Acta Pharm. 56 (2006) 231–244 Original research paper

Synthesis and biological evaluation of some pyrimidine, pyrimido[2,1-*b*][1,3]thiazine and thiazolo[3,2-*a*]pyrimidine derivatives

HAYAM H. SAYED*
AHMED H. SHAMROUKH
AYMN E. RASHAD

Department of Photochemistry
National Research Center
Dokki, Cairo, Egypt

4,6-Diamino-1*H*-pyrimidine-2-thione (**1**) was used for the preparation of pyrimidine derivatives **2–5**. Compound **5** was cyclized to produce pyrimido[2,1-*b*][1,3]thiazine derivative **6** which was condensed with *p*-chlorobenzaldehyde to give compound **7**. The latter compound was reacted with hydroxylamine to give isoxazolo[4,5-*d*]thiazino[2,3-*a*]pyrimidine **8**. Compound **8b** was treated with 2-chloroethyl methyl ether to afford compound **9**. Similarly, compound **3** reacted with chloroacetic acid to give thiazolo[3,2-*a*]pyrimidine **10,** which was condensed with *p*-chlorobenzaldehyde to give compound **11**. Compound **11** was condensed with hydroxylamine to give isoxazolo-[4,5-*d*]thiazolo[2,3-*a*]pyrimidine **12**. Compound **12b** was treated with 2-chloroethyl methyl ether to afford compound **13**. Biological evaluation of some prepared products showed that many of them revealed promising antimicrobial activity.

Keywords: pyrimidine, pyrimidothiazine, thiazolopyrimidine, antimicrobial activity

Accepted January 30, 2006

It is well known that pyrimidine and fused heterocyclic pyrimidine derivatives are of great biological interest, especially as antiviral, antitumor and antimicrobial agents (1–12). Some series of pyrimido[2,1-*b*][1,3]thiazine and thiazolo[3,2-*a*]pyrimidine derivatives exhibited modest activity against gram-positive bacterial strains (3, 4). Also, some thiazolo[3,2-*a*]pyrimidine were tested for their anti-inflammatory activities, and exerted moderate anti-inflammatory activity (5). In conjunction with our previous work on the synthesis of pyrimidine thione derivatives for biological evaluations (13–18), a series involving a simple pyrimidine thione moiety is described.

* Correspondence, e-mail: hayamsayed@yahoo.com

EXPERIMENTAL

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Shimadzu). ^{1}H NMR spectra were recorded on a Varian Gemini 180 spectrometer (Varian, UK) and chemical shifts were expressed as (ppm) values against TMS as internal reference. Mass spectra were recorded on a Gc Ms-QP 1000 EX (Shimadzu).

Synthetic pathway is presented in Schemes 1 and 2 and physicochemical and spectral data for the synthesized compounds are given in Tables I and II.

*Synthesis of 4,6-diamino-1*H*-pyrimidine-2-thione (**1**)*

Compound **1** was prepared following the published procedure (19).

*Synthesis of 4,6-diamino-1,3,4-trihydropyrimidine-2-thione (**2**)*

To compound **1** (1.4 g, 0.01 mol) in glacial acetic acid, Zn dust (0.02 mol) was added stepwise for half an hour. The reaction mixture was poured into water and the precipitate was filtered off and recrystallized from methanol to give compound **2** as white crystals.

*Synthesis of 4,6-bis-(benzylidene-amino)-1,3,4-trihydropyrimidine-2-thione (**3a**) and 4,6-bis-[4-chlorobenzylidene)-amino]-1,3,4-trihydropyrimidine-2-thione (**3b**)*

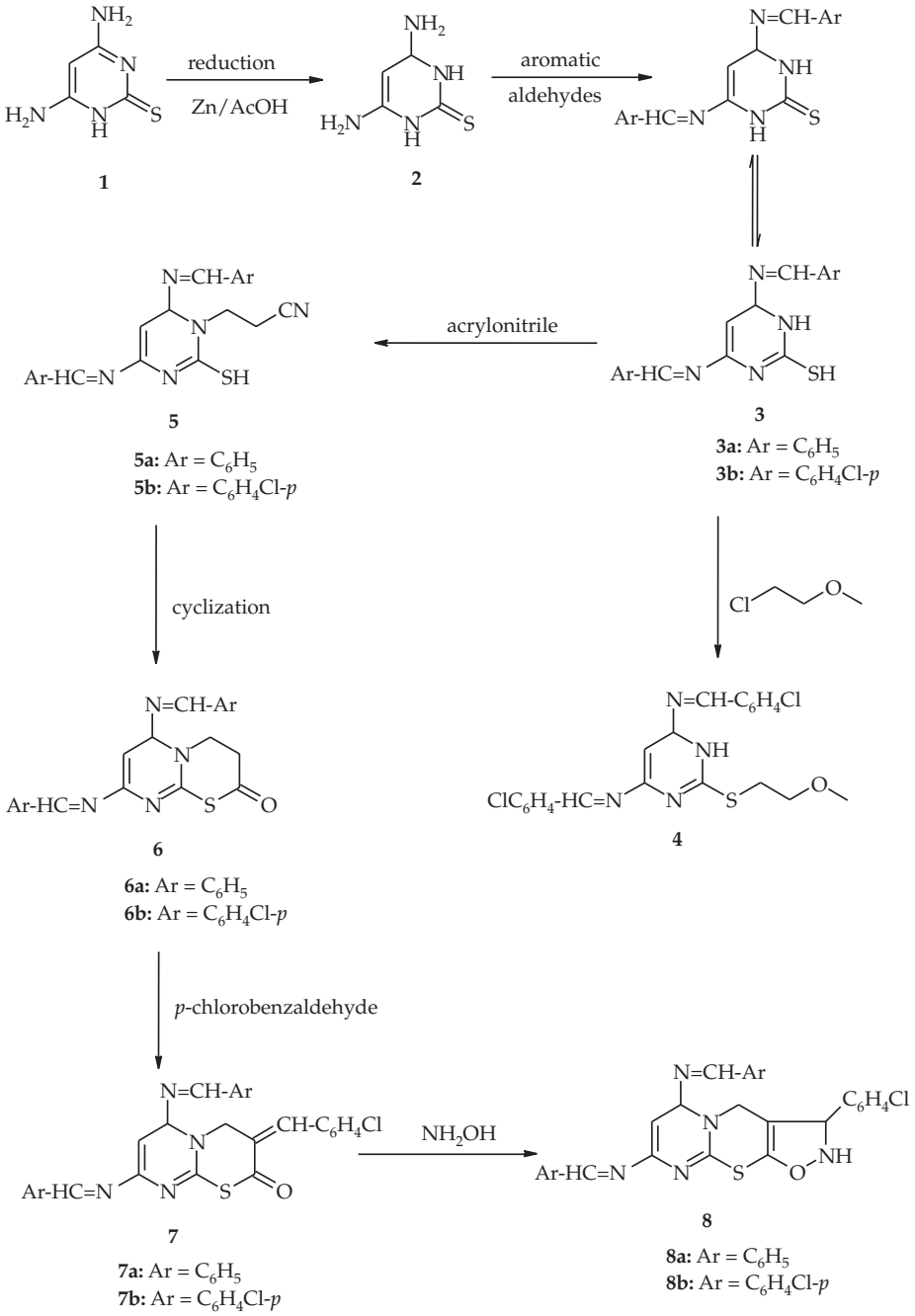
A mixture of compound **2** (1.44 g, 0.01 mol), benzaldehyde (2.12 mL, 0.02 mol) for **3a** or 4-chlorobenzaldehyde (2.8 mL, 0.02 mol) for **3b** and NaOH (1 g) in ethanol was refluxed for 4 h. The solvent was concentrated under reduced pressure, poured into water (100 mL) and neutralized with dilute HCl (1 mL, 25%). The precipitate was filtered off and recrystallized from dioxane to give compounds **3a** and **3b**, respectively.

