SYNTHESIS OF PYRIMIDO [4,5-d] PYRIMIDINETHIONE DERIVATIVES AS BIOCIDAL AGENTS

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In the search for new antimicrobial compounds, several hew pyrimido [4,5-d] pyrimidinethiones 6-14 have been synthesized from β-arylidene malononitriles 1 with thiourea followed by cyclocondensation with nitrogen and oxygen compounds. Structure of the new products was deduced form elemental analysis and spectral data [UV,IR, ^{1}H NMR and mass]. Effect of the newly compounds on the activity of bacterial strains in comparison with Streptomycin and Gentamicin was studied.

1- INTRODUCTION

The chemistry of pyrimidines is receiving much attention in recent years due to the physical and chemical properties of such derivatives **[1-3],** which have gained wide applications as biocidal agents **[4-9].** This together with our intrerest in this area, prompted us to investigate the synthesis of some new pyrimido [4,5-d] pyrimidinethione derivatives of potential biological activity. A relation between structure activity was deduced.

2- INVESTIGATION AND RESULTS

2-1 Chemistry

The starting compound, 4-amino-6-aryl-5-cyanopyrimidin-2(3H)-thiones 2a-e, was obtained by refluxing β-arylidene malononitrile 1a-e with thiourea in the presence of anhydrous K_2CO_3 **[10]** (Scheme1). The structures of compounds 2a-e were deduced from elemental analysis and spectral data. The IR spectrum of 2a showed absorption bands in the region, 3304, 3117,2211, 1600, and 1177cm^{-1} characteristic for NH_2, NH, CN, C-H aromatic and C-S groups respectively. The ^{1}H NMR spectrum of 2e showed signals at δ3.3, 3.5-4, 6.5,7.2, 10.5, 12 and 13 ppm attributed to OCH_3, aromatic, NH and NH_2 protons. MS fragmentation of 2a exhibited an M^+ ion at m/z 234 (23.75%) which underwent fragmentation typically exhibited by thiopyran moiety as base peak at m/z 97 (100%), followed by loss CN, NH_2 and fission of pyrimidinethione which confirmed that elimination of the side chain was observed with the pyrimidinethione **[11]** (Scheme3).

Alkylation of compound 2a,c,e with 3-chloro-5,6-diphenyl-1,2,4-triazine 3 in DMF yielded 6-aryl-5-cyano-4-[5,6-diphenyl-1,2,4-triazin-3-ylamino]-pyrimidin-2(3H)-thiones 4a-c presence of cyano group in compound 4a was established by the acidic hydrolysis with dilute HCl to give 4-[5,6-diphenyl- 1,2,4-triazin-3-ylamino]-6-[thiophen-2-yl]-pyrimidin-2(3H)-thione 5. The structure of 4 was confirmed from IR spectral data which exhibited the absorption bands due to bonded-NH-,CN in additional of C-H,C-N and C-S for aromatic and hetero groups, while that of 5 revealed the absence of only CN group. UV spectrum of both 4 and 5 give a well established structure **[12]**. Thus, UV spectrum of 4 recorded λ_{max}at 331.8 (∈, 2.88) and 270.3 (∈,3.44)nm while that of 5 recorded only λ_{max} at 272.4 (∈,3.03)nm. The reactivity of the adjacent amino and cyano groups in compound 2 was arrived us to synthesize new fused heterobicyclic systems, in order to give as conclusive proof for structure 2. This has been carried out by fusion of compound 4 with thiourea, compound 2 with formic acid and or with phenyl isocyanate in DMF to give

