimethyl formamide, phosphorus pentachloride, phosphorus oxychloride, *p*-aminoacetophenone hydrazine hydrate, glycine, n-butanol, glacial acetic acid, acetic anhydride, trimethylorthoformate, diethyloxalate, and *p*-nitrobenzaldehyde.

2.2. Animals

Female Swiss Albino mice from the animal house of Cairo Cancer Institute, weighing 18-22 gm were used. Animals were maintained on standard pellet diet and water.

2.3. Tumor cell lines

Ehrlich ascites (E.A.C), maintained in the laboratory by weakly interperitoneal transplantation in female Swiss Albino mice[34,35].

3. METHODS

3.1 Chemistry

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus (Shimadzu,Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA) . ^{1}HNMR spectra (300MHz) were recorded in dimethyl sulfoxide (DMSO) by employing tetramethyl silane (TMS) as an internal standard on Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) and the chemical shifts (δ) were expressed as ppm against TMS as internal standard. Mass spectra were recorded on a 70 ev EI Ms-QP 1000 (Shimadzu,Japan). Microanalyses were operated using vario, Elementar apparatus (Shimadzu). The progress of all the reactions was monitored by TLC on silica gel 60 for TLC (Merck) using chloroform-meanol (3:1) as mobile phase and spots were visualized by iodine vapours or by irradiation with UV-light (254nm).

Synthesis of 4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonylchloride: as in literature[15]

Synthesis of N-(2,3 or 4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonamides (3a – c).

A mixture of 2-thiouracil-5-sulphonamide 2 (1.13g, 0.005 mole), isomers of anisidine (0.6g,0.005 mole) and pyridine (0.4 mL, 0.005 mole) in absolute ethanol (50 mL) was heated under reflux for 12h, then cooled, filtered off, dried and recrystallized from dimethyl formamide (DMF)/water.

Yield, 3a (0.8 g, 67%), m.p 273-275 ^{o}C . 3b (0.6 g, 61%), mp 256-258 ^{o} C. 3c (0.5 g, 58%), mp 282-284^{o}C.

IR(KBr): 3300-3020(3NH, very broad), 1660 (CO), 1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d_6): δ 4.1 (s,3H,OCH_3), 7.1–7.3 (m,4H,ArH), 8.2 (s,1H,pyrimidine), 9.8, 10.2, 10.5 (s,3NH, D_2O-exchangeable). Ms m/z (10.43 %) 313.12 [M^+] . Analysis for C, H and N, $C_{11}H_{11}N_3O_4S_2$ Calcd: C, 42.17; H, 3.51; N, 13.42. Found: C, 42.31; H, 3.72; N, 13.55.

Synthesis of 4-Chloro-N-(2,3 or 4-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-sulphonamide (4a– c).

A mixture of 3a – c (0.01 mole) and phosphorus pentachloride (0.01 mole) in phosphorus oxychloride (20 mL) was heated on a steam bath for 3hrs and the reaction mixture poured gradually on to crushed ice. The precipitate was filtered off, dried, then crystallized from DMF/water.

Yield,4a (0.6 g,61%), m.p 283-284 ^{o}C . 4b (0.7 g, 64%), mp 267-268^{o}C. 4c (0.8 g, 69%), mp 262-264^{o}C.

IR(KBr): 3310-3026(3NH, very broad), 1670 (CO), 1325,1135 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 4.2 (s,3H,OCH_3), 7.1–7.2 (m,4H,ArH), 8.1 (s,1H,pyrimidine), 9.7, 10.6 (s,2NH, D_2O-exchangeable). Ms m/z (17.45%) 331.57 [M^+], (5.71%) [$M+2^+$],. Analysis for C, H and N, $C_{11}H_{10}N_3O_3S_2Cl$, Calcd: C, 39.82 ; H, 3.02 ; N, 12.67. Found: C, 39.71 ; H, 3.29 ; N, 12.45.

Synthesis of 4-[(4-acetylphenyl)amino]-N-(2,3or4-methoxyphenyl) -2-thioxo-1, 2-dihydropyrimidine -5-sulphonamides (5a – c).

A mixture of 4a – c (1 mole) and *p*-aminoacetophenone (1 mole) in pyridine (30 mL) was refluxed for 8-10 hrs and the reaction mixture poured gradually on water and then neutralized till acidification. The precipitate was filtered off, dried then crystallized from DMF/water.

Yield, 5a (0.7 g,66%), m.p 243-245^{o}C . 5b (0.8 g, 73%), mp 286-288^{o}C. 5c (0.8 g, 65%), mp 277-279^{o}C.

