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Synthesis of Thiazolo[4,5-d]pyrimidine Derivatives as Potential Antimicrobial Agents

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In this study, we report the synthesis and antimicrobial evaluation of several new thiazolo[4,5-d]pyrimidine derivatives, namely 7-substituted amino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thiones **4a**-**e**, **8**, **13**, **15**, ethyl 2-cyano-2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]-pyrimidin-2(3*H*)-ylidene)acetates **5a**-**b**, 2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3*H*)-ylidene)malononitriles **6a**-**b**, 5-methyl-7-morpholino-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-one **7,** and 7-[4-(1-substituted-5-phenyl-4,5-dihydro-1*H*-pyrazolin-3-yl)anilino]-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thiones **10**-**12**. Some of the tested compounds were more active against *C. albicans* than *E. coli* and *P. aeruginosa*, and all were inactive against *S. aureus.*

Key words: Thiazolo[4,5-d]pyrimidines**,** Pyrazolines, Antimicrobial

INTRODUCTION

The synthesis and pharmacological activity of condensed pyrimidine derivatives have been reported. Thiazolo [4,5-d]pyrimidine derivatives are the bioisosteric analogues of purines and are potentially bioactive molecules. Many derivatives with different substitution patterns display interesting pharmacological activities. Thiazolo[4,5-d]pyrimidine derivative **I** (Fig. 1) possesses antiviral activity against human cytomegalovirus (HCMV) (Revankar *et al*., 1998). Compounds **II**, **III** exhibit anticancer activity against 60 human tumor cell lines (Fahmy *et al*., 2003). Compound **IV** displays antipsychotic activity by antagonizing the activity of the corticotrophin releasing factor (Beck *et al*., 1999). In addition, thiazolo[4,5-d]pyrimidine derivative **V** exhibits dual antimicrobial and anti-inflammatory activity comparable to ampicillin and indomethacin *in vivo* with no or minimal ulcerogenic effects (Bekhit *et al*., 2003).

In light of these facts, the present work describes the synthesis of several 7-(substituted amino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione derivatives **4a**-**e**, **8**, and assesses their antimicrobial activity. The 2-thioxo group in compounds **4a**-**b** is further replaced by either (cyano,ethoxycarbonyl)methylidene or dicyanomethylidene moieties, and in compounds **5a**-**b**, **6a**-**b** and **7**, by an oxygen function (Fig. 1). Special emphasis was given to how these structural modifications would inform the development of new agents with enhanced antimicrobial activity.

Furthermore, since pyrazoline derivatives exhibit anti-inflammatory (Farghaly *et al*., 2000; Abdel-Aal *et al*., 2002), antimicrobial (Farghaly *et al*., 2001; Farghaly *et al*., 2002), and anticancer activity (Garcia-Lopez *et al.,*1979), we designed and synthesized new pyrazoline derivatives in combination with thiazolo[4,5-d]pyrimidines in a single molecular framework (compounds **10**-**12**) to obtain antimicrobial compounds with higher activity (Fig. 1). In addition, we attempted to prepare the polycyclic compounds **14** and **16** (Fig. 1) to study their antimicrobial activity compared with other thiazolopyrimidine derivatives **4**-**12**, but only we obtained the open chain derivatives, **13** and **15**. All products were screened *in vitro* for antimicrobial activity.

MATERIALS AND METHODS

Melting points were determined in open-glass capillaries on a Gallen-kamp melting point apparatus and were uncorrected. IR spectra were recorded for potassium bromide discs on a Perkin-Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were determined on a JNM-LA 400 FT NMR system (400 MHz), Faculty of Science, Assuit

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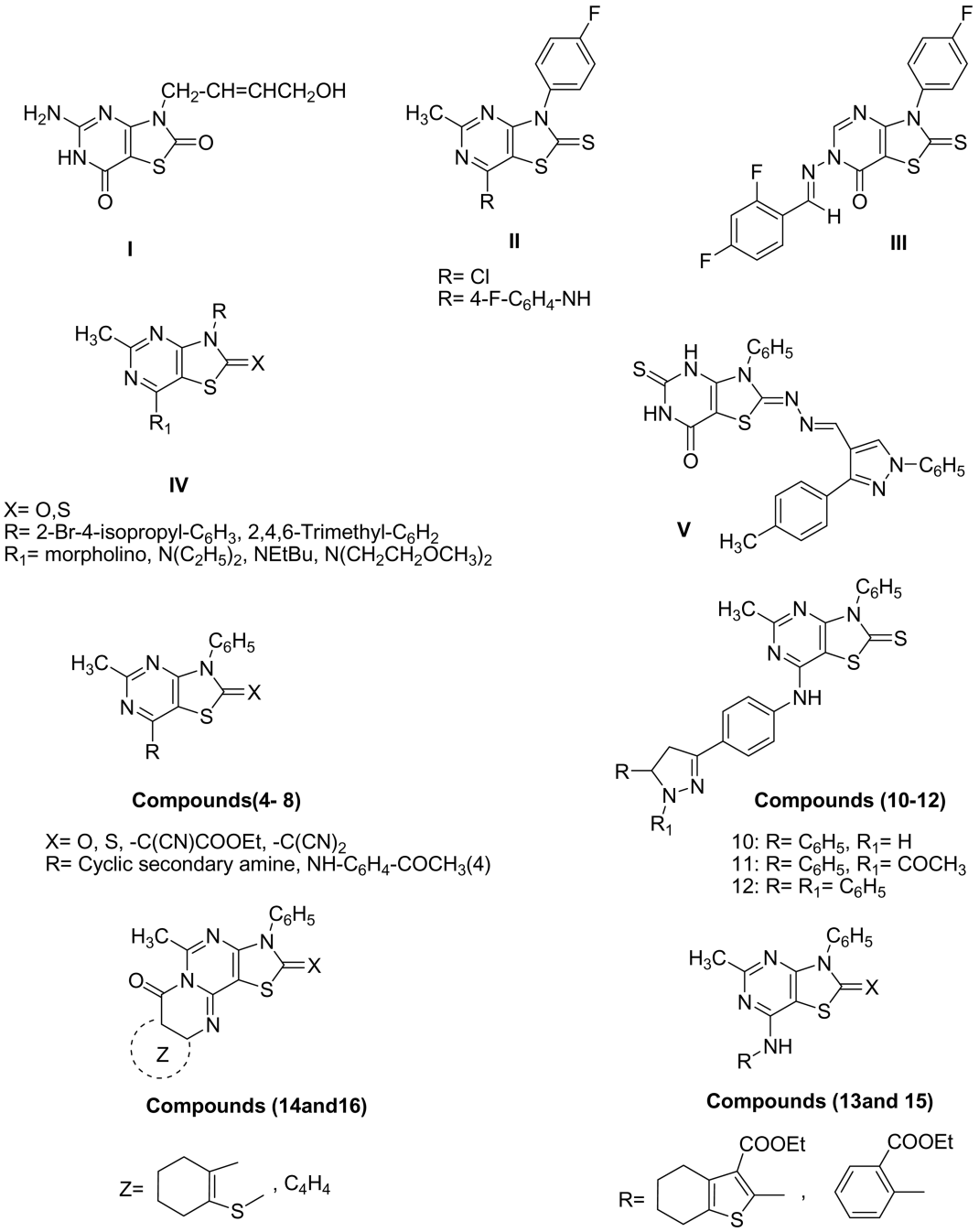


