SYNTHESIS, REACTIONS AND ANTITUMOR EVALUATION OF SOME POLYCONDENSED THIAZOLOPYRIMIDINE DERIVATIVES

Nermin A. M. El-Seadawy

Department of Chemistry, Faculty of Science (Girls), Al-Azhar University, Nasr City, Cairo, Egypt

ABSTRACT

Some novel thiazolo[3,2-a]pyrimidines, pyrimidino[1',2':3,2]thiazolo[4,5-b] pyridines,pyrimidino[1",2":3',2']thiazolo[4',5':2,3]pyrido-[2,3-d]pyrimidines, pyrimidino [1',2':3,2]thiazolo[4,5-d]pyrimidines, pyrimidino[1',2':3,2]thiazolo[4,5-d][1,2,4] triazolo[3,4-b]pyrimidines **(2-10)** were prepared starting with 2-(arylmethylene) trimethylthiazolo [3,2-a]pyrimidin-3-one**(2)**. Also, some, S-alkylated thiazolo[4,5-d] pyrimidine derivatives were synthesized via reaction of 4-substited-7,7,9-trimethyl-1,3,4-trihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidin-2-thione **(6b)** with different reagents. Furthermore, some of the prepared products were selected and tested for activity against HCT116 and MCF-7 (human colon carcinoma and human breast carcinoma).

Keywords: Antitumor evaluation, thiazolopyrimidines, triazolopyrimidines.

1. INTRODUCTION

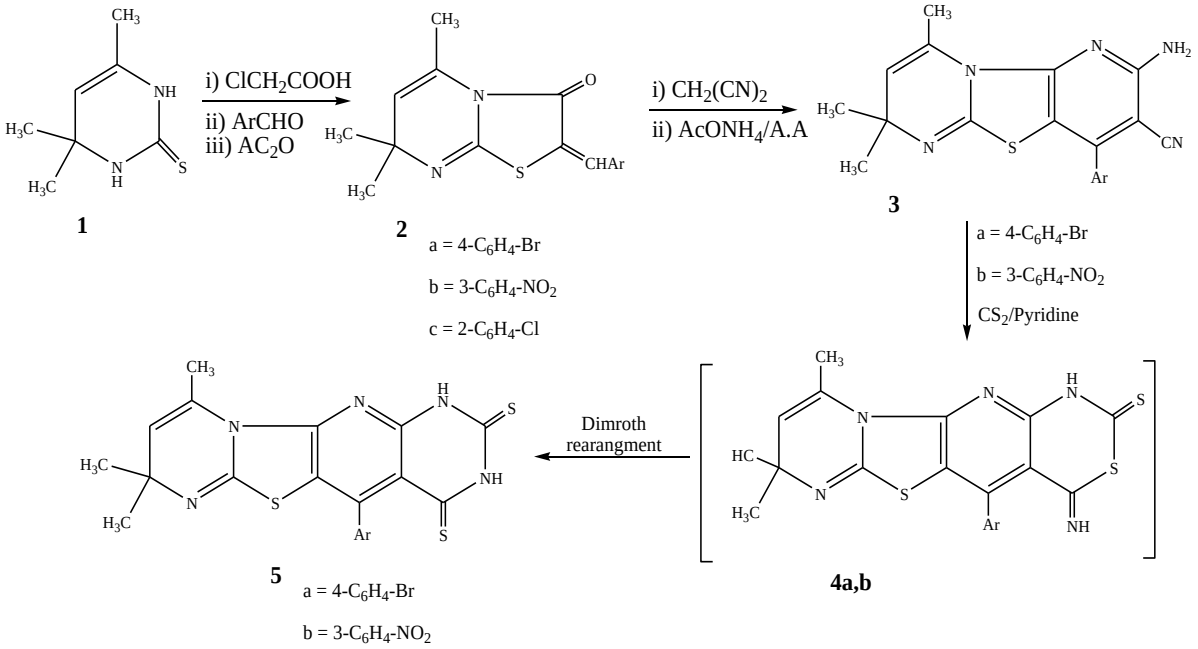
Fused thiazolo[4,5-d]pyrimidine derivatives are one of the most important classes of compounds because of their effective biological activity and their synthesis attracted great attention. **(Beek, *et al.*, 1999; Baxter, *et al.*, 2006; Walter, *et al.*, 2007).** They bear structural resemblance to purine and several substituted thiazolo[4,5-d]pyrimidine (a guanosine analogue) exhibited *in vivo* activity against a variety of RNA and DNA viruses **[Kini, *et al.*, 1991)** and human cytomegalovirus (HCMV) **(Lewis, *et al.*, 1995)**. Also, has antitumor antimetastatic properties **(Nagahara, K., 1990)**. Recently, many of their derivatives have been synthesized as potential anticancer **(Fahmy, *et al.*, 2003)**, anti-inflammatory activity **(Bekhit, *et al.*, 2003; Abdel-Megeid, *et al.*, 2005),** and antimicrobial activity **(Bekhit, *et al.*, 2003; Rashad, *et al.*, 2005, Rashad *et al.*, 2010).** Besides, prominent biological activities have been reported for pyrmidine derivatives, such as antiviral **(Hegab *et al.*, 2006; Rashad, *et al.*, 2007; Shamroukh, *et al.*, 2010)**; antihypertensive **(Shishoo, *et al.*, 2000);** antihistaminic **(Ammar, *et al.*, 2002);** analgesic**; (Amr, *et al.*, 2003; Alagrasamy, et al., 2007; Wagnat, *et al.*, 2008)**. In connection with our research program, for the synthesis of different fused hetrocyclic compounds having antitumor activity **(HO, *et al.*, 2003; Amr, *et al.*, 2006),** we describe here the synthesis of some new pyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine derivatives hoping to show promising antitumor activity.