*Synthesis of N^4,N^6-bis(4-chlorobenzylidene)-2-(2-methoxyethylsulfanyl)-3,4-dihydropyrimidine-4,6-diamine (**4**)*

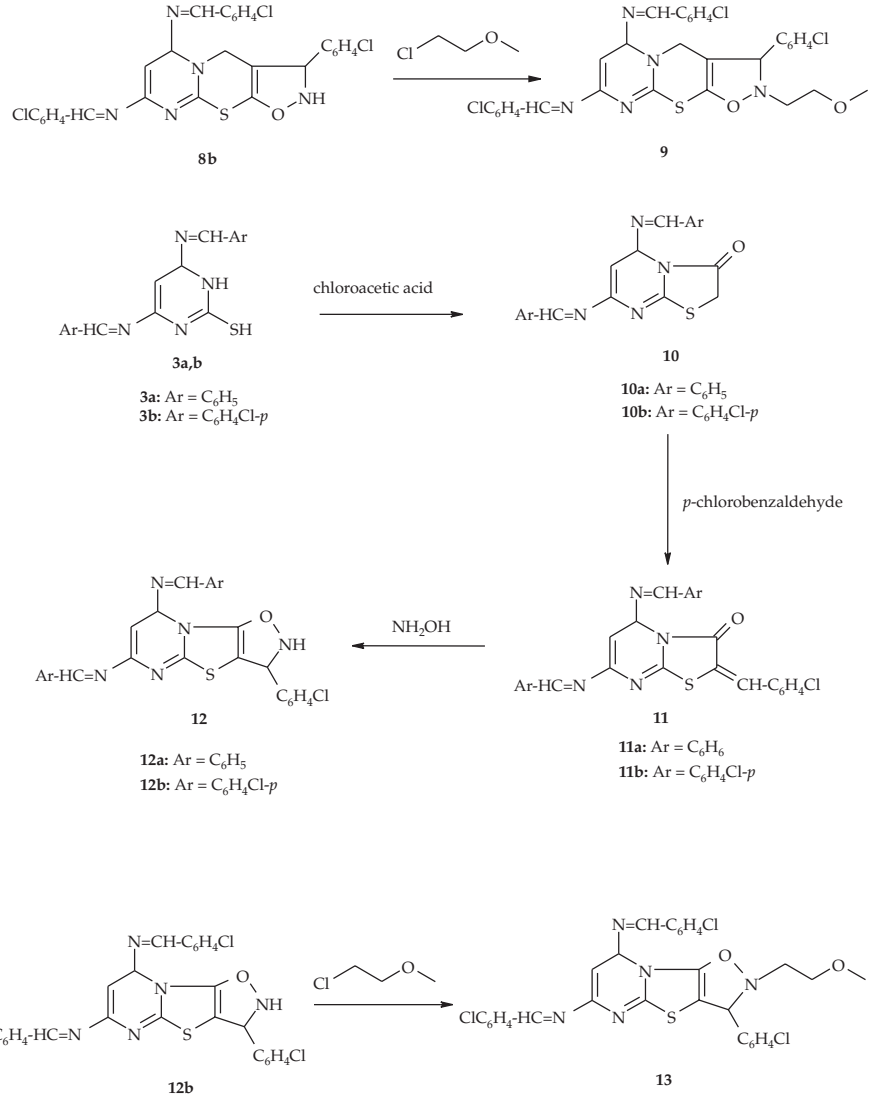
A mixture of compound **3b** (1.98 g, 0.005 mol) and 2-chloroethyl methyl ether (0.005 mol, 0.5 mL) was stirred at 60 °C in ethanol (50 mL) containing NaOH (0.5 g) for 6 h. The reaction mixture was poured into water (100 mL). The solid product was filtered off and recrystallized from ethyl acetate to give compound **4**.

*Synthesis of 3-[4,6-bis-(benzylidene-amino)-2-mercapto-6*H*-pyrimidin-1-yl]-propionitrile (**5a**)*

A mixture of compound **3a** (3.2 g, 0.01 mol) and acrylonitrile (3 mL) was refluxed in pyridine (50 mL) for 5 h. The reaction mixture was poured into water (150 mL). The precipitate was filtered off and recrystallized from ethanol acetate to give compound **5a**.


reduction
Zn/AcOH
aromatic
aldehydes
acrylonitrile
cyclization
p-chlorobenzaldehyde
NH2OH
1
2
3
3a: Ar = C6H5
3b: Ar = C6H4Cl-p
4
5
5a: Ar = C6H5
5b: Ar = C6H4Cl-p
6
6a: Ar = C6H5
6b: Ar = C6H4Cl-p
7
7a: Ar = C6H5
7b: Ar = C6H4Cl-p
8
8a: Ar = C6H5
8b: Ar = C6H4Cl-p

Scheme 1



Scheme 2

*Synthesis of 3-{4,6-bis-[(4-chlorobenzylidene)-amino]-2-mercapto-6*H*-pyrimidin-1-yl}-propionitrile (**5b**)*

A mixture of compound **3b** (3.9 g, 0.01 mol) and acrylonitrile (3 mL) was refluxed in pyridine (50 mL) for 6 h. The reaction mixture was poured into water (100 mL) and neutralized with dilute HCl (1 mL, 25%). The precipitate was filtered off and recrystallized from ethanol to give compound **5b**.