Fig. 1. Some selected models of thiazolopyrimidine derivatives possessing various pharmacological activities and the proposed design of the newly synthesized thiazolopyrimidine derivatives (**4**-**16**)

University, and on Jeol (500 MHz), Faculty of Science, Alexandria Univesity. Mass spectra were run on a Finnigan mass spectrometer model S SQ/7000 (70 ev), Faculty of Science, Cairo University. Microanalyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University, and The Microanalytical Unit, Faculty of Science, Assuit University. Follow-up of the reactions and checking the homogeneity of the compounds were performed by TLC. The spots were visualized by exposure to iodine vapor or UV light at λ 254 nm for a few seconds.

General procedure for the preparation of 7-substituted amino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thiones (4a-e)

To a solution of **3** (0.59 g, 2 mmol) in dry acetone, the appropriate amine (4 mmol) was added. The reaction mixture was heated under reflux for 3 h during which time the product was partially crystallized out. The product

obtained after cooling was filtered, dried, and recrystallized from ethanol (Table I). IR (KBr, Cm^{-1}) of **4a**: 1568, 1535, 1489 (C=N, C=C), 1535, 1298, 1086, 992 (N-C=S amide I, II, III and IV bands, respectively), 1272, 1041 (C-S-C), 1245, 1041 (C-O-C), 1120 (C-N). ^{1}H-NMR of **4a** ($CDCl_3$, 400 MHz): δ 2.60 (s, 3H, H_3CC=N), 3.91 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{3,5}$-H), 4.32 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{2,6}$-H), 7.24 (d, 2H, *J* = 6.9 Hz, C_6H_5-$C_{2,6}$-H), 7.48 (t, 1H, *J* = 6.9 Hz, C_6H_5-C_4-H), 7.81 (t, 2H, *J* = 6.9 Hz, C_6H_5-$C_{3,5}$-H). IR (KBr, cm^{-1}) of **4b**: 1569,1484(C=N, C=C), 1535, 1278, 1070, 976 (N-C=S), 1249, 1047 (C-S-C), 1109 (C-N). ^{1}H-NMR of **4b** ($CDCl_3$, 500 MHz): δ 1.68-1.75 (m, 6H, piperidine-$C_{3,4,5}$-H), 2.41 (s, 3H, H_3CC=N), 3.77-3.80 (m, 4H, piperidine-$C_{2,6}$-H), 7.33 (d, 2H, *J* = 7.7 Hz, C_6H_5-$C_{2,6}$-H), 7.53 (t, 1H, *J* = 7.7 Hz, C_6H_5-C_4-H), 7.58 (t, 2H, *J* = 7.7 Hz, C_6H_5-$C_{3,5}$-H). IR (KBr, Cm^{-1}) of **4c**: 1566, 1488 (C=N, C=C), 1538, 1249, 1045, 996 (N-C=S), 1268, 1070 (C-S-C), 1135 (C-N). ^{1}H-NMR of **4c** ($CDCl_3$, 500 MHz): δ 2.37 (s, 3H, H_3CC=N), 2.62-2.70 (m, 4H, piperazine-$C_{3,5}$-H), 3.64 (s, 2H, CH_2), 3.88-3.90 (m, 4H, piperazine-$C_{2,6}$-H), 7.32 (d, 2H, *J* = 7.7 Hz, NC_6H_5-$C_{2,6}$-H), 7.36-7.40 (m, 5H, C_6H_5), 7.53 (t, 1H, *J* = 7.7 Hz, N C_6H_5-C_4-H), 7.58 (t, 2H, *J* = 7.7 Hz, NC_6H_5-$C_{3,5}$-H). IR (KBr, Cm^{-1}) of **4d**: 1568, 1485 (C=N, C=C), 1542, 1276, 1070, 996 (N-C=S), 1249, 1046 (C-S-C), 1139 (C-N). ^{1}H-NMR of **4d** ($CDCl_3$, 400 MHz): δ 2.33 (s, 3H, CH_3C=N), 2.36 (s, 3H, N-CH_3), 2.50 (t, 4H, *J* = 4.6 Hz, piperazine $C_{3,5}$-H), 3.81 (t, 4H, *J* = 4.6 Hz, piperazine $C_{2,6}$-H), 7.31 (d, 2H, *J* = 6.0 Hz, C_6H_5-$C_{2,6}$-H), 7.50-7.58 (m, 3H, C_6H_5-$C_{3,4,5}$-H). IR (KBr, Cm^{-1}) of **4e**: 1567, 1493 (C=N, C=C), 1537, 1249, 1049, 963 (N-C=S), 1266, 1088 (C-S-C), 1129 (C-N). ^{1}H-NMR of **4e** ($CDCl_3$, 500 MHz): δ 1.33-1.37 (m, 4H, piperidine-$C_{3,5}$-H), 1.83-1.85 (m, 4H, piperidine-$C_{2,6}$-H), 2.49 (s, 3H, H_3CC=N), 3.11 (t, 1H, *J* = 13.0 Hz, piperidine-C_4-H), 4.54 (d, 2H, *J* = 13.0 Hz, CH_2), 7.15 (d, 2H, *J* = 7.6 Hz, C_6H_5-$C_{2,6}$-H), 7.22 (t, 1H, *J* = 7.6 Hz, C_6H_5-C_4-H), 7.29 (d, 2H, *J* = 7.7 Hz, N-C_6H_5-$C_{2,6}$-H), 7.31-7.33 (m, 2H, C_6H_5-$C_{3,5}$-H), 7.54 (t, 1H, *J* = 7.7 Hz, N-C_6H_5-C_4-H), 7.59 (t, 2H, *J* = 7.7 Hz, N-C_6H_5-$C_{3,5}$-H).