2. RESULTS AND DISCUSSION

With respect to the previous studies carried out in our laboratory, 2-thioxo-pyrimidine derivative **1,** precurosors **(Takeshima, *et al.*, 1968)** have been regarded as promising intermediates to produce thiazolopyrido pyrimidin-2,4-dithione derivatives **5,a,b** and thiazolo[4,5-d]pyrimidin-2-thione derivatives **6a-c** which bear some structural analogies with natural nucleobases. The interaction of pyrimidin-2-thione derivative **1** with

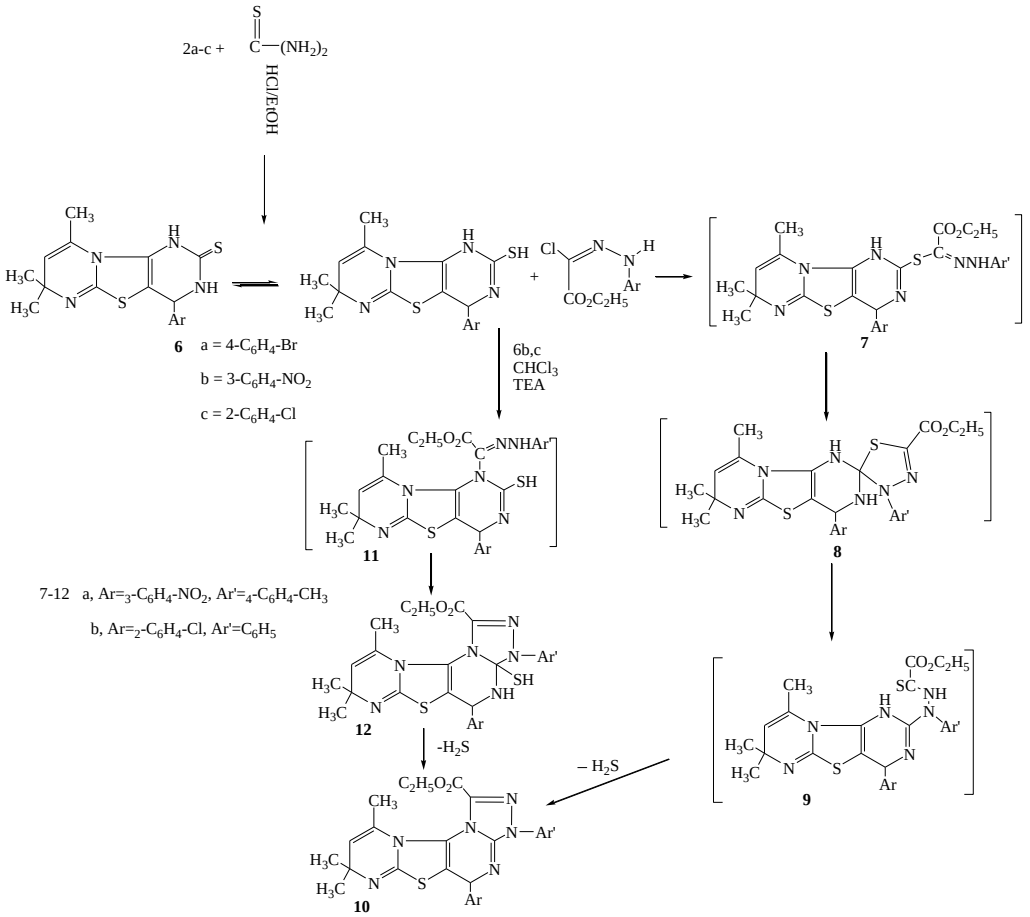
chloroacetic acid and aromatic aldehydes in the presence of acetic anhydride **(Hamman, *et al.*, 1981)** led to the formation of 2-(arylmethylene) trimethylthiazolidino[3,2-a]pyrimidin-3-one **2a-c** (Scheme 1). The structures of compounds **2a-c** were deduced from elemental analysis and spectral data. Compound **2b** as an example: its IR spectrum showed absorption band at 1706cm^{-1} (υ_{CO}); moreover, ^{1}H NMR spectrum showed signals at 7.36 ppm (s, 1H,=CH–Ar) and 7.80-7.99 (m, 4H, ArH).

Interaction of compound **2a,b** with malononitrile in acetic acid in the presence of ammonium acetate under reflux led to the formation of enaminonitrile derivatives **3a,b** (Scheme 1). The IR spectrum of compound **3a** showed the presence of bands characteristic for a cyano group at 2193cm^{-1} and amino group at 3209 & 3344 cm^{-1}. The pyrimidin-2,4-dithione derivatives **5a,b** were obtained by refluxing of **3a,b** with carbon disulfide in dry pyridine **(Hefez, *et al.* 2010)** as shown in (Scheme 1). The proposed structure was confirmed by spectral data. The IR spectrum of **5a** revealed the absence of a band characteristic for the cayno group, moreover, the ^{13}C NMR spectrum showed signal at δ 176.13 & 176.87 ppm corresponding to the two thione groups.