IR(KBr) : 3315-3028 (3NH, very broad), 1670 (CO), 1690 (CO) 1322,1145 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 2.2 (s,3H,CH_3), 4.2 (s,3H,OCH_3), 7.2–7.9 (m,8H,ArH), 8.3 (s,1H,pyrimidine), 6.7,9.6, 10.6 (s,3NH, D_2O-exchangeable). Ms m/z (9.85%) 430.32 [M^+]. Analysis for C, H and N, $C_{19}H_{18}N_4O_4S_2$ Calcd: C, 53.02; H, 4.19 ; N, 13.02. Found: C, 53.19 ; H, 3.99 ; N, 13.42.

Synthesis of 4-Hydrazinyl-N- (2,3 or 4-methoxyphenyl)- 2-thioxo-1,2-dihydropyrimidine-5-sulphonamides (6a – c).

A mixture of 4a – c (0.01 mole) and hydrazine hydrate (0.01mole) in methanol (10 mL) was stirred for 8 hrs. The precipitate was filtered off, dried then crystallized from DMF/water.

Yield,6a (0.5 g,55%), m.p 263-265^{o}C . 6b (0.6 g, 62%), mp 294-296^{o}C. 6c (0.8 g, 68%), mp 280-282^{o}C.

IR(KBr) : 3340-3044(3NH,NH_2 very broad), 1681 (CO), 1320,1148 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ, 4.1 (s,3H,OCH_3), 7.2–7.4 (m,4H,ArH), 8.1 (s,1H,pyrimidine), 6.7, 9.6, 10.6, 10.4 (s,NH_2, 3NH, D_2O-exchangeable). Ms m/z (18.45%) 325.13 [M^+]. Analysis for C, H and N,

$C_{11}H_{13}N_5O_3S_2$, Calcd: C, 40.62; H, 3.39; N, 21.54. Found: C, 40.47.19; H, 3.41; N, 21.42.

Synthesis of N-(2,3 or 4-methoxyphenyl)-3-oxo-5-thioxo-1,2,3,5,6,8a-hexahydroimidazo[1,2-c]pyrimidine-8-sulphonamides (7a – c).

A mixture of 4a – c (0.01 mole) and glycine (0.01 mole) in n-butanol (30 mL) was heated under reflux for 3 hr. The solid separated was refluxed with anhydrous acetic acid (5 mL) for 2 hr. The precipitate was filtered off, dried then crystallized from DMF/ water.
Yield,7a (0.6 g,57%), m.p 243-245^oC . 7b (0.7 g, 64%), mp 267-269^oC. 7c (0.8 g, 66%), mp 287-289^oC.
IR(KBr) : 3345-3034 (3NH, very broad), 1687 (CO), 1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 2.1(s,2H,CH_2), 4.2 (s,3H,OCH_3), 7.1–7.4 (m,4H,ArH), 8.2 (s,1H,pyrimidine), 9.7, 10.4, 10.5 (s,,3NH, D_2O-exchangeable). Ms m/z (11.45%) 352.08 [M^+]. Analysis for C, H and N, $C_{13}H_{12}N_4O_4S_2$ Calcd: C, 44.32; H, 3.41 ; N, 15.91. Found: C, 44.19 ; H, 3.48 ; N, 16.02.

Synthesis of N-(2,3 or 4-methoxyphenyl)-3-methyl-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-c]pyrimidine-8-sulphonamides (8a – c).

A mixture of 6a – c (0.01 mole) and acetic anhydride (30 mL) was heated under reflux for 4hr. The solid obtained was filtered off, dried and crystallized from DMF/water.
Yield,8a (0.6 g,58%), m.p 277-279^oC . 8b (0.7 g, 68%), mp 244-246^oC. 8c (0.8 g, 67%), mp 276-278^oC.
IR(KBr) : 3350-3037(2NH, very broad), 1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 2.3(s,3H,CH_3), 4.1 (s,3H,OCH_3), 7.1–7.4 (m,4H,ArH), 8.1 (s,1H,pyrimidine), 10.2,10.5 (s,,2NH, D_2O-exchangeable). Ms m/z (11.45%) 352.08 [M^+]. Analysis for C, H and N, $C_{13}H_{13}N_5O_3S_2$, Calcd: C, 44.44; H, 3.70 ; N, 19.94. Found: C, 44.19 ; H, 3.68 ; N, 19.77.