Table I. Analytical data of the newly prepared compounds

Compd. No.	Yield (%)	M. p. (°C)	Molecular formula (M_r)	Elemental analysis, found/calcd. (%)				
				C	H	N	Cl	S
2	80	263–264	$C_4H_8N_4S$	33.39	5.45	38.55		22.32
			(144.20)	33.32	5.59	38.85		22.24
3a	78	290–291	$C_{18}H_{16}N_4S$	67.56	4.94	17.61		9.99
			(320.41)	67.47	5.03	17.49		10.01
3b	82	313–314	$C_{18}H_{14}Cl_2N_4S$	55.32	3.68	14.48	18.24	8.19
			(389.30)	55.53	3.62	14.39	18.21	8.24
4	72	352–354	$C_{21}H_{20}Cl_2N_4OS$	56.48	4.56	12.44	15.80	7.12
			(447.38)	56.38	4.51	12.52	15.85	7.17
5a	70	217–219	$C_{21}H_{19}N_5S$	67.39	5.18	18.71		8.86
			(373.48)	67.53	5.13	18.75		8.95
5b	80	234–235	$C_{21}H_{17}Cl_2N_5S$	57.14	3.81	15.79		7.35
			(442.36)	57.02	3.87	15.83		7.25
6a	60	243–245	$C_{21}H_{18}N_4OS$	67.25	4.81	15.01		8.61
			(374.46)	67.36	4.85	14.96		8.56
6b	70	269–270	$C_{21}H_{16}Cl_2N_4OS$	56.94	3.71	12.52		7.33
			(443.35)	56.89	3.64	12.64		7.23
7a	75	273–275	$C_{28}H_{21}ClN_4OS$	67.72	4.31	11.35	7.07	6.35
			(497.01)	67.66	4.26	11.27	7.13	6.45
7b	75	297–299	$C_{28}H_{19}Cl_3N_4OS$	59.55	3.34	9.86	18.89	5.58
			(565.90)	59.43	3.38	9.90	18.79	5.67
8a	60	304–306	$C_{28}H_{22}ClN_5OS$	65.59	4.45	13.59	6.99	6.20
			(512.03)	65.68	4.33	13.68	6.92	6.26
8b	73	314–316	$C_{28}H_{20}Cl_3N_5OS$	57.96	3.54	11.99	18.26	5.64
			(580.92)	57.89	3.47	12.06	18.31	5.52
9	50	256–257	$C_{31}H_{26}Cl_3N_5O_2S$	58.33	4.05	11.08	16.55	5.12
			(638.99)	58.27	4.10	10.96	16.64	5.02
10a	60	233–234	$C_{20}H_{16}N_4OS$	66.77	4.50	15.49		8.86
			(360.43)	66.65	4.47	15.54		8.90
10b	65	263–265	$C_{20}H_{14}Cl_2N_4OS$	55.86	3.15	13.11	6.45	7.33
			(429.32)	55.95	3.29	13.05	16.52	7.47
11a	A:65	256–257	$C_{27}H_{19}ClN_4OS$	67.10	4.09	11.49	7.47	6.54
	B:58		(482.98)	67.14	3.97	11.60	7.34	6.64
11b	A:70	289–290	$C_{27}H_{17}Cl_3N_4OS$	58.84	3.19	10.02	19.19	5.84
	B:64		(551.87)	58.76	3.10	10.15	19.27	5.81
12a	60	312–313	$C_{27}H_{20}ClN_5OS$	65.28	4.10	13.98	7.27	6.34
			(498.00)	65.12	4.05	14.06	7.12	6.44
12b	65	345–346	$C_{27}H_{18}Cl_3N_5OS$	57.19	3.28	12.27	18.69	5.58
			(566.89)	57.21	3.20	12.35	18.76	5.66
13	60	317–318	$C_{30}H_{24}Cl_3N_5OS$	57.55	3.78	11.29	17.22	5.19
			(624.97)	57.65	3.87	11.21	17.02	5.13