Table I. Physical constants and elemental analyses of thiazolo[4,5-d]pyrimidine derivatives **4**-**15**

Comp No.	Yield %	M.P. °C	Mol. Formula (Mol. Wt.)	Microanalyses % calcd/found			
				C	H	N	S
4a	65	234 [(a)]	$C_{16}H_{16}N_4OS_2$ (344.46)	55.79 55.90	4.68 4.39	16.27 16.25	18.62 18.41
4b	70	185	$C_{17}H_{18}N_4S_2$ (342.49)	59.62 59.80	5.30 5.33	16.36 16.32	18.72 18.71
4c	68	150	$C_{23}H_{23}N_5S_2$ (433.60)	63.71 63.39	5.35 5.18	16.15 15.90	14.79 14.44
4d	65	186-188	$C_{17}H_{19}N_5S_2$ (357.50)	57.12 57.00	5.36 5.33	19.59 19.34	17.94 17.68
4e	73	148	$C_{24}H_{24}N_4S_2$ (432.61)	66.63 66.45	5.59 5.33	12.95 12.64	14.82 14.56
5a	62	>300	$C_{21}H_{21}N_5O_3S$ (423.50)	59.56 59.93	5.00 4.72	16.54 16.21	7.57 7.35
5b	65	178	$C_{22}H_{23}N_5O_2S$ (421.52)	62.69 62.33	5.50 5.11	16.61 16.30	7.61 7.22
6a	64	262	$C_{19}H_{16}N_6OS$ (376.44)	60.62 60.85	4.28 3.99	22.32 21.99	8.52 8.65
6b	61	164	$C_{20}H_{18}N_6S$ (374.47)	64.15 63.87	4.85 4.52	22.44 22.10	8.56 8.78
7	76	202	$C_{16}H_{16}N_4O_2S$ (328.40)	58.52 58.30	4.91 4.63	17.06 16.81	9.76 9.91
8	61	238-240	$C_{20}H_{16}N_4OS_2$ (392.50)	61.20 60.95	4.11 3.99	14.27 13.93	16.34 15.99
9	64	230	$C_{27}H_{20}N_4OS_2$ (480.61)	67.48 67.13	4.19 4.01	11.66 11.32	13.34 12.98
10	75	>300	$C_{27}H_{22}N_6S_2$ (494.64)	65.56 65.35	4.48 4.12	16.99 16.73	12.96 12.64
11	67	178	$C_{29}H_{24}N_6OS_2$ (536.68)	64.90 64.81	4.51 4.23	15.66 15.30	11.95 11.65
12	61	238	$C_{33}H_{26}N_6S_2$ (570.74)	69.45 69.24	4.59 4.20	14.72 14.89	11.24 10.94
13	76	258	$C_{23}H_{22}N_4O_2S_3$ (482.65)	57.24 56.94	4.59 4.30	11.61 11.75	19.93 19.71
15	78	238	$C_{21}H_{18}N_4O_2S_2$ (422.53)	59.70 58.98	4.29 4.19	13.26 13.57	

a = reported m.p. 255-257°C (Badawey *et al*., 1993)

Ethyl 2-cyano-2-(7-substituted-5-methyl-3-phenyl-thiazolo[4,5-d]-pyrimidin-2(3*H*)-ylidene)acetates (5a-b), 2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3*H*)-ylidene)malononitriles (6a-b)

To a solution of compound **4a** or **b** (10 mmol) in dry acetonitrile (20 mL), dimethyl sulphate (3.8 g, 2.8 mL, 30 mmol) was added. The reaction mixture was heated under reflux for 1 h and then cooled. Ethyl cyanoacetate or malononitrile (30 mmol) and triethylamine (3 mL) were added while stirring. Stirring was continued over a boiling water bath for 30 minutes during which the product was partially crystallized out. The reaction mixture was allowed to cool, and the product was filtered, washed with ethanol, dried, and crystallized from ethanol (Table I). IR (KBr, cm^{-1}) of **5a**: 2202 (C≡N), 1664 (C=O ester conj.), 1626 (C=N), 1573, 1494 (C=C), 1275, 1068 (C-S-C),1247, 1223, 1033 (C-O-C), 1116 (C-N). ^{1}H-NMR of **5a** ($CDCl_3$, 400 MHz): δ 1.30 (t, 3H, *J* = 8.0 Hz, CH_2CH_3), 2.40 (s, 3H, H_3C-C=N), 3.79 (t, 4H, *J* = 7.0 Hz, morpholine-$C_{3,5}$-H), 3.84 (t, 4H, *J* = 7.0 Hz, morpholine-$C_{2,6}$-H), 4.70 (q, 2H, *J* = 8.0 Hz, -CH_2CH_3), 7.40 (d, 2H, *J* = 7.5 Hz, C_6H_5-$C_{2,6}$-H), 7.45 (t,