Scheme 1

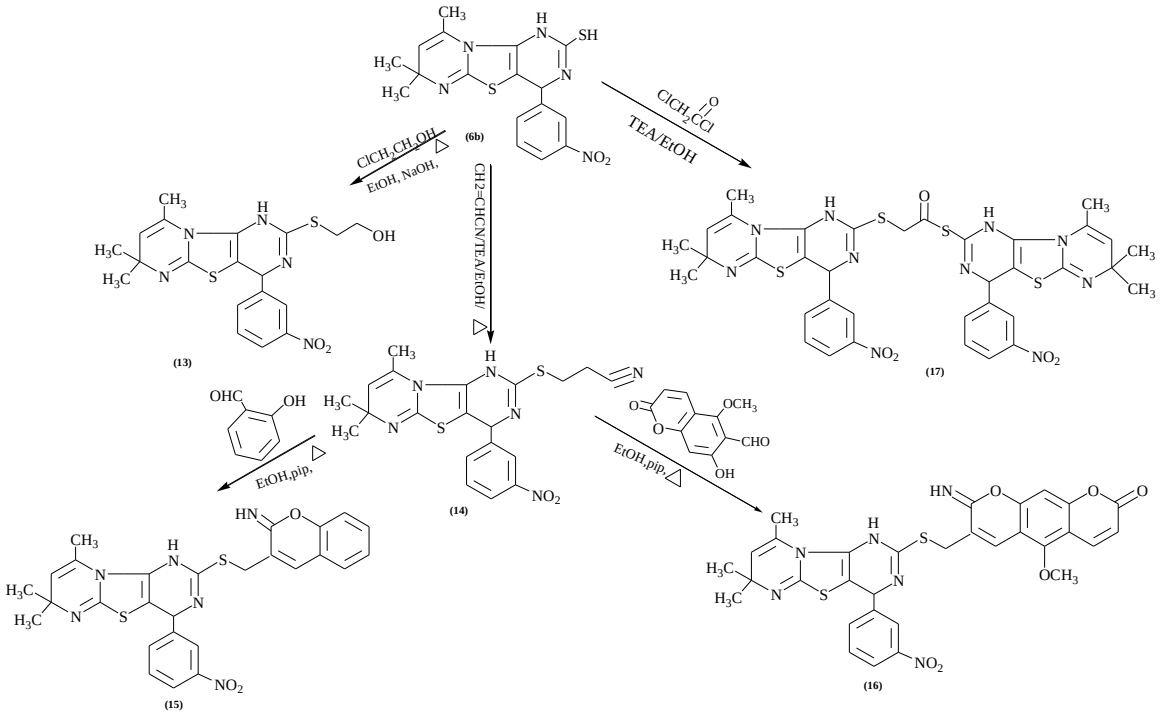
2-Arylmethyelene thiazolidinopyrimidin-3-one **2a-c** was considered as the versatile precursor to synthesis of other 2-thioxopyrimidine derivatives in good yield. When compounds **2a-c** were treated with thiourea in ethanol under reflux, it afforded the corresponding thiazolo[4,5-d]pyrimidin-2-thione derivatives **6a-c.** (Scheme 2).



Scheme 2

The structures of these compounds were confirmed with elemental analysis and spectral data. Compound **6a** as an example: its IR spectrum showed absorption bands at 3166.8 and 3273.8 cm^{-1}(2NH); ^{13}C NMR spectrum of **6b** revealed the presence of (C=S) at 173.43 ppm. Compounds **6a-c** were found to be useful for the syntheses of the interesting [1,2,4]triazolopyrimidines. Thus , two possible pathways can be accounted for the formaiotn of compound **10:1)**. the 1,3-dipolar cycloaddition of the thiol tautomer **6** to the nitrilium imide, generated in situ from hydrazonyl halides and triethylamine in dry chloroform gave the thiohydrazonate ester **7** which underwent nucleophilic cyclization to yield the spiro compound **8** followed by opening ring to give **9** which cyclized to yield **10** by loss of hydrogen sulfide: **2**)1,3-dipolar cycloaddition of the nitrilium imide to the C=S double bond of **6** can give hydrazontester **11** which then cyclized to give intermediate **12** which in turn gave compound **10** by loss of hydrogen sulfide (scheme 2). All attempts to isolate any intermediates were unsuccessful. Structures of **10a,b** were elucidated on the basis of elemental analysis, spectral data and alternative synthetic route **(Abdelhamid, *et al.*, 2007; Hafez, *et al.*, 2010)**. Compound **10a** as an example, IR spectrum showed absorption band at 1748 cm^{-1} (C=O ester) and ^{1}H-NMR spectrum revealed signals at δ 1.24 ppm (t, J = 7.5Hz, 3H, CH_3), 4.17 (q, J = 7.5 Hz, 2H, CH_2) (c.f. Experimental). Furthermore, alkylation of compound **6b** with chloroethanol in alcoholic sodium hydroxide gave 2-(4-(m-nitrophenyl)-7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidin-2-ylsulfanyl)ethanol **(13)**. (Scheme 3). The structure of the formed compound was established by its elemental analysis and spectral data (IR & ^{1}H NMR). ^{1}H NMR spectrum showed signals at δ 4.19-4.21 ppm (m, 4H, $2CH_2$), 5.01 (s, 1H, OH, exchangeable with D_2O). Cyanoethylation of compound **6b** with acrylonitrile in ethanol and triethyl amine afforded

Michael type adduct 2-(2-cyanoethylsulfanyl)-7,7,9-trimethyl-4-(m-nitrophenyl)-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine **(14).** Cyclization of the latter compound with salicyladehyde or 7-hydroxy-5-methoxy-2-oxo-benzopyran-6-carbox-aldehyde in ethanolic piperidine yielded the cyclized 2-iminobenzopyran and 2-iminobenzodipyran derivatives **(15)** and **(16)** respectively (scheme 3). The structures of compounds **14-16** were confirmed on the basis of their elemental and spectral data. Their IR spectra showed the absence of the absorption band at 2243 cm^{-1} characteristic for (C≡N) of compound **14** and presence (NH/C=O) groups in IR spectrum of compound **16** and the NH-proton (D_2O exchangeable in the ^{1}H-NMR spectra of compounds **15** and **16** (c.f. Exp.)