Table II. Spectral data of the newly prepared compounds

Compd. No.	Mass (*m/z*)	IR (ν, cm^{-1})	^1H NMR[a,b] (δ, ppm)
2	144 [M^+](100)	3350–3100 (NH_2 + NH)	10.9 (s, 1H, NH, exchangeable), 10.5 (s, 1H, NH, exchangeable), 8.8 (brs, 2H, NH_2, exchangeable), 4.6 (d, 1H, C_5-H), 4.3 (m, 1H, C_4-H), 3.4 (brs, 2H, NH_2 exchangeable)
3a	320 [M^+] (70)	3320, 3350 (2 NH)	11.0 (s, 1H, NH, exchangeable), 9.2 (s, 1H, NH, exchangeable), 8.2 (s, 2H, 2 Schiff base), 7.7–7.2 (m, 10H, Ar-H), 5.8 (d, 1H, C_5-H), 5.0 (m, 1H, C_4-H)[b].
3b	393 [M+4] (21), 391 [M+2](10), 389 [M^+] (33)	3320, 3350 (2 NH)	11.0 (s, 1H, NH, exchangeable), 9.3 (s, 1H, NH, exchangeable), 8.2 (s, 2H, Schiff base), 7.6–7.2 (m, 8H Ar-H), 5.8 (d, 1H, C_5-H), 5.0 (m, 1H, C_4-H)[b]
4	451[M+4](7), 449[M+2](3), 447 [M^+](12)	3150 (NH)	10.6 (brs, 1H, NH, exchangeable), 8.1 (s, 2H, Schiff base), 7.6–7.2 (m, 8H, Ar-H), 5.7 (m, 1H, C_5-H), 4.8 (m, 1H, C_4-H), 3.5–3.3 (m, 5H, CH_2 and OCH_3), 2.8 (t, 3H, CH_2)[a]
5a	373 [M^+](17)	2500–2510 (SH stretching), 2225 (CN)	8.2 (s, 2H, Schiff base), 7.9–7.1 (m, 11H, Ar-H and SH, exchangeable), 5.9 (d, 1H, C_5-H), 5.0 (d, 1H, C_6-H), 3.7 (t, 2H, CH_2), 3.0 (t, 2H, CH_2-CN)[a]
5b	446 [M+4](29), 444 [M+2](15), 442 [M^+](43)	2500–2510 (SH stretching), 2225 (CN)	8.1 (s, 2H, Schiff base), 7.7–7.3 (m, 9H, Ar-H and SH, exchangeable), 5.7 (d, 1H, C_5-H), 5.1 (d, 1H, C_6-H), 3.7 (t, 2H, CH_2), 2.5 (t, 2H, CH_2-CN)[a]
6a	374 [M^+](21)	1690 (C=O)	8.1 (s, 2H, Schiff base), 7.8–7.1 (m, 10H, Ar-H), 5.8 (d, 1H, C_7-H), 4.9 (d, 1H, C_6-H), 2.8 (t, 2H, CH_2), 2.5 (t, 2H, CH_2)[a]
6b	447 [M+4](38), 445 [M+2](20), 443 [M^+](62)	1696 (C=O)	8.3 (s, 2H, Schiff base), 7.8–7.2 (m, 8H, Ar-H), 5.7 (d, 1H, C_7-H), 5.0 (d, 1H, C_6-H), 2.9 (t, 2H, CH_2), 2.6 (t, 2H, CH_2)[b]
7a	499 [M+2](7), 497 [M^+] (18)	1705 (C=O)	8.3 (s, 2H, Schiff base), 8.0–7.2 (m, 15H, Ar-H and 1H aryl methylene), 5.9 (d, 1H, C_7-H), 4.9 (d, 1H, C_6-H), 3.3 (s, 2H, CH_2)[b]
7b	572 [M+6](45), 570 [M+4](31), 568 [M+2](15), 566 [M^+](43)	1705 (C=O)	8.2 (s, 2H, Schiff base), 7.6–7.1 (m, 13H, Ar-H and 1H aryl methylene), 5.8 (d, 1H, C_7-H), 5.0 (d, 1H, C_6-H), 3.3 (s, 2H, CH_2)[b]
8a	515 [M+2](19), 512 [M^+] (61)	3175 (NH stretching)	10.7 (s, 1H, NH, exchangeable), 8.2 (s, 2H, Schiff base), 7.9–7.0 (m, 14H, Ar-H), 5.9 (d, 1H, C_7-H), 5.0 (d, 1H, C_6-H), 4.7 (s, 1H, C_3-H), 3.3 (s, 2H, CH_2)[b]
8b	587 [M+6](70), 585 [M+4](44), 583 [M+2](23), 581 [M^+](77)	3175 (NH stretching)	10.7 (s, 1H, NH, exchangeable), 8.1 (s, 2H, Schiff base), 7.8–7.0 (m, 12H, Ar-H), 5.9 (d, 1H, C_7-H), 5.0 (d, 1H, C_6-H), 4.7 (s, 1H, C_3-H), 3.2 (s, 2H, CH_2)[b]
9	645 [M+6](20), 643 [M+4](13), 641 [M+2](6), 639 [M^+](22),	absence of NH group	8.3 (s, 2H, Schiff base), 7.9–7.0 (m, 12H, Ar-H), 5.9 (d, 1H, C_7-H), 5.0 (d, 1H, C_6-H), 4.6 (s, 1H, C_3-H), 3.7–3.3 (m, 5H, CH_2 and OCH_3), 3.1 (s, 2H, CH_2 thiazine), 2.7 (t, 2H, CH_2)[b]

Table II. contind.

Compd. No.	Mass (*m/z*)	IR (ν, cm^{-1})	^1H NMR[a,b] (δ, ppm)
10a	360 [M^+](100)	1710, (C=O)	8.1 (s, 2H, Schiff base), 7.7–7.2 (m, 10H, Ar-H), 6.0 (d, 1H, C_5-H), 5.8 (d, 1H, C_6-H), 3.5 (s, 2H, CH_2)[a]
10b	431 [M+2](32), 429 [M^+](98)	1715, (C=O)	8.2 (s, 2H, Schiff base), 7.7–7.2 (m, 8H, Ar-H), 6.0 (d, 1H, C_5-H), 5.7 (d, 1H, C_6-H), 3.6 (s, 2H, CH_2)[a]
11a	485 [M+2](28), 483 [M^+](87)	1695, (C=O)	8.2 (s, 2H, Schiff base), 7.8–7.2 (m, 15H, Ar-H and 1H aryl methylene), 6.0 (d, 1H, C_5-H), 5.7 (d, 1H, C_6-H)[b]
11b	557 [M+6](69), 555 [M+4](42), 553 [M+2](21), 551 [M^+](75)	1695, (C=O)	8.3 (s, 2H, Schiff base), 7.8–7.2 (m, 13H, Ar-H and 1H aryl methylene) 6.1 (d, 1H, C_5-H), 5.7 (d, 1H, C_6-H)[b]
12a	500 [M+2](29), 498 [M^+](88)	3175 (NH)	10.5 (s, 1H, NH, exchangeable), 8.3 (s, 2H, Schiff base), 7.7–7.0 (m, 14H, Ar-H), 5.8 (d, 1H, C_5-H), 5.4 (d, 1H, C_4-H), 4.6 (s, 1H, C_9-H)[b]
12b	573 [M+6](67), 571 [M+4](38), 569 [M+2](19), 567 [M^+](71)	3156 (NH)	10.7 (s, 1H, NH, exchangeable), 8.2 (s, 2H, Schiff base), 7.8–7.0 (m, 12H, Ar-H), 5.8 (d, 1H, C_5-H), 5.0 (d, 1H, C_4-H), 4.7 (s, 1H, C_9-H)[b]
13	631 [M+6](10), 629 [M+4](9), 627 [M+2](4), 625 [M^+](12)	absence of NH group	8.1 (s, 2H, Schiff base), 7.7–7.1 (m, 12H, Ar-H), 5.7 (d, 1H, C_5-H), 5.1 (d, 1H, C_4-H), 4.6 (s, 1H, C_9-H), 3.6–3.2 (m, 5H, CH_2 and OCH_3), 2.7 (t, 2H, CH_2)[b]

[a] In $CDCl_3$
[b] In DMSO-d_6

*Synthesis of 6,8-bis-(benzylidene-amino)-3,4,6-trihydropyrimido[2,1-b][1,3]thiazin-2--one (**6a**)*

Compound **5a** (3.74 g, 0.01 mol) in glacial acetic acid/hydrochloric acid (30+10 mL) was refluxed for 4 h. The solution was concentrated by evaporation under reduced pressure, then the solid was filtered off and recrystallized from acetic acid to give compound **6a**.