1H, *J* = 7.5 Hz, C_6H_5-C_4-H), 7.52 (t, 2H, *J* = 7.5 Hz, C_6H_5-$C_{3,5}$-H). IR (KBr, Cm^{-1}) of **5b**: 2201 (C≡N), 1663 (C=O ester conj.), 1626 (C=N) 1571, 1496 (C=C), 1286, 1075 (C-S-C), 1251, 1045 (C-O-C), 1136 (C-N). ^{1}H-NMR of **5b** ($CDCl_3$, 400 MHz): δ 1.30 (t, 3H, *J* = 7.5 Hz, -CH_2CH_3), 1.64-1.80 (m, 6H, piperidine-$C_{3,4,5}$-H), 2.40 (s, 3H, H_3C-C=N), 3.72-3.91 (m, 4H, piperidine-$C_{2,6}$-H), 4.27 (q, 2H, *J* = 7.5 Hz, -CH_2CH_3), 7.33 (t, 2H, *J* = 7.0 Hz, C_6H_5-$C_{3,5}$-H), 7.54 (t, 1H, *J* = 7.0 Hz, C_6H_5-C_4-H), 7.57 (d, 2H, *J* = 7.0 Hz, C_6H_5-$C_{2,6}$-H). IR (KBr, Cm^{-1}) of **6a**: 2205 (C≡N), 1626 (C=N), 1570, 1510, 1469 (C=C), 1273, 1069 (C-S-C), 1245, 1040 (C-O-C), 1121 (C-N). ^{1}H-NMR of **6a** ($CDCl_3$, 500 MHz): δ 2.49 (s, 3H, H_3CC=N), 3.81 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{3,5}$-H), 3.87 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{2,6}$-H), 7.32 (d, 2H, *J* = 6.9 Hz, C_6H_5-$C_{2,6}$-H), 7.54 (t, 1H, *J* = 6.9 Hz, C_6H_5-C_4-H), 7.59 (t, 2H, *J* = 6.9 Hz, C_6H_5-$C_{3,5}$-H). IR (KBr, Cm^{-1}) of **6b**: 2201 (C≡N), 1625 (C=N), 1571, 1501 (C=C), 1252, 1073 (C-S-C), 1110 (C-N). ^{1}H-NMR of **6b** ($CDCl_3$, 500 MHz): δ 1.68-1.74 (m, 6H, piperidine-$C_{3,4,5}$-H), 2.41 (s, 3H, H_3CC=N), 3.77-3.79 (m, 4H, piperidine-$C_{2,6}$-H), 7.33 (d, 2H, *J* = 8.4 Hz, C_6H_5-$C_{2,6}$-H), 7.52 (t, 1H, *J* = 7.6 Hz, C_6H_5-C_4-H), 7.58 (t, 2H, *J* = 7.6 Hz, C_6H_5-$C_{3,5}$-H).

5-Methyl-7-morpholino-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-one (7)

To a solution of compound **4a** (3.44 g, 10 mmol) in acetonitrile (20 mL), dimethyl sulphate (3.8 g, 2.8 mL, 30 mmol) was added, and the reaction mixture was heated under reflux for 1 h and then cooled. Triethylamine (3 mL) and few drops of water were added while stirring, and stirring was continued over a boiling water bath for 30 minutes during which time the product was partially crystallized out. The reaction mixture was allowed to cool, and the product was filtered, dried, and crystallized from ethanol (Table I). IR (KBr, Cm^{-1}) of **7**: 1699 (C=O), 1673 (C=N), 1570, 1499 (C=C), 1250, 1036 (C-O-C), 1109 (C-N), 1064 (C-S-C). ^{1}H-NMR of **7** ($CDCl_3$, 500 MHz): δ 2.42 (s, 3H, H_3CC=N), 3.79 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{3,5}$-H), 3.82 (t, 4H, *J* = 7.7 Hz, morpholine-$C_{2,6}$-H), 7.39 (d, 2H, *J* = 9.1 Hz, C_6H_5-$C_{2,6}$-H), 7.46 (t, 1H, *J* = 7.7 Hz, C_6H_5-C_4-H), 7.53 (t, 2H, *J* = 7.7 Hz, C_6H_5-$C_{3,5}$-H). MS m/z (%) of **7**: 330 (6) [M^+ +2], 328 (50) [M^+], 300 (100) [M^+-CO].

7-(4-Acetylanilino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione (8)

A mixture of **3** (2.94 g, 10 mmol) and 4-aminoacetophenone (1.35 g, 10 mmol) in 30 mL *n*-butanol was refluxed for 5 h. The product obtained after cooling was filtered, dried, and recrystallized from ethanol (Table I). IR (KBr, Cm^{-1}) of **8**: 3308, 3204, 3103, (NH), 1660 (C=O), 1627 (C=N), 1606, 1499 (C=C), 1553 (δ NH), 1553, 1257, 1073, 956 (N-C=S), 1241, 1045 (C-S-C). ^{1}H-NMR of **8** (DMSO, 400 MHz): δ 2.33 (s, 3H, CH_3C=N), 2.45 (s, 3H, CH_3C=O), 7.35 (d, 2H, *J* = 6.8 Hz, C_6H_5-$C_{2,6}$-H), 7.49 (t, 1H, *J* = 6.8 Hz, C_6H_5-C_4-H), 7.54 (t, 2H, *J* = 6.8 Hz, C_6H_5-$C_{3,5}$-H), 7.84 (d, 2H, *J* = 8.8 Hz, NH-C_6H_4-$C_{2,6}$-H), 7.91 (d, 2H, *J* = 8.8 Hz, NH-C_6H_4-$C_{3,5}$-H), 10.02 (s, 1H, NH, D_2O exchangeable).

5-Methyl-3-phenyl-7-[4-(1-phenyl-3-oxopropenyl) anilino]thiazolo[4,5-d]pyrimidine-2(3*H*)-thione (9)

Method A

A mixture of **8** (3.92 g, 10 mmol), anhydrous K_2CO_3 (1.38 g, 10 mmol), and benzaldehyde (0.1 mL, 10 mmol) was refluxed in dry dioxane (20 mL) for 10 h and filtered while hot. The product separated after cooling was filtered, dried, and recrystallized from dioxane (Table I).