Scheme (3)

On the other hand, alkylation of compound **6b** with chloroacetyl chloride in the presence of triethylamine and dimethyl formamide gave 4-(m-nitrophenyl)-7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine-2-ylsulfanyl)-2-(4-m-nitrophenyl) -7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine-2-ylsulphanyl) ethanethioate **(17).** Dehydrohalogenation took place joining two molecules of compound **6b.** Inspection of the IR spectrum of the reaction product **17** revealed the presence of the C=O group and its mass spectrum exhibited a molecular ion peak M^+ at m/z: 814 (12.7%) (c.f. Experimental).

PHARMACOLOGY

Antitumor effect

CONCLUSION

The newly synthesized compounds **2b** and **13** were tested for antitumor activity and they exhibited a high significant anticancer activity against both of HCT 116cell line and MCF-7 cell line (human colon carcinoma cell and human breast carcinoma cell). The results are summarized in Figures. 1-4.

Evaluation of cytotoxicity effect 2-(m-nitrobenzylidine)-5,7,7-trimethylthizolidino[3,2-a] pyrimidin-3-one (2b) against HCT 116 cell line

Sample conc. (µg)	Viability %
50	30.93
25	63.32
12.5	77.01
6.25	84.98
3.125	96.54
1.56	98.83
0	100.00

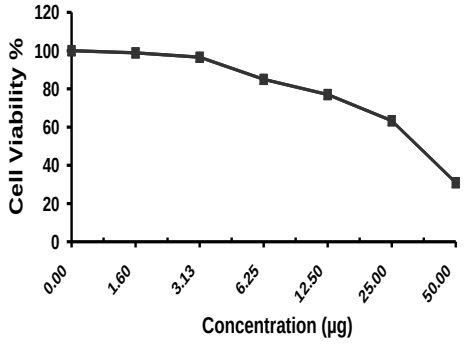


Fig (1)

Comment:

Inhibitory activity against* colon carcinoma *cells was detected under these experimental conditions with IC_{50}=35.3 µg.

Evaluation of cytotoxicity effect 2-(m-nitrobenzylidine)-5,7,7-trimethylthizolidino [3,2-a] pyrimidin-3-one (2b) against MCF-7 cell line

Sample conc. (µg)	Viability %
50	47.55
25	76.96
12.5	88.58
6.25	93.14
3.125	97.52
1.56	100
0	100.00

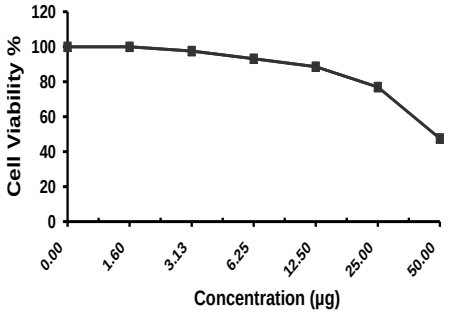


Fig (2)

Comment:

Inhibitory activity against* breast carcinoma *cells was detected under these experimental conditions with IC_{50} = 47.9µg.

Evaluation of cytotoxicity effect 2-(4-(m-nitrophenyl)-7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine-2-yl sulfanyl)ethanol (13) against HCT 116 cell line

Sample conc. (µg)	Viability %
50	6.44
25	14.95
12.5	22.70
6.25	35.68
3.125	47.42
1.56	55.39
0	100.00

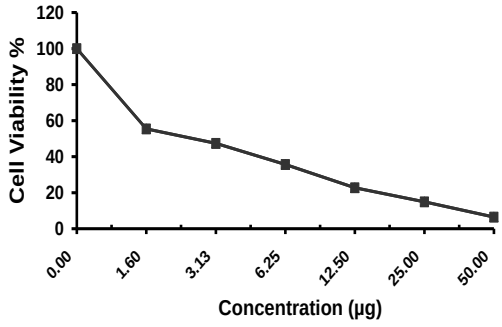


Fig. (3)

Comment:

***Inhibitory activity against* colon carci[illegible]**

conditions with IC_{50}=2.2 µg.

Evaluation of cytotoxicity effect of 2-(4-(m-nitrophenyl)-7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d]pyrimidine-2-yl sulfanyl)ethanol (13) against MCF-7 cell line

Sample conc. (µg)	Viability %
50	8.94
25	17.21
12.5	28.97
6.25	34.66
3.125	45.18
1.56	56.32
0	100.00

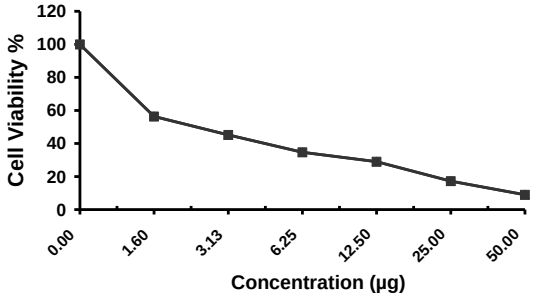


Fig. (4)

Comment:

Inhibitory activity against* breast carcinoma *cells was detected under these experimental conditions with IC_{50} = 2.4 µg.