*Synthesis of 6,8-bis-[(4-chlorobenzylidene)-amino]-3,4,6-trihydropyrimido[2,1-b][1,3]-thiazin-2-one (**6b**)*

Compound **5b** (2.2 g, 0.005 mol) in glacial acetic acid/hydrochloric acid (15+10 mL) was refluxed for 3 h. The solution was concentrated by evaporation under reduced pressure. Then the solid was filtered off and recrystallized from dioxane to give compound **6b**.

*Synthesis of 6,8-bis-(benzylidene-amino)-3-(4-chlorobenzylidene)-4,6-dihydropyrimido[2,1-b][1,3]thiazin-2-one (**7a**)*

A mixture of compound **6a** (3.74 g, 0.01 mol), 4-chlorobenzaldehyde (1.4 g, 0.01 mol) in glacial acetic acid/acetic anhydride (30+10 mL) and anhydrous sodium acetate (2 g) was refluxed for 3 h. The reaction mixture was allowed to cool and was poured into water (150 mL). The precipitate was filtered off and recrystallized from benzene to give compound **7a**.

*Synthesis of 3-(4-chlorobenzylidene)-6,8-bis-[(4-chlorobenzylidene)-amino)-3-(4-chlorobenzylidene)-4,6-dihydropyrimido[2,1-b][1,3]thiazin-2-one (**7b**)*

A mixture of compound **6b** (2.2 g, 0.005 mol), 4-chlorobenzaldehyde (0.7 g, 0.005 mol) in glacial acetic acid/acetic anhydride (30+10 mL) and anhydrous sodium acetate (2 g) was refluxed for 3 h. The reaction mixture was allowed to cool and was poured into water (150 mL). The precipitate was filtered off and recrystallized from dioxane to give compound **7b**.

*Synthesis of N^6,N^8-bis-dibenzylidene-3-(4-chlorophenyl)-2,3,4,6-tetrahydroisoxazolo-[4,5-d]thiazino[2,3-a]pyrimidine-6,8-diamine (**8a**)*

A mixture of compound **7a** (2.48 g, 0.005 mol), hydroxylamine hydrochloride (0.2 g, 0.005 mol) and anhydrous sodium acetate (2 g) was refluxed in glacial acetic acid for 6 h. The reaction mixture was allowed to cool and was poured into water (150 mL). The solid substance was filtered off and recrystallized from dioxane to give compound **8a**.

*Synthesis of N^6,N^8-bis-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2,3,4,6-tetrahydroisoxazolo[4,5-d]thiazino[2,3-a]pyrimidine-6,8-diamine (**8b**)*

A mixture of compound **7b** (2.83 g, 0.005 mol), hydroxylamine hydrochloride (0.2 g, 0.005 mol) was refluxed in glacial acetic acid for 6 h. The reaction mixture was allowed to cool and was poured into water (150 mL). The solid substance was filtered off and recrystallized from DMF to give compound **8b**.

*Synthesis of N^6,N^8-bis-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2-(2-methoxy-ethyl)-2,3,4,6-tetrahydroisoxazolo[4,5-d]thiazino[2,3-a]pyrimidine-6,8-diamine (**9**)*

To a solution of compound **8b** (2.90 g, 0.005 mol) in anhydrous DMF (30 mL), sodium hydride (0.2 g) was added under stirring at 60 °C for 1 h, then 2-chloroethyl methyl ether (0.1 mL, 0.01 mol) was added and the reaction mixture was stirred at 40 °C for 8 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by column chromatography (chloroform/petroleum ether, 4:1) to give compound **9** as orange crystals.

*Synthesis of 5,7-bis-(benzylidene-amino)-5*H*-thiazolo[3,2-a]pyrimidin-3-one (**10a**)*

A mixture of compound **3a** (3.2 g, 0.01 mol), chloroacetic acid (1.07 g, 0.01 mol) and anhydrous sodium acetate (2 g) was refluxed in glacial acetic acid/acetic anhydride (30+10 mL) for 3 h. The reaction mixture was allowed to cool and poured into water (100 mL). The precipitate was filtered off and recrystallized from methanol to give compound **10a**.

*Synthesis of 5,7-bis-[(4-chlorobenzylidene)-amino]-5*H*-thiazolo[3,2-a]pyrimidin-3-one (**10b**)*

A mixture of compound **3b** (1.94 g, 0.005 mol), chloroacetic acid (0.51 g, 0.005 mol) and anhydrous sodium acetate (2 g) was refluxed in glacial acetic acid/acetic anhydride (30+10 mL) for 3 h. The reaction mixture was allowed to cool and was poured into water (100 mL). The precipitate was filtered off and recrystallized from methanol to give compound **10b**.

*Synthesis of 5,7-bis-(benzylidene-amino)-2-(4-chlorobenzylidene)-5*H*-thiazolo[3,2-a]-pyrimidin-3-one (**11a**) and 2-(4-chlorobenzylidene)-5,7-bis-[(4-chlorobenzyliden)-amino]-5*H*-thiazolo[3,2-a]pyrimidin-3-one (**11b**)*

Method A. – A mixture of compound **10a** or **10b** (0.005 mol), 4-chlorobenzaldehyde (0.7 g, 0.005 mol) and anhydrous sodium acetate in glacial acetic acid/acetic anhydride (30+10 mL) was refluxed for 3 h. The reaction mixture was allowed to cool and was poured into water (100 mL). The solid substance was filtered off and recrystallized from dioxane to give compounds **11a,b**.

Method B. – A mixture of compound **3a** or **3b** (0.01 mol), aromatic aldehyde (0.01 mol) and anhydrous sodium acetate (2 g) in glacial acetic acid/acetic anhydride (30+10 mL) was refluxed for 4 h. The reaction mixture was allowed to cool and was poured into water. The precipitate was filtered off and recrystallized from dioxane. Compounds **11a** and **11b** prepared by this method are identical in all respects (physical and spectral data) to those prepared by method A.