Method B

A mixture of **8** (3.92 g, 10 mmol) and benzaldehyde (0.1 mL, 10 mmol) was refluxed in acetic anhydride for 3 h during which time the product was partially crystallized out. After cooling, the product was completely crystallized, filtered, washed with cold water, dried, and recrystallized from dioxane. Yield (3.2 g, 66%), m.p., and IR were similar to those prepared by method A. A mixed m.p. with compound **9** prepared by method A gave no depression. IR (KBr, cm^{-1}) of **9**: 3287 (NH), 1698 (C=O), 1637 (C=N), 1617, 1509, 1495 (C=C), 1559, 1288, 1073, 994 (N-C=S), 1255, 1041 (C-S-C). ^{1}H-NMR of **9** ($CDCl_3$, 400 MHz): δ 2.50 (s, 3H, CH_3), 7.35 (d, 1H, *J* = 15.0 Hz, CH=CH), 7.40-7.66 (m, 10H, Ar-H), 7.84 (d, 1H, *J* = 15.0 Hz, CH=CH), 7.60 (d, 2H, *J* = 8.8 Hz, NH-C_6H_4-$C_{2,6}$-H), 8.05 (d, 2H, *J* = 8.8 Hz, NH-C_6H_4-$C_{3,5}$-H), 10.01 (s, 1H, NH, D_2O exchangeable). MS m/z (%) of **9**: 482 (14) [M^++2], 480 (100) [M^+].

7-[4-(5-Phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)anilino]-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione (10)

To a suspension of **9** (0.48 g, 1 mmol) in absolute ethanol, hydrazine hydrate (99%) (0.1 mL, 2 mmol) was added. The reaction mixture was refluxed for 3-4 h until a crystalline precipitate was separated, filtered, washed with H_2O, and crystallized from ethanol (Table I). IR (KBr, Cm^{-1}) of **10:** 3285 (N-H), 1636 (C=N), 1607, 1513 (C=C), 1560, 1255, 1097, 934 (N-C=S), 1281, 1049 (C-S-C). ^{1}H-NMR of **10** (DMSO, 500 MHz): δ 2.32 (s, 3H, H_3CC=N), 2.80 (dd, 1H, *J* = 16.2, 10.3 Hz, pyrazoline-C_4-H), 3.62 (dd, 1H, *J* = 16.2, 10.7 Hz, pyrazoline-C_4-H), 4.06 (s, 1H, NH, D_2O exchangeable), 4.80 (dd, 1H, *J* = 10.7, 10.3 Hz, pyrazoline-C_5-H), 7.24 (t, 1H, *J* = 6.9 Hz, C_6H_5-C_4-H), 7.32 (t, 2H, *J* = 7.6 Hz, N-C_6H_5-$C_{3,5}$-H), 7.36 (d, 2H, *J* = 6.9 Hz, C_6H_5-$C_{2,6}$-H), 7.37 (d, 2H, *J* = 7.6 Hz, N-C_6H_5-$C_{2,6}$-H), 7.50 (t, 1H, *J* = 7.6 Hz, N-C_6H_5-C_4-H), 7.55 (t, 2H, *J* = 6.9 Hz, C_6H_5-$C_{3,5}$-H), 7.61 (d, 2H, *J* = 8.4 Hz, NH-C_6H_4-$C_{2,6}$-H), 7.65 (d, 2H, *J* = 8.4 Hz, NH-C_6H_4-$C_{3,5}$-H), 9.86 (s, 1H, pyrazoline-NH,

D_2O exchangeable). MS m/z (%) of **10**: 496 (24) [M^+ +2], 494 (89) [M^+], 77 (100).

7-[4-(1-Acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) anilino]-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione (11)

To a solution of hydrazine hydrate (99%) (0.l mL, 2 mmol) in glacial acetic acid (5 mL), compound **9** (0.48 g, 1 mmol) was added, and the reaction mixture was refluxed for 3 h. On cooling, the precipitated solid was filtered, washed with water, and crystallized from benzene/petroleum ether 60-80°C (Table I). IR (KBr, cm^{-1}) of **11**: 3290, 3188 (NH), 1655 (C=O), 1626 (C=N), 1607, 1515, 1494 (C=C), 1554, 1256, 1074, 956 (N-C=S), 1045 (C-S-C). ^{1}H-NMR of **11** ($CDCl_3$, 500 MHz): δ 2.44 (s, 3H, H_3CC=N), 2.57 (s, 3H, CH_3C=O), 3.13 (dd, 1H, *J* = 17.9, 4.6 Hz, pyrazoline-C_4-H), 3.72 (dd, 1H, *J* = 17.9, 12.0 Hz, pyrazoline-C_4-H), 5.56 (dd, 1H, *J* = 12.0, 4.6 Hz, pyrazoline-C_5-H), 6.88-7.54 (m, 10H, Ar-H), 7.95 (d, 2H, *J* = 6.8 Hz, NH-C_6H_4-$C_{2,6}$-H), 7.97 (d, 2H, *J* = 6.8 Hz, NH-C_6H_4-$C_{3,5}$-H), 8.42 (s, 1H, NH, D_2O exchangeable). MS m/z (%) of **11**: 538 (17) [M^++2], 536 (89) [M^+], 77(100).

7-[4-(1,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)anilino]-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2 (3*H*)-thione (12)

To a solution of phenylhydrazine (0.22 g, 2 mmol) in glacial acetic acid (5 mL), compound **9** (0.48 g, 1 mmol) was added. The reaction mixture was refluxed for 4-5 h, cooled, and poured on to ice-water. The obtained precipitate was collected by filtration washed with water, dried, and crystallized from ethanol (Table I). IR (KBr, cm^{-1}) of **12**: 3259 (NH), 1643 (C=N), 1598, 1517, 1497 (C=C), 1566, 1256, 1069, 1000 (N-C=S), 1289, 1041 (C-S-C). ^{1}H-NMR of **12** ($CDCl_3$, 500 MHz): δ 2.46 (s, 3H, H_3CC=N), 3.17 (dd, 1H, *J* = 17.0, 7.3 Hz, pyrazoline-C_4-H), 3.87 (dd, 1H, *J* = 17.0, 13.0 Hz, pyrazoline-C_4-H), 5.33 (dd, 1H, *J* = 13.0, 7.3 Hz, pyrazoline-C_5-H), 6.79 (t, 1H, *J* = 7.0 Hz, N-C_6H_5-C_4-H), 6.87 (s, 1H, NH, D_2O exchangeable), 7.18 (t, 1H, *J* = 7.5 Hz, C-C_6H_5-C_4-H), 7.34 (d, 2H, *J* = 6.0 Hz, NH-C_6H_4-$C_{2,6}$-H), 7.36 (d, 2H, *J* = 6.0 Hz, NH-C_6H_4-$C_{3,5}$-H), 7.10-7.80 (m, 13H, Ar-H). MS m/z (%) of **12**: 570 (12) [M^+], 77 (100).