Synthesis of 8-(-N-(2,3 or 4-methoxyphenyl)- -5-thioxo-5,6-dihydro [1,2,4]triazolo [4,3-c]pyrimidine-8-sulphonamides (9a – c).

A mixture of 6a–c (0.01 mole) and trimethylorthoformate (30 mL) was heated under reflux for 6 hr. The solid obtained was filtered off, dried, and crystallized from DMF/water.
Yield, 9a (0.6 g,58%), m.p 283-285^oC . 9b (0.8 g, 69%), mp 276-278^oC. 9c (0.8 g, 68%), mp 297-299^oC.
IR(KBr) : 3335-3027 (2NH, very broad),1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 2.1(s,1H,CH), 4.1 (s,3H,OCH_3), 7.2–7.4 (m,4H,ArH), 8.1 (s,1H,pyrimidine), 10.2,10.4 (s,,2NH, D_2O-exchangeable). Ms m/z (11.45%) 337.24 [M^+]. Analysis for C, H and N,$C_{12}H_{11}N_5O_3S_2$, Calcd: C, 42.73; H, 3.26 ; N, 20.77. Found: C,42.87 ; H, 3.38 ; N, 20.67.

Synthesis of N-(2,3 or 4-methoxyphenyl)-3,4-dioxo-6-thioxo-3,4,6,7-tetrahydro-2H-pyrimido[6,1-c][1,2,4]triazine-5-sulphonamides (10a – c).

A solution of 6a – c (0.01 mole) and diethyl oxalate (0.01 mole) in absolute ethanol (40 mL) was heated under reflux for 12 hr. The solid obtained was filtered off, dried and crystallizefromDMF/water. Yield,10a (0.5 g,52%), m.p 267-269 ^oC . 10b (0.6 g, 64%), mp 256-8^oC. 10c (0.6 g, 66%), mp 277-279^oC. IR (KBr) : 3322-3037(2NH, very broad), 1680, 1687 (2 CO), 1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 4.1 (s,3H,OCH_3), 7.2–7.5 (m,4H,ArH), 8.2 (s,1H,pyrimidine),6.8 10.2,10.3 (s,,3NH, D_2O-exchangeable). Ms m/z (13.87%) 381.24 [M^+]. Analysis for C, H and N,$C_{13}H_{11}N_5O_5S_2$, Calcd: C, 40.94; H, 2.89; N, 18.37. Found: C,40.87; H, 2.99; N,18.45.

Synthesis of N-(2,3 or 4-methoxyphenyl)-4-[(2E)-2-(4-nitrobenzylidene) hydrazinyl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonamides (11a – c).

A mixture of 6a – c (0.01 mole) and *p*-nitrobenzaldehyde (0.01 mole) in ethanol (30 mL) was heated under reflux for 8 hr. The solid obtained was filtered off, dried and crystallized from DMF/water.
Yield,11a (0.7 g,70%), m.p 257-259 ^oC . 11b (0.5 g, 54%), mp 277-278^oC. 11c (0.8 g, 78%), mp 258-259^oC. IR(KBr) : 3332-3027(3NH, very broad),1320,1140 (SO_2) cm^{-1}. ^{1}HNMR (DMSO-d6): δ 4.1 (s,3H,OCH_3),6.8(s,1H), 7.2–7.4 (m,8H,ArH), 8.2 (s,1H,pyrimidine),6.9 10.3,10.5 (s,,3NH, D_2O-exchangeable). Ms m/z (8.37%) 460.12 [M^+]. Analysis for C, H and N, $C_{18}H_{16}N_6O_5S_2$, Calcd: C, 46.96; H, 3.48 ; N, 18.26. Found: C,46.85 ; H, 3.56 ; N,18.33.

3.2.Evaluation of cytotoxic activity:

A line of Ehrlich ascites carcinoma (E.A.C) obtained from Cairo Cancer Institute was used in this study for evaluation of the newly prepared compounds for their antitumor activity.

3.3.In vitro test for cytotoxic effect

The biological evaluation of some selected compounds was carried out at the National Cancer Institute in Cairo. A set of sterile test tubes were used, where $2.5x10^5$ tumor cells per mL were suspended in phosphate buffer saline (0.1M), then 25, 50, 100 µg/mL of the tested compounds were added to the suspension kept at 37^oC for 2hr. Trypan blue dye exclusion test was then carried out to calculate the percentage of non viable cells[36].