*Synthesis of N^4,N^6-dibenzylidene-9-(4-chlorophenyl)-1,4,9-trihydroisoxazolo[4,5-d]-thiazolo[2,3-a]pyrimidine-4,6-diamine (**12a**)*

A mixture of compound **11a** (2.4 g, 0.005 mol), hydroxylamine hydrochloride (0.04 g, 0.005 mol) and anhydrous sodium acetate (1 g) was refluxed in glacial acetic acid for 5 h. The reaction mixture was allowed to cool and was poured into water (100 mL). The solid substance was filtered off and recrystallized from DMF to give compound **12a**.

*Synthesis of N^4,N^6-bis-(4-chlorobenzylidene)-9-(4-chlorophenyl)-1,4,9-trihydroisoxazolo [4,5-d]thiazolo[2,3-a]pyrimidine-4,6-diamine (**12b**)*

A mixture of compound **11b** (0.55 g, 0.001 mol) and hydroxylamine hydrochloride (0.008 g, 0.001 mol) was refluxed in glacial acetic acid for 4 h. The reaction mixture was allowed to cool and was poured into water (100 mL). The solid substance was filtered off and recrystallized from DMF to give compound **12b**.

Synthesis of N^4,N^6-bis-(4-chlorobenzylidene)-9-(4-chlorophenyl)-1-(2-methoxy-ethyl)--4,9-dihydroisoxazolo[4,5-d]thiazolo[2,3-a]pyrimidine-4,6-dimine (13)

A mixture of compound **12b** (0.57 g, 0.001 mol) and sodium anhydride (0.2 g) was stirred in DMF at 70 °C for 1 h, cooled, and then 2-chloroethyl methyl ether (0.001 mol, 0.1 mL) was added. The reaction mixture was stirred at 70–80 °C overnight. The reaction mixture was evaporated under reduced pressure and chromatographed on silica gel column (chloroform/methanol 7:3) to give compound **13**.

Antimicrobial activity

The *in vitro* antimicrobial activity of the synthesized compounds was tested against several pathogenic representatives: *Escherichia coli*, *Bacillus subtilis*, *Mycobacterium phlei*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. All microorganisms used were obtained from the culture collection of the Department of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Disc diffusion sensitivity test was done in the manner identical to that of Bauer *et al.* (20). Media for disc sensitivity tests were nutrient agar and Muller-Hinton agar (MHA), purchased from Difco, (USA). The nonsterile powder of the tested compounds was dissolved in sterile DMSO to yield 2 μg mL^{-1} passed through 0.2 μm membrane filter (Millipore Corp., USA). The filtrates were dispensed as 2 mL samples into sterile, small screw-capped vials and kept stored at –15 °C. DMSO as a solvent showed no inhibition zones. The results were compared to streptomycin as a reference drug.

RESULTS AND DISCUSSION

4,6-Diamino-1*H*-pyrimidine-2-thione (**1**) (19) was used as a key compound for this study and for further syntheses of other fused heterocycles. Reaction of compound **1** with Zn dust in glacial acetic acid gave 4,6-diamino-3,4-dihydro-1*H*-pyrimidine-2-thione (**2**). The spectral data of compound **2** confirmed its structure (Table II).

Compound **2** was condensed with aromatic aldehydes in alkaline medium to give 4,6-bis-(arylidene-amino)-1,3,4-trihydropyrimidine-2-thione derivatives **3a,b** (Scheme 1). The IR spectra of the latter compounds showed the absence of amino groups; their MS gave the characteristic fragmentation pattern due to the presence of chlorine atoms in compound **3b** (Table II). Alkylation of **3b** with 2-chloroethyl methyl ether in the pres-

ence of alcoholic NaOH solution (21) gave N^4,N^6-bis(4-chlorobenzylidene)-2-(2-methoxyethylsulfanyl)-3,4-dihydropyrimidine-4,6-diamine (**4**). The IR spectra of the latter compound showed the absence of one NH group and its ^{1}H NMR spectrum in $CDCl_3$ revealed a signal for the methoxyethyl protons (Table II). Cyanoethylation of compounds **3a,b** with an equimolecular amount of acrylonitrile in pyridine gave 3-[4,6-bis-(benzylidene--amino)-2-mercapto-6*H*-pyrimidin-1-yl]-propionitrile derivatives **5a,b**. The IR spectra of the latter compounds showed the presence of cyano group (Table II). Cyclization of compounds **5a,b** in glacial acetic acid/hydrochloric acid (3:1) produced 6,8-bis-[(4-chlorobenzylidene)-amino]-3,4,6-trihydropyrimido[2,1-*b*][1,3]thiazin-2-ones **6a,b**. The elemental analysis and spectral data of compounds **6a,b** confirmed their structures (Tables I and II).

Condensation of the aforementioned compounds with *p*-chlorobenzaldehyde gave the respective 6,8-bis-(arylidene-amino)-3-(4-chlorobenzylidene)-4,6-dihydropyrimido [2,1-*b*][1,3]thiazin-2-ones **7a,b** (Scheme 1). Compounds **7a,b** underwent a cycloaddition reaction with hydroxylamine hydrochloride in glacial acetic acid and anhydrous sodium acetate to produce N^6,N^8-bis-diarylidene-3-(4-chlorophenyl)-2,3,4,6-tetrahydroisoxazolo-[4,5-*d*]thiazino[2,3-*a*]pyrimidine-6,8-diamines **8a,b**. The IR spectra of the latter compounds showed the absence of C=O group and the presence of NH group. Also, MS spectra gave their molecular ion peaks (Table II).