7-[3-(Ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b] thieno-2-yl]amino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione (13)

A mixture of **3** (2.9 g, 10 mmol) and ethyl 2-amino-4,5,6,7-tetrahydro[1]benzothiophene-3-carboxylate (2.25 g, 10 mmol) was heated in an oil bath at 150-160°C for 30 minutes. The brittle mass that was obtained was powdered, washed with sodium carbonate solution (10%), dried, and crystallized from dioxane (Table I). IR (KBr, Cm^{-1}) of **13**: 3133 (NH), 1695 (C=O), 1654 (C=N), 1590, 1521, 1498 (C=C), 1570, 1226, 1097, 956 (N-C=S), 1277, 1074 (C-S-C), 1250, 1040 (C-O-C). ^{1}H-NMR of **13** ($CDCl_3$, 400 MHz): δ 1.39 (t, 3H, *J* = 7.5 Hz, -CH_2CH_3), 1.55-1.78 (m, 2H, tetrahydrobenzothiophene-C_5-H), 1.79-1.99 (m, 2H, tetrahydrobenzothiophene-C_6-H), 2.54 (s, 3H, CH_3), 2.65-2.67 (m, 2H, tetrahydrobenzothiophene-C_4-H), 2.68-2.78 (m, 2H, tetrahydrobenzothiophene-C_7-H), 4.35 (q, 2H, *J* = 7.5 Hz, -CH_2CH_3), 7.37 (d, 2H, *J* = 8.0 Hz, C_6H_5-$C_{2,6}$-H), 7.52 (t, 1H, *J* = 8.0 Hz, C_6H_5-C_4-H), 7.58 (t, 2H, *J* = 8.0 Hz, C_6H_5-$C_{3,5}$-H), 11.39 (s, 1H, NH, D_2O exchangeable). MS m/z (%) of **13**: 484 (10) [M^+ + 2], 482 (40) [M^+], 436 (100) [M^+ -OC_2H_5 – H].

7-[2-(Ethoxycarbonyl)anilino]-5-methyl-3-phenylthiazolo[4,5-d]-pyrimidine-2(3*H*)-thione (15)

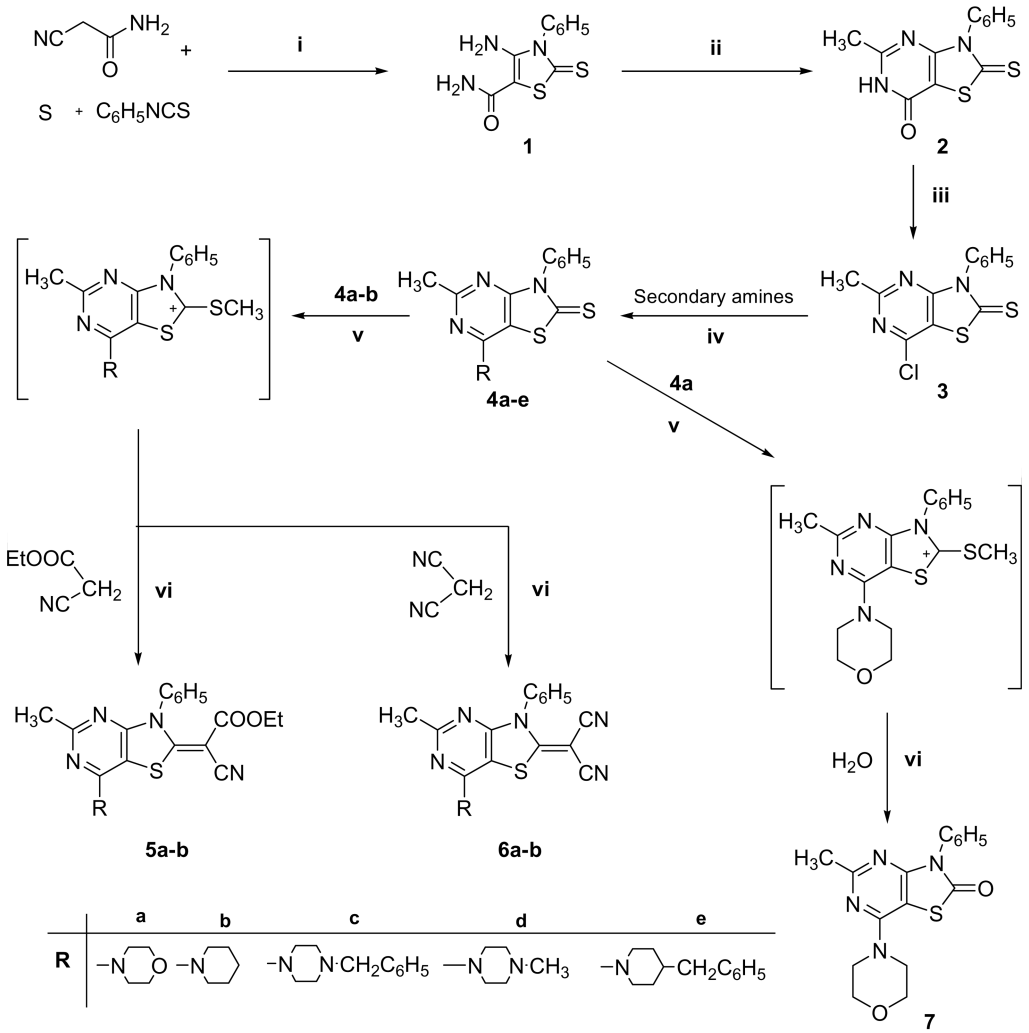
A mixture of **3** (2.9 g, 10 mmol) and ethyl anthranilate (1.6 g, 1.4 mL, 10 mmol) was heated in an oil bath at 150-160°C for 30 minutes. The brittle mass that was obtained was powdered, washed with sodium carbonate solution (10%), dried, and crystallized from ethanol (Table I). IR (KBr, Cm^{-1}) of **15**: 3154, 3102 (NH), 1689 (C=O), 1627 (C=N), 1603, 1516 (C=C), 1568, 1281, 1079, 990 (N-C=S), 1275, 1070 (C-S-C), 1256, 1048 (C-O-C). ^{1}H-NMR of **15** ($CDCl_3$, 400 MHz): δ 1.50 (t, 3H, *J* = 7.0 Hz, -CH_2CH_3), 2.50 (s, 3H, H_3CC=N), 3.90 (q, 2H, *J* = 7.0 Hz, -CH_2CH_3), 7.07-7.58 (m, 7H, Ar-H), 8.07 (dd, 1H, *J* = 8.0, 2.5 Hz, NH-$C_6H_4COOC_2H_5$-C_6-H), 8.90 (dd, 1H, *J* = 8.0, 2.5 Hz, NH-$C_6H_4COOC_2H_5$-C_3-H), 10.93 (s, 1H, NH, D_2O exchangeable).