Table (1) Characterization data of compounds 2-17.

Comp. No.	M.P. [°C]	Yield [%] solvent	Mol formula (mol. Wt)	C% calcd./found	Analysis H % calcd./found	N%	S%	Br
2a	231-232	85 B	$C_{16}H_{15}N_2OBrS$ 363.27	52.90 52.87	4.16 4.14	7.71 7.69	8.83 8.80	22.0 21.97
2b	247-249	80 M	$C_{16}H_{15}N_3O_3S$ 329	58.34 58.37	4.59 4.65	12.76 12.78	9.74 9.75	--
2c	211-212	65 B	$C_{16}H_{15}N_2OSCl$ 318.82	60.28 60.15	4.74 4.70	8.79 8.85	10.06 10.12	11.12(Cl) 11.07
3a	192-193	90 B	$C_{19}H_{16}N_5BrS$ 426.33	53.53 53.49	3.78 3.76	16.43 16.48	7.52 7.49	18.74 18.82
3b	181-183	95 B	$C_{19}H_{16}N_6O_2S$ 392.43	58.15 58.12	4.11 4.19	21.42 21.39	8.17 8.14	--
5a	243-244	70 E	$C_{20}H_{16}N_5BrS_3$ 502.47	47.81 47.83	3.21 3.18	13.94 13.90	19.14 19.16	15.90 15.88
5b	251-253	75 M	$C_{20}H_{16}N_6O_2S_3$ 468.58	51.26 51.24	3.44 3.41	17.94 17.90	20.53 20.50	--
6a	172-173	85 B	$C_{17}H_{17}N_4BrS_2$ 421.38	48.46 48.44	40.70 4.91	13.30 13.73	15.22 15.20	18.96 18.98
6b	187-188	90 B	$C_{17}H_{17}N_5O_2S_2$ 387.48	52.69 52.76	4.42 4.39	18.07 18.04	16.55 16.52	--
6c	169-170	70 B	$C_{17}H_{17}N_4ClS_2$ 376.93	54.17 54.14	4.55 4.56	14.86 14.80	17.01 17.07	9.41 9.48
10a	231-334	65 E	$C_{28}H_{27}N_7O_4S$ 557.63	60.31 60.37	4.88 4.85	17.58 17.55	5.75 5.72	--
10b	249-251	79 E	$C_{27}H_{25}N_6O_2ClS$ 533.04	60.84 60.81	4.73 4.70	15.77 15.78	6.02 6.00	6.65 (Cl) 6.61
13	265-267	70 A	$C_{19}H_{21}N_5O_3S_2$ 431.53	52.88 52.89	4.91 4.88	16.23 16.20	14.86 14.19	--
14	243-245	85 E	$C_{20}H_{20}N_6O_2S_2$ 440.11	54.53 54.50	4.58 4.60	19.08 19.15	14.56 14.53	--
15	169-170	75 B	$C_{27}H_{24}N_6O_3S_2$ 544.65	59.54 59.41	4.44 4.41	15.43 15.40	11.77 11.74	--
16	178-179	80 B	$C_{31}H_{26}N_6O_6S_2$ 642.7	57.93 57.96	4.08 4.15	13.08 13.19	9.89 9.91	--
17	181-183	75 E	$C_{36}H_{34}N_{10}O_4S_4$ 814.96	52.60 51.67	4.70 4.67	17.81 17.77	18.12 18.09	--

Solvent of crystallization A: Acetone , B : benzene , M : methanol, E: ethanol

EXPERIMENTAL

Melting points were recorded on an electrothermal IA 9100 digital melting point apparatus and were uncorrected. IR spectra (V_{max} in cm^{-1}) were recorded on a Shimadzu FT-IR 8300 spectrophotometer using KBr pellets technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker WM-400 spectrophotometer using DMSO-d_6 as the solvent and TMS as the internal reference (chemist shifts in ppm). The mass spectra were run at 70 eV with a finnigan SSQ7000 spectrophotometer (thermo-instrument system incorporation, USA) Elemental analysis were operated using Mario El Mentar apparatus, Organic microanalysis unit. Elemental analysis and the above spectra were measured the at National Research Center. Pharmacology was carried out in the Regional Center for Mycology & Biotechnology, Al-Azhar University.

2-Arylidine-5,7,7-trimethyl thiazolidino[3,2-a]pyrimidin-3-one (2a-c).

General procedure: A mixture of compound **1** (0.01 mol), chloroacetic acid (0.01 mol), aromatic aldehyde (0.01 mol) and 2 gm of fused sodium acetate in 10 mL of acetic acid and 5 mL of acetic anhydride was refluxed for 1h . The reaction mixture was allowed to cool and then poured into cold water, the product obtained was filtered, dried and recrystalized from the proper solvent to give **2a-c**.

2-(p-Bromobenzylidine)-5,7,7-trimethyl thiazolidino[3,2-a] pyrimidine-3-one (2a).

It was obtained from **1** and p-bromobenzaldehyde; IR (cm^{-1}, υ): 1703 (CO); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.31, 1.37,1.69 (3s, 9H, C_{-7} $2CH_3$, $C_{-5}CH_3$), 5.19 (s, 1H, $C_{-6}H$), 7.41-7.81(m, 5H, 1H=CH– + 4HArH).