Alkylation of compound **8b** with 2-chloroethyl methyl ether produced N^6,N^8-bis-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2-(2-methoxy-ethyl)-2,3,4,6-tetrahydroisoxazolo [4,5-*d*]thiazino[2,3-*a*]pyrimidine-6,8-diamine (**9**) (Scheme 2). The IR spectra of the latter compound showed the absence of NH group and its ^{1}H NMR spectrum in DMSO-d_6 revealed a signal for the methoxyethyl protons (Table II). Also, when compounds **3a,b** were treated with chloroacetic acid in glacial acetic acid/acetic anhydride in the presence of sodium acetate, 5,7-bis-(arylidene-amino)-5*H*-thiazolo[3,2-*a*]pyrimidin-3-ones **10a,b** were obtained. The IR spectra of the latter compounds showed the presence of C=O group (Table II). Condensation of thiazolopyrimidine derivatives **10a,b** with 4-chlorobenzaldehyde under reflux afforded 5,7-bis-(arylidene-amino)-2-(4-chlorobenzylidene)--5*H*-thiazolo[3,2-*a*]pyrimidin-3-one derivatives **11a,b** (Experimental section, Table II and Scheme 2). Compounds **11a,b** reacted with hydroxylamine hydrochloride in glacial acetic acid and anhydrous sodium acetate to produce N^4,N^6-diarylidene-9-(4-chlorophenyl)-1,4,9-trihydroisoxazolo[4,5-*d*]thiazolo[2,3-*a*]pyrimidine-4,6-diamines **12a,b** (Scheme 2). Compound **12b** was alkylated with 2-chloroethyl methyl ether to produce N^4,N^6-bis--(4-chlorobenzylidene)-9-(4-chlorophenyl)-2-(2-methoxy-ethyl)-4,9-dihydroisoxazolo[4,5-*a*]-thiazolo[2,3-*a*]pyrimidine-4,6-dimine **13**. The IR spectra of the latter compound showed the absence of NH group and its ^{1}H NMR spectrum in DMSO-d_6 revealed a signal for the methoxyethyl protons (Table II).

The antimicrobial activity of some newly synthesized compounds (**3a,b, 4, 5b, 6b, 7b, 8b, 10a,b** and **12b)** were tested and the results are shown in Table III. Evaluation of the new compounds established that compounds **3a,b, 4, 5b, 6b,** and **7b** were slightly active against the tested microorganisms. It was noticed that compound **12b** demonstrated the highest inhibitory activity among the tested compounds. On the other hand, it was found that compounds **5b** and **8b** revealed antibacterial activity more effective than the other tested compounds while compound **10b** revealed antifungal activity more effective than the other tested compounds and the reference drug.

Table III. Antimicrobial activity of some synthesized compounds[a]

Compound	Disc diffusion test (mm)					
	E. coli	*S. aureus*	*M. phlei*	*B. subtilis*	*C. albicans*	*A. niger*
Streptomycin[b]	+++	+	++	+++	+++	+
3a	–	+	+	–	+	++
3b	+	–	–	+	++	++
4	++	–	+	+	+	++
5b	++	++	+	++	–	–
6b	–	+	–	–	+	+
7b	–	+	+	–	++	++
8b	++	+	+	++	+	+
10a	–	–	–	–	+++	+
10b	–	–	–	+	+++	+++
12b	+	++	+	++	+++	+++

[a] γ = 2 μg mL^{-1} in DMSO.
[b] γ = 25 μg mL^{-1} in DMSO Lot. 30730, Bioanalyse (Turkey).
+++ highly sensitive (14–16 mm), ++ fairly sensitive (12–14 mm), + slightly sensitive (10–12 mm), – not sensitive.

CONCLUSIONS

The substituted pyrimidine derivatives, 4,6-bis-(benzyldidene-amino)-1,3,4-trihydropyrimidine-2-thione (**3a**), 4,6-bis-[(4-chlorobenzylidene)-amino]-1,3,4-trihydropyrimidine--2-thione (**3b**) and N^4,N^6-bis(4-chlorobenzylidene)-2-(2-methoxyethylsufanyl)-3,4-dihydropyrimidine-4,6-diamine (**4**) revealed promising antimicrobial activity. However, fusing a thiazine or a thiazole ring to the pyrimidine derivatives as in compounds 6,8-bis-[4-chlorobenzylidene)-amino]-3,4,6-trihydropyrimido[2,1-*b*] [1,3]thiazin-2-one (**6b**), 3-(4-chlorobenzylidene)-6,8-bis-[4-chlorobenzylidene)-amino)-3-(4-chlorobenzylidene)-4,6-dihydropyrimido[2,1-*b*][1,3]thiazin-2-one (**7b**), 5,7-bis-(benzylidene-amino)-5*H*-thiazolo[3,2-*a*]pyrimidin-3-one (**10a**) and 5,7-bis-[(4-chlorobenzylidene)-amino]-5*H*-thiazolo [3,2-*a*]pyrimidin-3-one (**10b**) decreased the antimicrobial activity. On the other hand, increased antimicrobial activity was achieved by fusing an isoxazole ring with the pyrimidothiazine or thiazolopyrimidine ring system in compound N^6,N^8-bis-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2,3,4,6-tetrahydroisoxazolo[4,5-d]thiazine[2,3-*a*]pyrimidine-6,8-diamine (**8b**) and N^4,N^6-bis-(4-chlorobenzylidene)-9-(4-chlorophenyl)-1,4,9-trihydroisoxazolo[4,5-*d*]thiazolo[2,3-a]pyrimidine-4,6-diamine (**12b**).

Acknowledgement. – The authors are grateful to Dr. A. A. Fahmy, Department of Microbial Products, Ain Shams University, Cairo, Egypt for biological evaluation. Also, the authors thank the Central Services Unit (National Research Centre, Cairo, Egypt) for operating the spectral data (microanalytical data, IR, MS and ^{1}H NMR).