4-amino-1-[5,6-diphenyl-1,2,4-triazin-3-yl]-5-(3,4,5-trimethoxyphenyl)-2-thioxo-pyrimido[4,5-d] pyrimidin-7(8H)-thione **6** 5-aryl-4(3H)-oxo-pyrimido [4,5-d] pyrimidin-7 (8H)-thiones **7a,b** and 5-aryl-4-imino-3- phenyl-2(1H)-oxo-pyrimido[4,5-d] primidin-7(8H)- thiones **8a,b** respectively (Scheme 1). The lack of absorption in the region 2200-2300cm^{-1} (C=N) in the IR spectra of **6-8** suggested the cyclic structure. The apprarance of a signal at δ**3.7-3.9** (OMe) **6.8-7** (C_6H_5), **7.2-7.5** (aryl), **7.8,8.5** (NH, NH) 10 (OH) and **12.7** ppm (=NH) in the PMR spectrum of **8a** established the postulated structrue. High resolution mass spectrum was recorded for **7a** and **8b.** The molecular ion appcars with mild abundance indicating its stability under electron impact. The base peak of **7a** at m/z 222 and 93 for **8b.** These fragmentation processes are in agreement with these reported earlier for similar compunds **[13]**.

The original objective of the present work was the formation of fused pyrimido-pyrimidinethiones in view of biological activity. Thus, the fusion of compound **2c** with urea and or thiourea resulted 4-amino-5-(2,4-dichlorophenyl)-2(1H)-oxo-pyrimido[4,5-d]pyrimidin-7(8H)-thione**9** and or 4-amino-5-(2,4-dichlorophenyl) -2(1H)-thioxo-pyrimido [4,5-d] pyrimidin-7(8H)-thione **10** (Scheme 2). The structure for compounds **9** and **10** was confirmed by spectral data. The IR spectra of **9** and **10** showed the presence of strong bands at γ 3300, 3100, 1750, 1600 and 1200 cm^{-1} due to NH_2 NH, C=O, N=N and C-S functional groups. The MS of **9** was characterized by the appearance of a molecular ion peak at m/z 340 (1.36%) and the base peak at m/z 301(100%), while that of **10b** recorded the molecular ion peak at m/z 363 (0.9%) with the base peak at m/z 299(100%). The observed ions at high abundance for compounds **9** and **10** is due to the stability of fused pyrimido [4,5-d] pyrimidinedithione (Scheme 4).

On the other hand, the synthesis of 2-amino-5-aryl-pyrimido [4,5-d]pyrimidin-7(8H)- thiones **11a,b** have been accomplished by reacting of compound **2d,e** with formamide (Scheme 2). The structure of **11** was confirmed from IR spectrum which exhibited the absorption bands at γ 3300, 3100, 1600,1175 and 1050cm^{-1} due to NH_2, NH, C=N, C-S, and C-O-C functional groups ^{1}HNMR spectrum of **11** showed a signals due to OMe, aromatic and hetero C-H and NH protons.

The target 5-(3,4,5-trimethoxyphenyl)-1,3-dihydro-2,4-dithioxo-pyrimido[4,5-d] pyrimidin-7(8H)-thione **12** has been obtained from the interaction between compound **2e** with CS_2 in DMF. Alkylation of compound **12** with monochloroacetic acid in the presence of DMFafforded5- (3,4,5-trimethoxyphenyl)-2,4,7-tricarboxymethylthio-pyrimido [4,5-d] pyrimidine **13** which on heating above its melting point yielded 2,4,7-trimethylthio-5-(3,4,5-trimethoxyphenyl)- pyrimido[4,5-d]pyrimidine (**14**). Compound **14** was also obtained from treatment of compound **12** with MeI in aqueous KOH which confirmed the fine structrue of both **12** and /or **14.** ^{1}H NMR spectrum of **13** recorded a signals at δ 3.3-4, 6.6-7.0, 9.5,9.9,10.2 and 10.7 ppm assigned to the OMe, 3CH_2, aromatic and hetero C-H and 3 COOH protons. The structure of **14** was also supported from MS which give a molecular peak ion at m/z 346 (1.14%) which underwent fragmentation to give a well established fragment trimethoxy benzyl at m/z 181 (100%).