Table (1) Effect of some pyrimidine derivatives on the viability of tumor cells in vitro

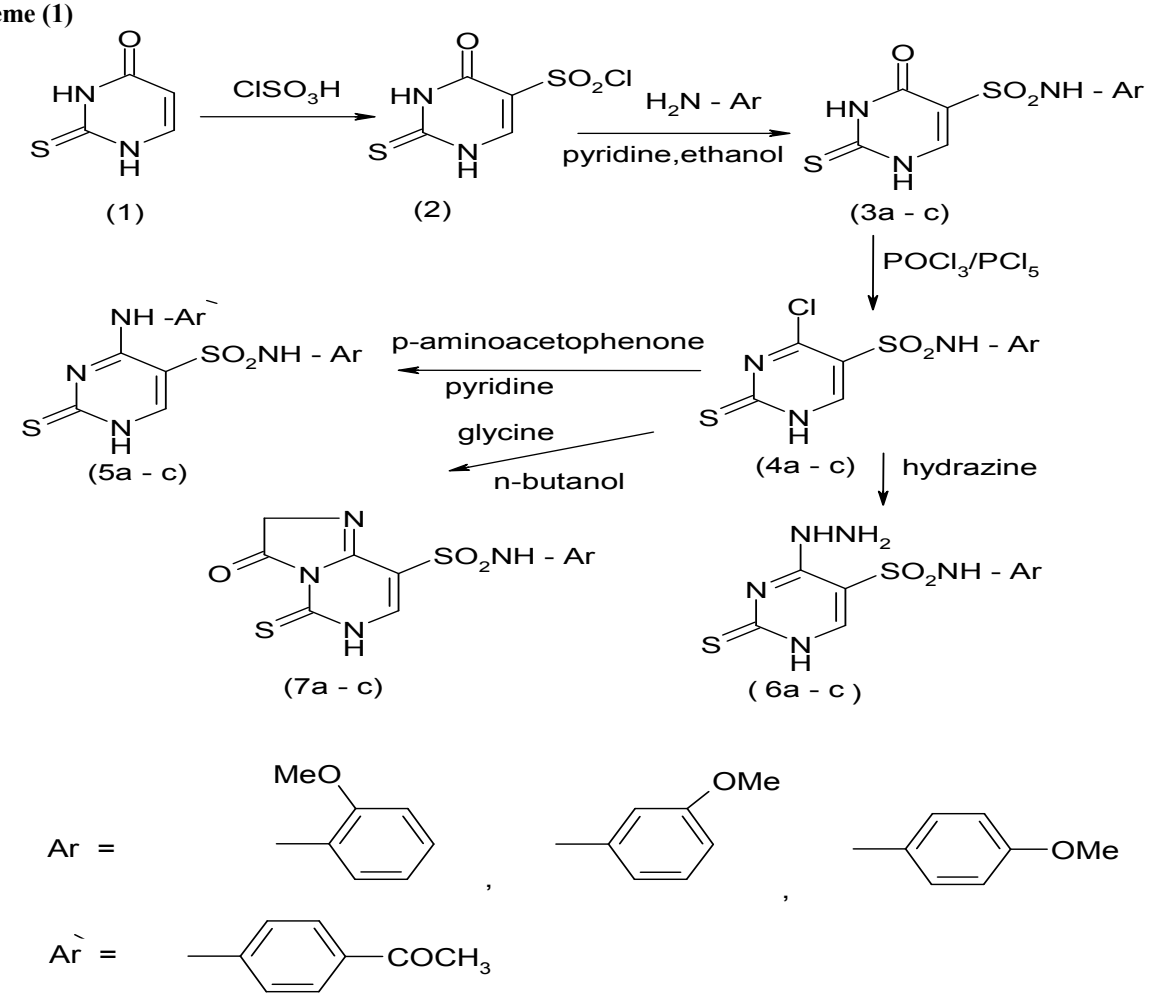
Sample NO.	% Inhibition of cell viability µg/ mL		
	100	50	25
3a	70	50	10
4a	60	30	10
5a	Zero	Zero	Zero
6a	Zero	Zero	Zero
7a	70	50	10
8a	80	60	10
9a	70	40	10
10a	60	40	10
11a	Zero	Zero	Zero