RESULTS AND DISCUSSION

Chemistry

Compounds were synthesized as depicted in Schemes 1, 2 and 3. The starting material, 7-chloro-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione **3**, was prepared by reacting cyanoacetamide, sulphur, and phenylisothiocyanate in the presence of triethylamine, according to the procedure reported by Gewald (Gewald *et al*., 1966), to give 4-amino-5-carbamoyl-3-phenylthiazole-2(3*H*)-thione **1**. Cyclization of this amino amide by heating under reflux in acetic anhydride followed by treatment of the product with phosphorous oxychloride gave the required chloro derivative **3** in good yield (Badawey *et al*., 1993) (Scheme 1). Nucleophilic substitution of the chlorine atom by reaction of **3** with the appropriate amine in boiling dry acetone gave 7-(substituted amino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thiones **4a**-**e**. The ^{1}H-NMR spectra of **4a**-**e** showed signals for the protons of the substituted amino function at the 7-position in addition to the signals for the 5-methyl and 3-phenyl groups of thiazolopyrimidine at their expected chemical shifts. Treatment of **4a**-**b** with

dimethyl sulphate in boiling acetonitrile, followed by the reaction of the produced 2-methylthiothiazolium salt with malononitrile or ethyl cyanoacetate in the presence of triethylamine, following Gewald's method (Gewald *et al*., 1981), gave ethyl 2-cyano-2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3*H*)-ylidene)acetates **5a-b** or 2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3*H*)-ylidene)malononitrile derivatives **6a-b**, respectively. The process of the reaction to form compounds **5a-b** and **6a-b** was assumed to involve the initial formation of a carbanion of either malononitrile or ethyl cyanoacetate due to abstraction of a proton from their active methylene group by triethylamine. At high temperature, the formed carbanion attacks the positively charged thiazolium salt at its 2-position, replacing the good-leaving, S-methyl group to give the desired compounds **5a-b** and **6a-b**. The IR spectra of compounds **5a-b** and **6a-b** showed the characteristic absorption band of the CN group at 2201-2205 cm^{-1}. In addition, the IR spectra of compounds **5a-b** showed the C=O band characteristic of the ester group at 1663-1664 cm^{-1}. ^{1}H-NMR spectra of compounds **5a-b** showed characteristic signals of the ethyl ester protons as triplets at 1.30 ppm and quartets at 4.27 and 4.70 ppm, in addition to the other signals characteristic for the thiazolopyrimidine nucleus. In addition, 5-methyl-7-morpholino-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-one **7** was prepared from **4a** through subsequent action of dimethyl sulphate and triethylamine in the presence of few drops of water (Scheme 1). The IR spectrum of compound **7** showed the characteristic absorption band of the C=O



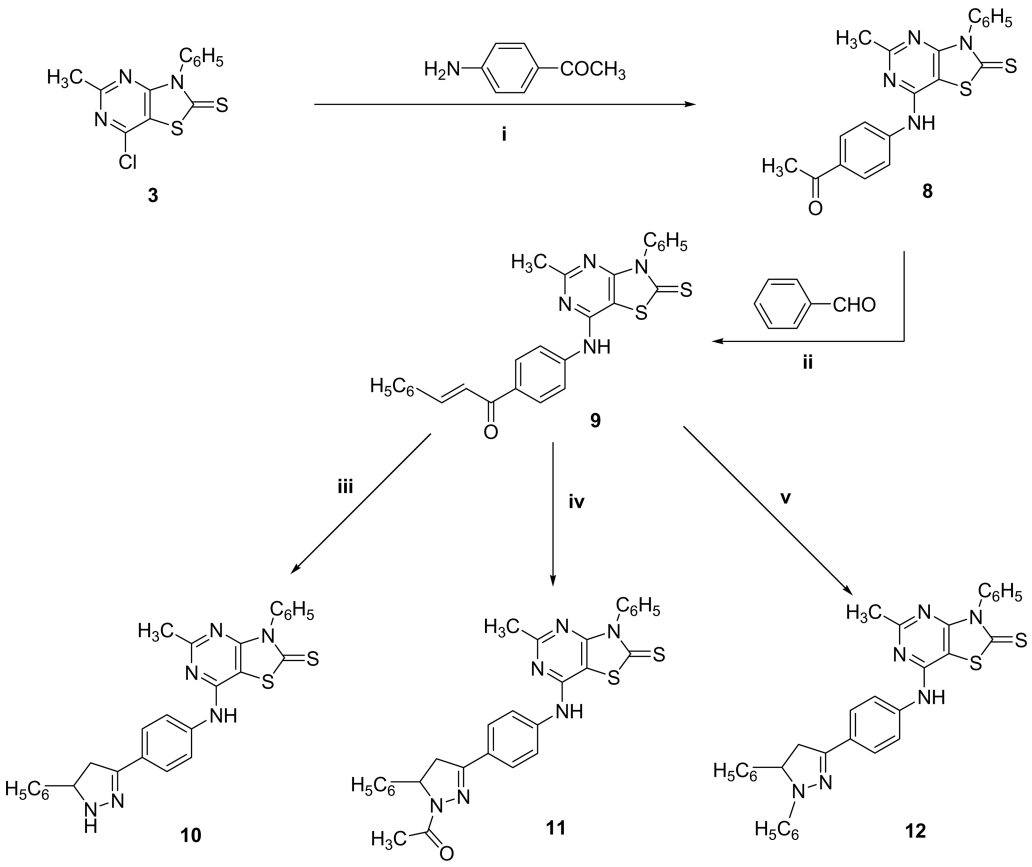
Reaction conditions: i: $N(C_2H_5)_3$, DMF, stirr, 50°C, 63%; **ii:**$(CH_3CO)_2O$, reflux, 3h, 80%; **iii**: $POCl_3$, reflux, 5h, 89%; **iv:** dry acetone, reflux, 3h, 65-73%; **v:** $(CH_3)_2SO_4$, acetonitrile, reflux, 1h; **vi:** $N(C_2H_5)_3$ stirr, boiling water bath, 30 min., 61-65%.