2-2 Antimicrobial activity

The antimicrobial activity of these compounds were investigated against gram positive bacteriums (*Staphyloccus aureus* and *Bacillus subtilis*) and gram negative bacteriums (*Escherichia coli* and *Pseudomonas aeruginosa*) by the disc method [14]. In each plate, one filter disc was saturated with DMF as a negative control. Streptomycin (10 μg/disc) and Gentamicin (10 μg/disc) discs were used as positive controls. The plates were then incubated at 37° for 24h at different concentrations and growth inhibition calculated with reference to control. The results of in vitro antibacterial screening of tested compounds are shown in Tabel 1.

The results obtained showed that the first group involves pyrimidin-2(3H)-thione ring having amino and cyano groups at 4-and 5-positions displayed a wider spectrum activity compared with other gorup, and showed significant activity against *E,coli*, *P. aeruginosa* and S. *aureus.*

In the other group, compounds **4b**, **5a**, **6** and **7a** displayed marginal activity against all tested bacterial species. Undetectable activity were noticed when compounds **7**-**14** were applied at the two concentrations used except, compound **11** which exhibited significant activity against all bacterial species tested except *P. aeruginosa* In addition compound **10a** showed a promising effect on the microbial growth especially against *E. coli and S. aureus.*

Quantitative structure activity relationship for the tested compounds towards *E.coli, S. aureus* and B. *subtilis* compared with Streptomycin and Gentamicin shown that the relative inhibitions for the compouns **2a**, **7a** and **11** equivalent and more than antibiotic used which confirmed that pyrimido [4,5-d] pyrimidinethione derivatives have a broad biocidal spectrum [15] (Table 2).

3- Experimental

M.p.s reported were uncorrected. UV spectra were recorded in absolute EtOH on a Perkin-Elmer, Lambda 4B Controller Accessory Interface, UV-VIS spectrophotometer in (λ_{max} nm). IR spectra in KBr were recoreded in a Perkin-Elmer, 1430 Ratio Recording spectrophotometer (γ in cm^{-1}) ^1H NMR spectra were recorded on Bruker 200 MHz/52 MM spectrometer using DMSO-d_6 as a solvent and TMS as internal reference (Chemical shift in δ,ppm). Mass spectra were recorded using Finningan SSQ 700 spectrometer (70eV).

3.1. Synthesis of 4-amino -6-aryl -5-cyanopyrimidin-2(3H)-thiones (2a-e).

A mixture of **1a-e** (0.01 mol), thiourea (0.01 mol), anhydrous K_2CO_3 (0.03 mol) in acetone (30 ml) was heated under reflux for 3h. The precipitiate obtained was washed with water and crystallized to give **2a-e** (Table3).

3.2 Alkylation of 2a,c,e : Formation of 6-aryl-5-cyano-4-[5,6-diphenyl-1,2,4-triazin-3-ylamino]-pyrimidin-2(3H) -thiones (4a-c)

To a solution of **3** (0.01 mol) in DMF, compound **2a, c, e** (0.01 mol) was added and the reaction mixture was refluxed for 30min and poured into ice. The solid obtained was filtered, washed with cold H_2O and recrystallized to give **4a-c** (Table3).

3.3 Acidic hydrolysis of 4a:Formation of 4-[5,6-diphenyl-1,2,4-triazin-3-ylamino]-6-[thiophen-2-yl]-pyrimidin-2 (3H)- thione (5).

Compound **4a** (1gm) was added to a solution of HCl (10%, 20 ml) and the reaction mixture was refluxed for 4h. cooled and poured into ice. The solid obtained was recrystallized to give **5** (Table 3).

3.4 Fusion of 4c with thiourea : Formation of 4-amino-1- [5,6-diphenyl-1,2,4-triazin-3-yl]-5-[3,4,5-trimethoxyphenyl]-2-thioxo-pyrimido[4,5-d]pyrimidin-7(8H)-thione (6).

A mixture of compound **4c** (0.01 mol) and thiourea (0.01mol) was heated under reflux for 2h cooled and treated with cold water. The solid obtained was crystallized to give **6** (Table 3).