4. RESULTS AND DISCUSSION

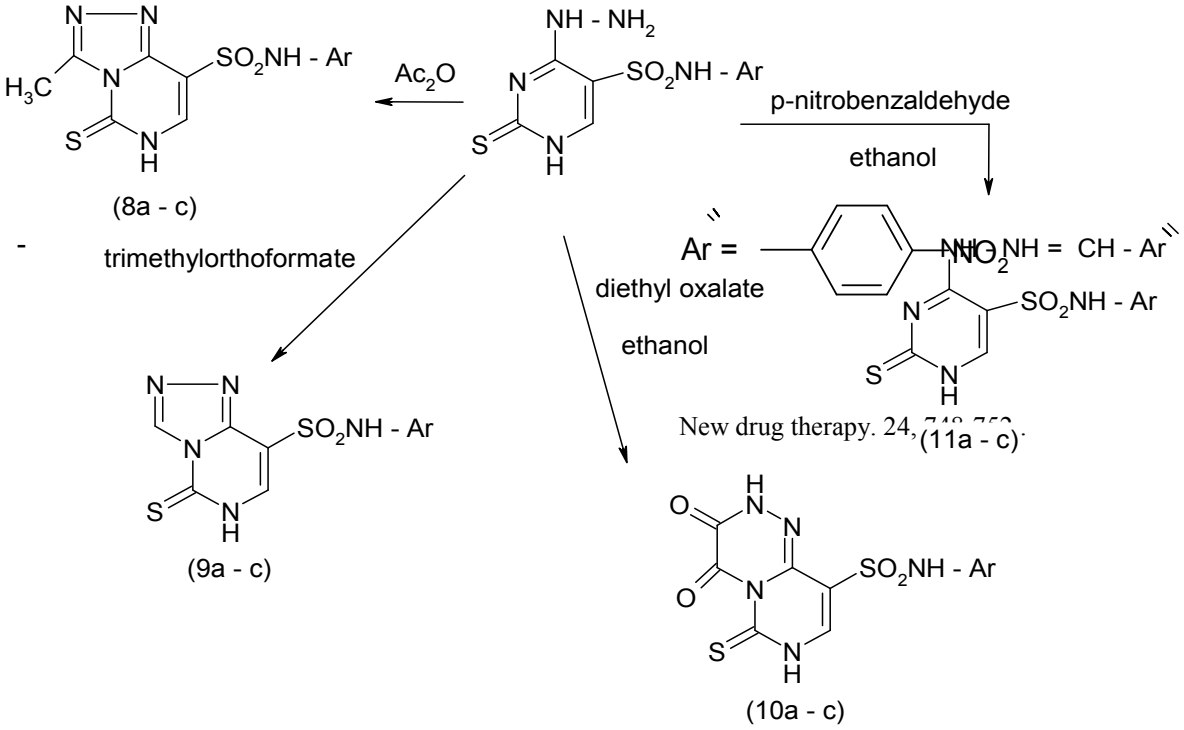
In fact, pyrimidine itself is un reactive toward electrophilic substitution reactions due to the –I and –M effects of the two nitrogens, while 2-thiouracil is active due to the electron releasing effect of OH and SH groups[38], thus, 2-thiouracil could be chlorosulphonated in a good yield as reported by Fathalla et al[15] to give an active sulfonyl chloride (2) which in turn could be reacted with isomers of anisidine (*o*,*m*,and *p*-anisidine) in presence of an acid binder such as pyridine in ethanol as a solvent yielding sulphonamide derivatives (3a – c) which in turn could be chlorinated using $POCL_3/PCl_5$ mixture giving active chloropyrimidine derivatives (4a – c), these chloro compounds could be reacted with *p*-aminoacetophenone in pyridine as a solvent and an acid binder yielding compounds (5a–c), also they could be hydrazinolysed by NH_2NH_2 giving hydrazinopyrimidines (6a–c). In another pathway they could be cyclocondensed with glycine in butanol as a solvent giving imidazopyrimidines (7a–c). In another remarkable reactions, hydrazinopyrimidines (6a–c) were cyclocondensed with Ac_2O into triazolopyrimidines (8a–c), also they could be reacted with trimethylorthoformate giving triazolopyrimidines (9a–c) and reacted with diethyloxalate yielding pyrimidotriazines (10a–c), finally they could be condensed with *p*-nitrobenzaldehyde giving Schiff 's bases (11a – c).

The data of selected pyrimidine derivatives 3a, 4a, 5a, 6a, 7a, 8a, 9a, 10a and 11a showed that 2-thiouracil-5-sulphonamide is active, when chlorinated, it is still active but when chlorine is replaced by NH group, the activity is abolished as in compounds 5a, 6a and 11a, while cyclo-condensation of chloro derivative 4a retains the activity as in compounds 7a, 8a, 9a, and 10a.

Scheme (1)



Scheme (2)



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