REFERENCES

1. P. Pecorari, M. Rinaldi, L. Costantino, A. Provvisionato,C. Cermelli and M. Portolani, Synthesis and biological activity of pyrimido[2,1-*b*][1,3]thiazine, [1,3]thiazino[3,2-*a*]purine and [1,2,3]triazolo[4,5-*d*][1,3]thiazino[3,2-*a*] pyrimidine derivatives and thiazole analogues, *Farmaco* **46** (1991) 899–911.
2. M. S. Al-Thebeiti, Synthesis of some new derivatives of thiazolo-[3,2-*a*] pyrimidine-3,5,7(2*H*)-trione of potential biological activity, *Boll. Chim. Farm.* **140** (2001) 221–223.
3. B. Tozkoparan, M. Ertan, P. Kelicen and R. Demirdamar, Synthesis and anti-inflammatory activities of some thiazolo[3,2-*a*]pyrimidine derivatives, *Farmaco* **54** (1999) 588–593.
4. M. S. A. El-Gaby, S. G. Abdel-Hamide, M. M. Ghorab and S. M. El-Sayed, Synthesis and anticancer activity *in vitro* of some new pyrimidines, *Acta Pharm.* **49** (1999) 149–158.
5. C. G. Dave, D. R. Shah, G. K. Shah, P. S. Pandya, K. C. Dave and V. J. Patel, Pyridopyrimidines part III. Synthesis and analgesic activity of 4-aminopyrido[2,3-*d*]pyrimidines, *Indian J. Pharm. Sci.* **48** (1986) 75–77.
6. K. Stulik and V. Pacakova, High performance liquid chromatography of biologically important pyrimidine derivatives with ultraviolet-voltametric-polarographic detection, *J. Chromatogr.* **1** (1983) 77–86.
7. P. Zimmermann, J. Senn-Bilfinger, B. Kohl, G. Hanauer, S. Postius, W. Opferkuch and G. Grundler, *Preparation of Imidazopyridazines for Control of Helicobacter Bacteria*, PCT Int. Appl. Wo 98 28, 299, 2 Jul 1998; ref. *Chem. Abstr.* **129** (1998) 109095t.
8. M. Bos, T. Godel, C. Riemer and A. Sleight, *Sulfonamide for Treatment of Central Nervous Disorders*, Eur. Pat. Appl. EP 815861, 7 Jan 1998; ref. *Chem. Abstr.* **128** (1998) 145382x.
9. C. G. Dave, P. R. Shah, V. B. Desai and S. Srinivasan, Synthesis and biological activity of some pyridylthioureas and pyridopyrimidinethiones, *Indian J. Pharm. Sci.* **44** (1982) 83–85.
10. D. Bozsing, G. Kovanyi Lax, E. Berenyi Poldermann, K. Magyar, S. Tuboly and A. Mandi, *Preparation and Testing of Aminoiminoheterocyclopyrimidines as Immunostimulants*, Ger. Offen. DE 3, 743, 935, 14 Jul 1988; ref. *Chem. Abstr.* **110** (1989) 75547g.
11. S. Shigeta, S. Mori, F. Watanabe, K. Takahashi, T. Nagata, N. Koike, T. Wakayama and M. Saneyoshi, Synthesis and antiherpes virus activities of 5-alkyl-2-thiopyrimidine nucleoside analogues, *Antivir. Chem. Chemother.* **13** (2002) 67–82.
12. A. E. Rashad and M. A. Ali, Synthesis and antiviral screening of some thieno[2,3-*d*]pyrimidine nucleosides, *Nucleosides, Nucleotides* **25** (2006) 17–28.
13. N. M. Yousif, F. A. Gad and H. H. Sayed, Synthesis and reactions of some arylmethylene-1-indanone derivatives of expected biological activity, *Egypt. J. Chem.* **35** (1992) 101–110.
14. N. M. Yousif, F. A. Gad**,** and H. H. Sayed, Synthesis and reactions of β-ketoanilides for biological activity, *Egypt. J. Pharm. Sci.* **37** (1996) 145–156.
15. H. H. Sayed, Reactions with α,β-spiroepoxyalkanones part – VI. Reactions of spirononanones with amines and thiourea, *Indian J. Chem.* **37**B (1998) 1054–058.
16. H. H. Sayed and F. A. Fahmy, Uses of 2-aryl-3-(tetral-1-one) oxirane in synthesis of spiro and condensed heterocyclic compounds, *Egypt. J. Chem.* **44** (2001) 365–372.
17. A. E. Rashad, H. H. Sayed, A. H. Shamroukh and H. M. Awad, Preparation of some fused pyridopyrimidine and pyridothienotriazine derivatives for biological evaluation, *Phosphorus Sulfur* **180** (2005) 2767–2777.
18. F. M. E. Abdel-Megeid, N. A. Hassan, M. A. Zahran and A. E. Rashad, Synthesis of 5,6-dihydronaphtho[1',2':4,5]thieno[2,3-*d*]pyrimidines, 5,6-dihydro-naphtho[1',2':4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines and some of their nucleosides, *Sulfur Lett.* **21** (1998) 269–284.

19. V. M. Berezovskii and A. M. Yurkevich, An improved synthesis of adenine, *Zh. Obshch. Khim*. **32** (1962) 1655–1659; ref. *Chem. Abstr.* **58** (1963) 4566d.
20. A. W. Bauer, M. M. Kirby, J. C. Sherris and M. Turck, Antibiotic susceptibility testing by a standardized single disc method, *Am. J. Clin. Pathol.* **45** (1996) 493–496.
21. T. H. Brown, R. C. Blakemore, G. J. Durant, J. C. Emmett, C. R. Ganeline and M. E. Parsons, D. A. Rawlings and T. F. Walker, Isocytosine H_2-receptor histamine – antagonists part I. Oxmetidine and related compounds, *Eur. J. Med. Chem*. **23** (1988) 53–62.

S A Ž E T A K

Sinteza i biološka evaluacija nekih pirimidina, pirimido[2,1-*b*][1,3]tiazina i tiazolo[3,2-*a*]pirimidin derivata

HAYAM H. SAYED, AHMED H. SHAMROUKH i AYMN E. RASHAD

4,6-Diamino-1*H*-pirimidin-2-tion (**1**) upotrebljen je kao ishodni spoj u sintezi derivata pirimidina **2–5**. Spoj **5** je cikliziran u pirimido[2,1-*b*][1,3]tiazin derivat **6**, koji je kondenziran s *p*-klorbenzaldehidom u spoj **7**. Produkt **7** je u reakciji s hidroksilaminom dao izoksazol[4,5-*d*]tiazino[2,3-*a*]pirimidin **8**. Spoj **8b** je u reakciji s 2-kloretil-metil-eterom dao spoj **9**. Slično je spoj **3** reagirao s kloroctenom kiselinom i dao tiazol[3,2-*a*]pirimidin **10,** koji je kondenziran s *p*-klorbenzaldehidom u produkt **11**. Spoj **11** je kondenzacijom s hidroksilaminom dao izoksazol[4,5-*d*]tiazolo[2,3-*a*]pirimidin **12**. Spoj **12b** je s 2-kloretil-metil-eterom dao produkt **13**. Biološka evaluacija pokazuje da neki od sintetiziranih produkata imaju antimikrobno djelovanje.

Ključne riječi: pirimidin, pirimidotiazin, tiazolopirimidin, antimikrobno djelovanje

Department of Photochemistry, National Research Center, Dokki, Cairo, Egypt