2-(m-Nitrobenzylidine)-5,7,7-trimethyl thiazolidino[3,2-a]pyrimidin 3-one (2b)

It was obtained from compound **1** and m-nitrobenzaldehyde; IR (cm^{-1}, υ) 1706(CO); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.46, 1.50, 1.78 (3s, 9H,C_{-7} $2CH_3$, $C_{-5}CH_3$), 5.7 (s, 1H, $C_{-6}H$), 7.36 (s, 1H, =CH–Ar), 7.80-7.99 (m, 4H, Ar–H).

2- (o-Chlorobenzylidine)-5,7,7-trimethyl thiazolidino[3,2-a]pyrimidin -3-one (2c).

It was obtained from **1** and o-chlorobenzaldehyde; ^{1}H NMR (DMSO-d_6, δ, ppm) 1.50, 1.62, 1.80 (3s, 9H, $C_{-7}2CH_3$, $C_{-5}CH_3$), 5.54 (s, 1H, $C_{-6}H$), 7.25(s, 1H, =CH–Ar), 7.81-7.87 (m, 4H, ArH)

2-Amino-4-substituted-7,7,9-trimethylpyrimidino [1',2':3,2] thiazolo[4,5-b]pyridin-3-carbonitrile (3a,b).

General procedure:

A mixture of compound **2a or 2b** (0.01 mol), malononitrile (0.01 mol) and ammonium acetate (0.02 mol) in glacial acetic acid (30 mL) was heated under reflux for 6h., left at room-temperature, then poured into cold water. The solid product was filtered off, washed with water, dried and recrystallized to give compound **3a,b**.

2-Amino-4-(p-bromophenyl)-7,7,9-trimethyl pyrimidino[1',2':3,2] thiazolo[4,5-b] pyridine-3-carboitrile (3a).

IR (cm^{-1}, υ): 2193 (C≡N), 3209 & 3344 (NH_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.25 (br, 6H, C_{-7} $2CH_3$), 1.41(s, 3H, $C_{-9}CH_3$), 4.9 (br, 2H,NH_2, D_2O exchangeable), 5.31 (s, 1H, $C_{-8}H$), 7.25-7.69 (m, 4H, ArH).

2-Amino-4-(m-nitrophenyl)-7,7,9-trimethyl pyrimidino[1',2':3,2] thiazolo[4,5-b]pyridine-3-carbonitrile (3b)

IR (cm^{-1}, υ): 2189 (C≡N), 3130 & 3210 (NH_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.46 (br, 6H, $C_{-7}2CH_3$), 1.62 (s, 3H, $C_{-9}CH_3$), 4.46 (br, 2H, NH_2, D_2O exchangeable), 5.17 (s, 1H, $C_{-8}H$), 7.64-7.79 (m, 4H, ArH).

5-Substituted -8,8,10-trimethyl pyrimidino[1'',2'':3',2'] thiazolo[4',5': 2,3]pyrido[2,3-d] pyrimidin-2,4-[1H, 3H]dithione derivatives (5a,b) .

General procedure: To a solution of o-aminonitrile **3a** or **3b** (0.01 mol) in pyridine (15 mL), carbon disulfide (0.05 mol) was added and the reaction mixture was heated on a water bath for 10h. After cooling, ethanol was added and the separated solid was collected by filtration, dried and recrystallized to give compound **5a,b**.

5-(p-Bromophenyl)-8,8,10-trimethyl pyrimidino[1'',2'':3',2']thiazolo [4',5': 2,3] pyrido [2,3-d]pyrimidine-2,4-[1H, 3H)dithione (5a).

IR (cm^{-1}υ): 3405 (br, 2NH), 1230,1239(2C=S); ^{1}H-NMR-d_6, δ, ppm): 1.24 (br, 6H, C_{-8} $2CH_3$), 1.63 (s, 3H, $C_{-10}CH_3$), 5.20 (s, 1H, $C_{-9}H$), 7.36-7.70 (m, 4H, ArH), 12.77 (br, 2H, 2NH, D_2O exchangeable)., ^{13}C NMR (DMSO-d_6, δ, ppm): 28.55, 28.79, 30.57 ($3CH_3$), 65.55 (C_{-8}), 78 (C_{-9}), 124.17, 124.34, 125.98, 126.58, 128.56, 129.32 (ArC), 130. 80 (C-4a), 131.40 (C-5a), 134.71 (C-10), 135.35 (C-5), 142.55 (C-4b), 144.79 (C_{-5b}), 148.19 (C-6a), 176.13, 176.87 (2C=S).

5-(m-Nitrophenyl)-8,8,10-trimethyl pyrimidino[1'',2'':3',2']thiazolo [4',5':2,3]pyrido [2,3-d] pyrimidin-2,4[1*H*,3*H*] dithione (5b).

IR (cm^{-1}, υ): 3409(br, 2NH), 1233, 1241 (2C=S); MS m/z (%): 468 (71).

4-Substituted-7,7,9-trimethyl-1,3,4-trihydropyrimidino [1',2':3,2]thiazolo[4,5-d] pyrimidin-2-thione (6a-c).

General procedure: A mixture of compound **2a-c** (0.01 mol) and thiourea (0.01 mol) was refluxed in absolute ethanol 30 mL and 5 mL of concentration hydrochloric acid for 6h. The solid product was filtered off dried and recrystallized to give compounds **6a-c.**

4-(p-Bromophenyl)-7,7-9-trimethyl-1,3,4-trihydropyrimidino[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-thione (6a).