3.5 Action of formic acid on 2 : Formation of 5-aryl-4(3H)-oxo-pyrimido[4,5-d] pyrimidin-7 (8H)-thiones (7a,b).

A solution of 2c,d (0.02 mol) in formic acid (15 ml) was heated under reflux for 6h. The excess of formic acid was removed. The residue was crystallized to give 7a,b (Table 3).

3.6. Addition of phenyl isocyanate to 2 : Synthesis of 5-aryl-4-imino-3- phenyl-2(1H)-oxo-pyrimido [4,5-d] pyrimidin-7(8H)-thiones (8a,b).

To a solution of 2b,e (0.01 mol) in DMF (20 ml), phenyl isocyanate (0.01 mol)was added dropwise and reaction mixture was refluxed for 4h, cooled and poured into ice. The solid obtained was recrystallized to give 8a,b (Table 3).

3.7. Fusion of amides with 2 : Synthesis of 4-amino-5-[2,4-dichlorophenyl]-2(1H) -oxo-pyrimido [4,5-d] pyrimidin-7(8H)-thione (9) and 4-amino-5-[2,4-dichlorophenyl]- 2(1H)-thioxo-pyrimido [4,5-d] pyrimidin-7(8H)-thione (10).

A mixture of 2c (0.01 mol) urea or thiourea (0.01 mol) was heated under reflux for 2h, cooled and washed with cold H_2O. The solid obtained was recrystallized to give 9 and 10 (Table 3).

3.8. Reaction of 2 with formamide : Synthesis of 4-amino-5-aryl-pyrimido [4,5-d] pyrimidin-7 (8H)-thiones (11a,b)

A mixture of 2c,d (0.01 mol), formamide (15 ml) and formic acid (2 drops) was heated under reflux for 6 h. After cooling, the reaction mixture, water had been added, the solid obtained was collected, and crystallized to give 11a,b (Table 3)

3.9. Reaction of 2 with carbon disulfide : Synthesis of 5-[3,4,5-trimethoxyphenyl]-2,4,7-trithioxo-1,3,8-trihydro-pyrimido[4,5-d] pyrimidine (12).

To a solution of 2e (0.01 mol) in DMF (30 ml), CS_2 (20 ml) was added. The reaction mixture was heated under reflux on a water-bath for 12h. The solvent was evaporated and the residue was treated with H_2O and crystallized to give 12 (Table 3).

3.10. Reaction of 12 with monochloroacetic acid : Synthesis of 5-[3,4,5-Trimethoxyphenyl]-2,4,7-tricarboxymethylthio-pyrimido[4,5-d]pyrimidine (13).

A mixture of 12 (0.01 mol) and monochloroacetic acid (0.03 mol) in DMF (20 ml) was refluxed for 3h, cooled and poured into crushed ice. The solid produced was filtered off and crystallized to give 13 (Table 3).

3.11. Preparation of 5-[3,4,5-trimethoxyphenyl]-2,4,7-trimethylthio-pyrimido [4,5d] pyrimidine (14).

(A) Compound 13 (lg) was heated above its melting point for 15min, cooled and triturated with methanol to give 14 (Table 3).

(B) To a solution of 2e (0.01 mol) in aqueous KOH (1%, 20 ml), was added MeI (0.01 mol) and stirred for 24h at room temperature then triturated with methanol followed poured into ice-HCl. The solid thus obtained was filtered and washed with cold water and crystallized. Melting and mixed melting points of the solid which obtained from methods (a) and (b) gave no depression.

4- ACKNOWLEDGEMENT

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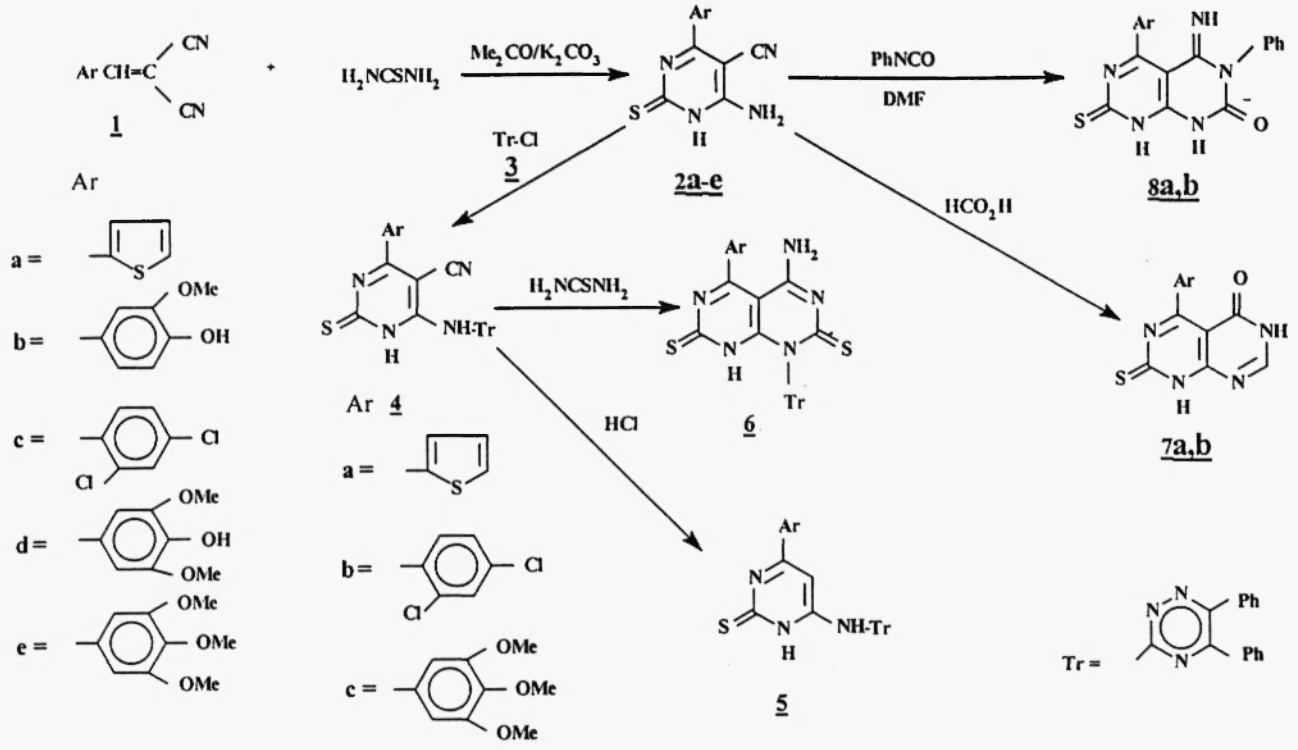
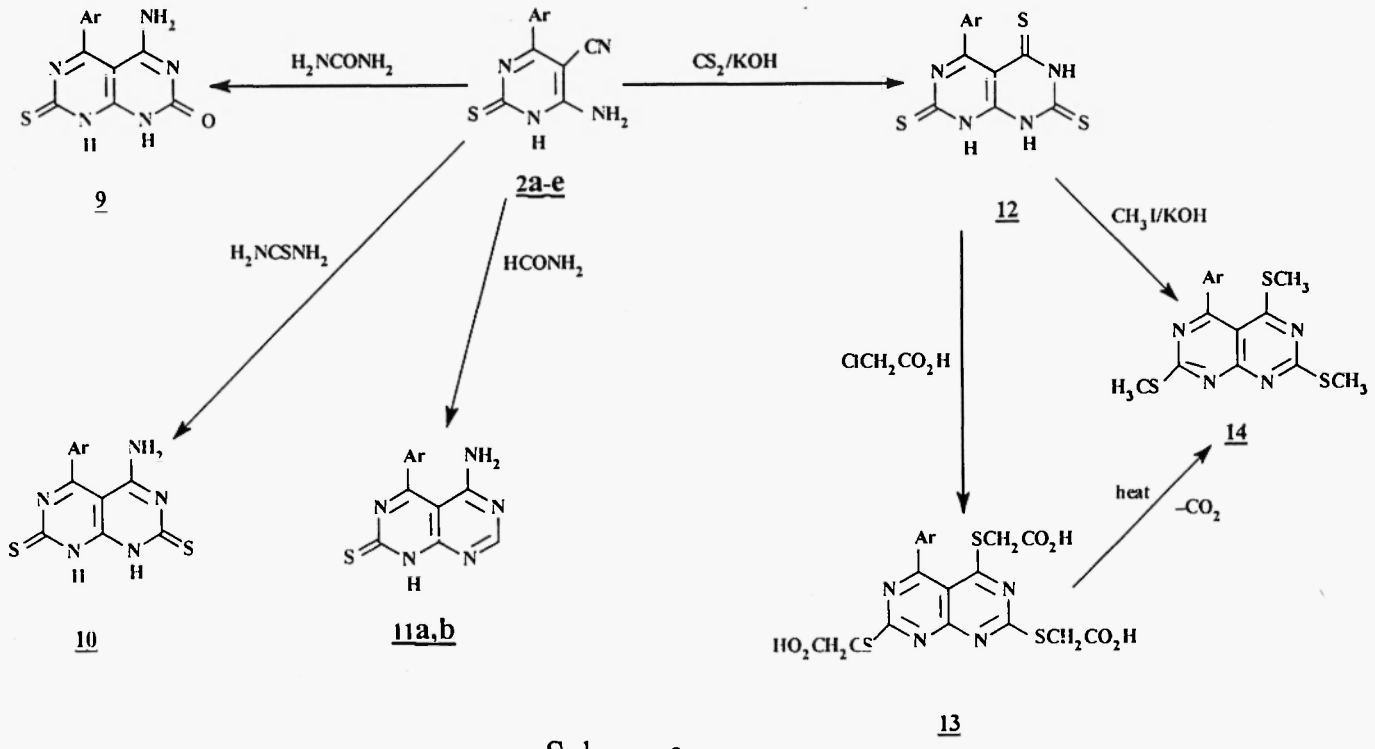