IR (cm^{-1}, υ): 3166.8, 3273.8 (2NH), 1255 (C=S); ^{1}H-NMR (DMESO-d_6, δ, ppm): 1.24, 1.25, 1.5 (3s, 9H, $C_{-7}2CH_3$, $C_{-9}CH_3$), 4.99 (s, 1H-$C_{-4}H$) 5.30 (s, 1H, $C_{-8}H$), 7.47-7.61 (m, 4H, ArH), 8.87 and 8.93 (2s, 2H, 2NH, D_2O exchangeable); MS m/z (%): 421 (17).

4-(m-Nitrophenyl)-7,7,9-trimethyl-1,3,4-trihydropyrimidino[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-thione (6b).

^{1}H-NMR (DMSO-d_6, δ, ppm) 1.51,1.63,1.81 (3s, 9H, C_{-7} $2CH_3$, $C_{-9}CH_3$), 4.97 (s, 1H, $C_{-4}H$), 5.39 (s, 1H, $C_{-8}H$), 7.51-7.64 (m, 4H, ArH), 8.91 and 8.99 (2s, 2H, 2NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6, ppm): 24.01, 24.80, 26.95 ($3CH_3$), 58.65(C_{-7}), 72.53(C_{-8}), 123.76, 124.76, 125.39, 126.72, 128.01, 128.09 (ArC),128.31(C_{-4}) 128.54 (C_{-4a}), 128.82(C_{-5a}), 130.25 (C_{-4b}), 131.18 (C_{-9}), 173.43 (C=S).

4-(o-Chlorophenyl)-7,7,9-trimethyl-1,3,4-trihydropyrimidino[1',2':3,2]thiozolo[4,5-d] pyrimidine-2-thione (6c).

IR (cm^{-1}, υ): 3341 (br, 2NH), 1249.(C=S); ^{1}HNMR (DMSO-d_6, δ, ppm): 1.30, 1.41, 1.83 (3s, 9H, $C_{-7}2CH_3$, $C_{-9}CH_3$), 5.1 (s, 1H, $C_{-4}H$), 5.50 (s, 1H, $C_{-8}H$), 7.61-7.82 (m, 4H, ArH), 8.53 and 8.61 (2s, 2H, 2NH, D_2O exchangeable).

Ethyl 3,5-disubstituted-8,8,10-trimethyl pyrimidino[1',2':3,2] thiazolo [4,5-d][1,2,4] triazolo[3,4-b]pyrimidine-1-carboxylate (10a,b).

General procedure : A mixture of **6b,c** (0.01 mol) and hydrazonyl chlorides (0.01 mol) was stirred under reflux in dry chloroform (30 mL) and 0.5 mL of triethylamine for 15h. the solvent was evaporated and the solid produced was washed three times by 30 mL ethanol, and crystallized to give **(10a,b).**

Ethyl 3-(p-tolyl)-5-(m, nitrophenyl)-8,8,10-trimethyl pyrimidino [1',2':3,2]thiazolo[4,5-d]([1,2,4] triazolo)[3,4-b] pyrimidin-1-carboxylate (10a).

It was obtained from **6b** and ethyl chloro-(p-tolyl hydrazono) acetate, IR (cm^{-1}, υ): 1748(CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.24 (t, J=7.5Hz, 3H, CH_3), 1.42 (br, 6H, $C_{-8}2CH_3$), 1.81, 2.07 (2s, 6H, C_{-10} CH_3, CH_3), 4.17 (q, J=7.5, 2H, CH_2), 4.96 (s, 1H, C_5–H), 5.33 (s, 1H, C_9-H), 7.67-7.97 (m, 8H, 2ArH).

Ethyl-3-phenyl-5-(o-chlorophenyl)-8,8,10-trimethyl-pyrimidino[1',2':3,2]thiazolo[4,5-d] ([1,2,4]triazolo)[3,4-b]pyrimidine1-carboxyalte (10b).

It was obtained form **6c** and ethyl chloro(phenyl hydrazono)-acetate, IR (cm^{-1}, υ): 1741 (CO); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.26 (t, J=7.8 Hz, 3H, CH_3), 1.42 (br, 6H, $C_{-8}2CH_3$), 1.87 (s, 3H, C_{-10} CH_3), 4.19 (q, J = 8Hz, 2H, CH_2), 4.7 (s, 1H, C_5–H), 5.36(s, 1H, C_{-9}–H), 7.69-7.97 (m, 9H, 2ArH).

2-(4-(m-Nitrophenyl)-7,7,9-trimethyl-1,4-dihydro-pyrimidino[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-yl sulfanyl)-ethanol (13).

A solution of compound **6b** (0.01 mol) and sodium hydroxide (0.01 mol) in ethanol (30 mL) was treated with 2-chloroethanol (0.01 mol) and the reaction mixture was refluxed for 16h. the formed precipitate was filtered off, dried and recrystalized to give compound **(13**) IR (cm^{-1}, υ): 3200-3420 (NH/OH); ^{1}H-NMR (DMSO-d_6,δ,ppm): 1.60(br,6H,$C_{-7}2CH_3$), 2.07 (s, 3H, $C_{-9}CH_3$), 4.19-4.21 (m, 4H, $2CH_2$), 5.01 (s, 1H, OH, D_2O exchangeable), 5.1 (s, 1H, $C_{-4}H$), 5.5 (s, 1H, $C_{-8}H$), 7.6-7.73 (m, 4H, ArH), 8.37 (s, 1H, NH, D_2O exchangeable)

2-(2-Cyanoetheyl sulfanyl)-7,7,9-trimethyl -4-(m-nitrophenyl) 1,4-dihydropyrimidino [1',2':3,2]thiazolo[4,5-d]pyrimidine (14).