Scheme 1



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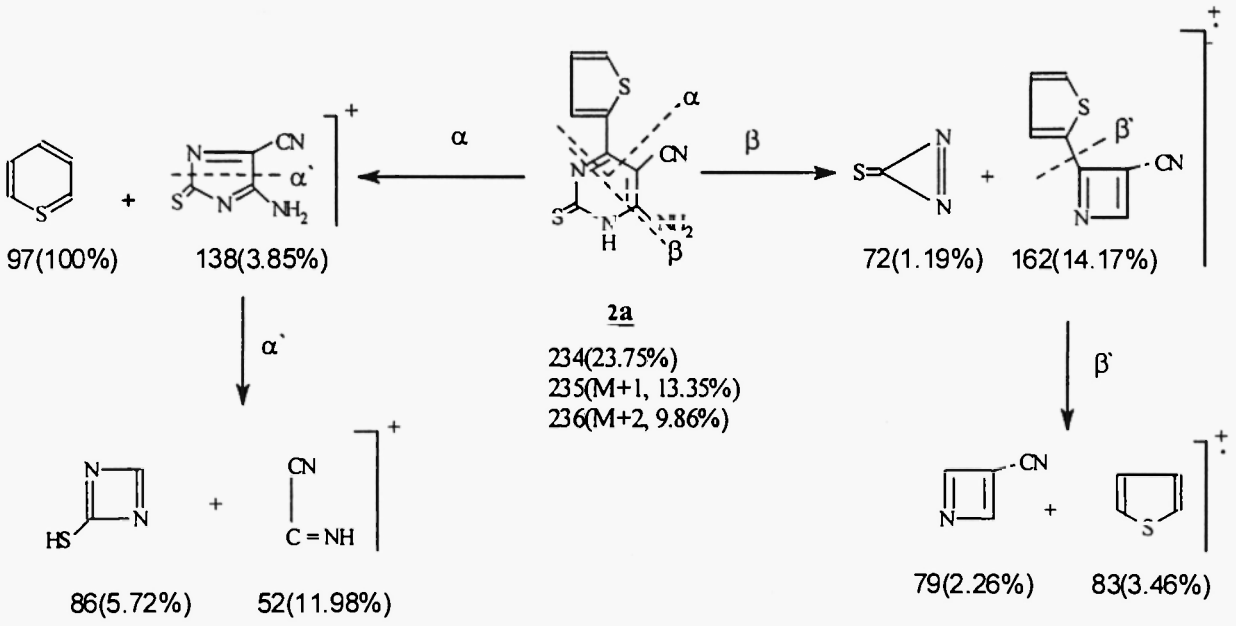
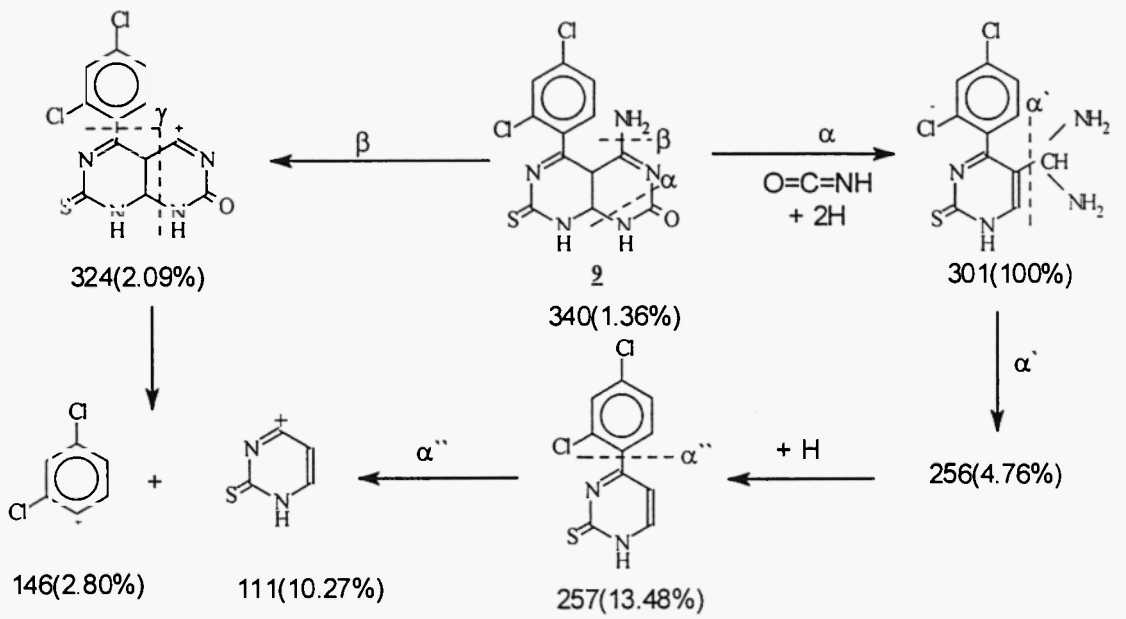


Scheme 3



Scheme 4

Table 1: Antimicrobial activity of some new compounds 2-14

	Diameter of inhibition zone (mm)							
Organism	*1*		*2*		*3*		*4*	
Conc. (mg/ml) compound	500	250	500	250	500	250	500	250
2a	8	8	8	8	9	7	0	0
2b	9	6	9	7	8	6	0	0
2c	6	5	8	6	6	6	0	0
2d	0	0	0	0	0	0	0	0
2e	8	7	0	0	0	0	0	0
4b	7	5	6	6	7	6	8	6
5a	9	6	8	6	8	5	9	7
6	5	4	5	4	4	4	9	6
7a	9	8	5	4	8	7	9	8
7b	0	0	0	0	0	0	0	0
8b	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10a	7	5	0	0	8	6	0	0
10b	0	0	0	0	0	0	0	0
11a	9	9	0	0	10	7	11	8
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
*S	10	10	5	5	6	6	4	4
**G	15	15	10	10	13	13	15	15

1: Escherichian coli; 2: Pseudomonas aeruginosa;
3: Staphyococcus aureuos; 4: Bacillus subtilis
* S: Streptomycin **G : Gentamicin
*** Agar plates containing 1 ml (10 bacterial/ml) of an overnight broth culture were prepared then incubated at 37°C for 24 h.

Table 2: Relative Inhibitions of Some Tested Compounds Towards used antibiotics

Compound	*E.coli*				*S.aureus*				*B.subtilis*			
	500		250		500		250		500		250	
	*S	**G	S	G	S	G	S	G	S	G	S	G
2a	80%	60%	80%	60%	150%	70%	110%	53%	-	-	-	-
7a	90%	60%	80%	60%	133%	61%	110%	53%	225%	60%	200%	60%
11	90%	60%	90%	60%	166%	76%	110%	533%	275%	73%	200%	60%

*S : * Streptomycin ** G: Gentamicin

Table 3: Characterization data of the synthesized compounds

Compd.*	Yield (%)	M.p. (°C)	Crystal. solvent	Mol. formula	M/e (M^+)	
2a	55	230-231	DMF	$C_9H_6N_4S_2$	234	336(M+2)
2b	40	210-212	EtOH	$C_{12}H_{10}N_4O_2S$	274	275(M+1)
2c	85	160-161	MeOH	$C_{11}H_6Cl_2N_4S$	297	300(M+3)
2d	70	250-251	EtOH	$C_{13}H_{12}N_4O_3S$	304	305(M+1)
2e	60	200-202	Dioxan	$C_{14}H_{14}N_4O_3S$	318	320(M+2)
4a	62	150-151	EtOH	$C_{24}H_{15}N_7S_2$	465	465(M+2)
4b	65	130-132	MeOH	$C_{26}H_{15}Cl_2N_7S$	528	530(M+3)
4c	60	162-164	EtOH	$C_{29}H_{23}N_7O_3S$	549	550(M+1)
5	75	185-186	DMF	$C_{23}H_{16}N_6S_2$	440	442(M+2)
6	85	180-181	DMF	$C_{30}H_{24}N_8O_3S_2$	608	610(M+2)
7a	72	150-151	DMF	$C_{12}H_6Cl_2N_4OS$	325	328(M+3)
7b	75	180-182	DMF	$C_{14}H_{12}N_4O_4S$	332	333(M+1)
8a	60	175-177	AcOH	$C_{19}H_{15}N_5O_3S$	393	394(M+1)
8b	55	240-241	AcOH	$C_{21}H_{19}N_5O_4S$	437	438(M+1)
9	80	210-211	Pet.ether	$C_{12}H_7ON_5Cl_2S$	340	343(M+3)
10	90	210-211	Dioxan	$C_{12}H_7Cl_2N_5S_2$	356	360(M+4)
11a	50	180-181	EtOH	$C_{14}H_{13}N_5O_3S$	331	332(M+1)
11b	55	200-202	Diaxon	$C_{15}H_{15}N_5O_3S$	345	346(M+1)
12	75	120-122	DMF	$C_{15}H_{14}N_4O_3S_3$	394	397(M+3)
13	68	200-201	EtOH	$C_{21}H_{20}N_4O_9S_3$	568	571(M+3)
14	50	170-171	MeOH	$C_{18}H_{20}N_4O_3S_3$	436	440(M+4)

* Satisfactory C,H,N,S and Cl analyses were obtained for all the compounds.

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