A mixture of compound **6b** (0.01 mol), acrylonitrile (0.01 mol) and triethylamine two drops in ethanol (50 mL) was heated under reflux for 3h, cooled, filtered, dried and recrystallized to give compound **14.** IR(cm^{-1}, υ): 2243 (C≡N), 3341(NH) groups; ^{1}H NMR (DMSO-d_6, δ, ppm) : 1.31, 1.41, 1.72(3s, 9H,$C_{-7}2CH_3$, $C_{-9}CH_3$), 3.2 (t, 2H, SCH_2), 3.82, (t, 2H, CH_2CN), 5.11 (s, 1H, $C_{-4}H$), 5.51 (s, 1H, $C_{-8}H$), 7.67-7.82 (m, 4H, ArH), 8.61 (s, 1H, NH, D_2O exchangeable).

Synthesis of compounds 15 and 16

Compound **6b** (0.01 mol) and salicyladehyde or 7-hydroxy-5-methoxy-2-oxo-benzopyran-6-carboxaldehyde (0.01 mol) were refluxed in ethanolic piperidine (30 ml) for 30 min. The solid product was filtered, dried and recrystalized to give compounds **15** and **16**.

3-(7,7,9-Trimethyl-1,4-dihydropyrimidino)[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-yl sulfanyl methyl)-2-iminobenzopyran(15).

IR (cm^{-1}, υ): 3415 (2NH); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.23 (br, 6H, C_{-7} $2CH_3$), 1.62 (s, 3H,$C_{-9}CH_3$), 2.39 (s, 2H, SCH_2), 4.98(s, 1H,C_{-4}H) 5.52 (s, 1H, C_{-8}H), 6.4 (s, 1H, pyran H), 7.36-7.70 (m, 9H, 2ArH + (NH, D_2O exchangeable), 11.2 (br, 1H, =NH, D_2O exchangeable)

3-(7,7,9-Trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-yl - sulfanyl methyl)-2-imino-5-methyoxybenzo[1,2-b:5,4-b']dipyran-8-one (16)

IR (cm^{-1}, υ): 1731(C=O), 3342(2NH); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.60, 1.62, 1.72 (3s, 9H, $C_{-7}2CH_3$, $C_{-9}CH_3$), 2.48 (s, 2H, SCH_2), 3.88 (s, 3H, OCH_3), 5.01 (S, 1H, C_{-4}H) 5.79 (s, 1H, C_{-8}H), 7.3 (s, 1H, C_{-4} pyran), 7.80-8.4 (m, 8H, ArH, C_{-6}H pyran + C_{-7} pyran + (NH, D_2O exchangeable), 10.14 (s, 1H,=NH, D_2O exchangeable).

4-(-m-Nitrophenyl)-7,7,9-trimethyl-1,4-dihydropyrmi-dino [1',2':3,2]thiazolo[4,5-d] pyrimidin-2-ylsulfanyl)-2-[4-(m-nitro-phenyl)-7,7,9-trimethyl-1,4-dihydropyrimidino[1',2':3,2]thiazolo[4,5-d] pyrimidin-2-yl sulfanyl)ethanethioate (17).

A mixture of compound **6b** (0.01 mol), chloroacetyl chloride (0.01 mol) and triethylamine two drops in dimethyl formamide (30 ml) was heated under reflux for 3h, the solid product was filtered, dried and recrystallized to give compound **17**. IR (cm^{-1},υ): 1671 (C=O) 3351(br, 2NH) ; MS: m/z (%): 814[M^+, 12.7].

Cytotoxicity assay:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50ug/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x 10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 *ig)* were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated **(Mosmann, 1983; Vijayan *et al.*, 2004)**.

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تشييد، تفاعلات ومضادات خلايا الأورام لبعض مشتقات السيازولو بريمدين

نرمين عبدالله مرزوق السعداوي

قسم الكيمياء – كلية العلوم (بنات) – جامعة الأزهر – مدينة نصر - القاهرة – مصر

في هذا البحث تم تحضير السيازولو**[3,2-a]**بيرميدينات، بيريميدنو**[3,2 :1',2'']**سيازولو **[4,5- b]**بيريدينات،بيريميدنو ['**1'',2':3',2**]**[4',5':2,3]**سيازولو**[2,3-d]**بيريدو[بيريميدينات،بيريميدنو**[3,2':3,2 :1',2']**سيازولو**[4,5 - d]** **[**بريمدينات،بريمدينو**(1',2':3,2)** سيازولو **[4,5-d]**] [١ ،٢ ،٤] تراي ازولو**[3,4-b]** بريميدنات **(2-10)** بتفاعل ٢- (أريل ميثلين) تراي ميثيل سيازلو [3,2-a]بريميدين -٣- أون **(2)**. أيضا تم تشييد بعض مشتقات الكلة -S–سيازولو **[4,5-d]** بريميدن عن طريق تفاعل ٤- مستبدل **٩.٧.٧** – تراي ميثيل **٤.٣.١**- ترايهيدروبريميدنيو**[3,2 :1',2']** سيازولو[d -**4,5**] بريميدين -٢- ثيون **(6_b)** مع كواشف مختلفة. وعلاوة على هذا تم اختبار النشاط المضاد لأورام القولون والثدي لبعض المركبات المختارة وجد أن بعض هذه المركبات لها فاعلية ضد نشاط الأورام.