Scheme 1. 7-Substituted amino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thiones (**4a-e**), ethyl 2-cyano-2-(7-substituted-5-methyl-3-phenylthiazolo [4,5-d]pyrimidin-2(3*H*)-ylidene)acetates (**5a-b**), 2-(7-substituted-5-methyl-3-phenylthiazolo[4,5-d]-pyrimidin-2(3*H*)-ylidene)malononitriles (**6a-b**) and 5-methyl-7-morpholino-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-one (**7**)

group at 1699 cm^{-1}, beside the absorption bands due to C=N, C=C, C-O-C, and C-S-C functions.

Furthermore, refluxing the 7-chlorothiazolopyrimidine derivative **3** with 4-aminoacetophenone in *n*-butanol gave 7-(4-acetylanilino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione **8** (Scheme 2). The IR spectrum of **8** showed the characteristic absorption bands of the NH group at 3308, 3204, 3103 cm^{-1}, and the absorption band of the C=O group at 1660 cm^{-1}. ^{1}H-NMR spectrum of **8** showed an additional singlet for the CH_3-C=O protons at 2.45 ppm and a deuterium-exchangeable signal for the NH proton at 10.02 ppm, in addition to the other signals at their expected chemical shifts.

Condensation of 4-acetylanilino derivative **8** with benzaldehyde by refluxing in dioxane in the presence of anhydrous potassium carbonate, under Claisen-Schmidt reaction conditions (Solmons *et al*., 1994), gave 5-methyl-3-phenyl-7-[4-(1-phenyl-3-oxopropenyl)anilino]thiazolo[4,5-d]-pyrimidine-2(3*H*)-thione **9.** Alternatively, **8** was condensed with benzaldehyde by refluxing in acetic anhydride, according to the method described by Philipis (Philipis *et al.,* 1959), to afford the same chalcone **9** in the same yield but with a shorter reaction time. The ^{1}H-NMR spectrum of the chalcone **9** lacked the CH_3-C=O protons at 2.45 ppm, and instead showed the characteristic signals of the CH=CH protons as two doublets at 7.35 and 7.84 ppm, J = 15.0 Hz, which indicates a trans arrangement, in addition to the other signals at their expected chemical shifts. Cyclization of the chalcone **9** by heating with hydrazine hydrate in either ethanol or acetic acid or with phenylhydrazine in acetic acid gave the corresponding 5-phenylpyrazoline **10**, 1-acetyl-5-phenylpyrazoline **11**, or 1,5-diphenylpyrazoline derivative **12**, respectively. The IR spectra of **10** and **12** were characterized by the disappearance of the C=O band at 1698 cm^{-1}, which was present in the precursor, while the IR spectrum of **11** showed a C=O absorption band at 1655 cm^{-1}. The ^{1}H-NMR spectrum of compound **12** is characterized by the presence of three doublet of doublet signals at 3.17, 3.87, and 5.33 ppm due to pyrazoline-C_4-H and C_5-H, in addition to the other signals at their expected chemical shifts. The ^{1}H-NMR spectrum of **10** also showed two deuterium exchangeable



Reaction conditions: i: n-butanol, reflux, 5h, 61%; **ii:** two methods: **a:** anhyd.K_2CO_3, dry dioxane, reflux 10h, 64%; **b:** $(CH_3CO)_2O$, reflux, 3h, 66%; **iii:** NH_2NH_2(99%), EtOH(absolute), reflux, 3-4h, 75%; **iv:** NH_2NH_2(99%), HAc(glacial), reflux, 3h, 67%; **v:** $C_6H_5NHNH_2$, HAc(glacial), reflux, 4-5h, 61%.

Scheme 2. Synthesis of 7-[4-(1-Substituted-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)anilino]-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3*H*)-thione derivatives (**10**-**12**)

singlets for NH and pyrazoline-NH at 4.06 and 9.86 ppm, respectively, while the ^{1}H-NMR spectrum of compound **11** showed a singlet at 2.57 ppm due to acetyl protons.

Fusion of **3** with either ethyl 2-amino-4,5,6,7-tetrahydro [1]benzo-thiophene-3-carboxylate or ethyl anthranilate afforded [3-(ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b]thieno-2-yl]amino derivative **13** or 2-(ethoxycarbonyl)aniline derivative **15**, respectively, instead of the cyclized products **14** and **16** (Scheme 3). The presence of the ester C=O absorption band at 1695 and 1689 cm^{-1} for compounds **13** and **15**, respectively, in the IR spectra, in addition to the presence of a triplet and a quartet characteristic for the ester moiety and a deuterium exchangeable signal due to NH in their ^{1}H-NMR spectra, indicated the uncyclized structures of compounds **13** and **15**.

Antimicrobial activity

All products were evaluated *in vitro* for their antimicrobial activity against *Staphyloccus aureus,* a gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, which are gram-negative bacteria, and for antifungal activity against *Candida albicans* using the cup diffusion technique (Jain *et al*., 1971). Compounds showing inhibition zones of at least18 mm were considered active and were further evaluated for their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values using the two-fold serial dilution method (Scott *et al.*, 1989). Ampicillin was used as a standard antibacterial agent, and clotrimazole was used as a standard antifungal agent. Dimethylformamide as a control showed no antimicrobial activity.

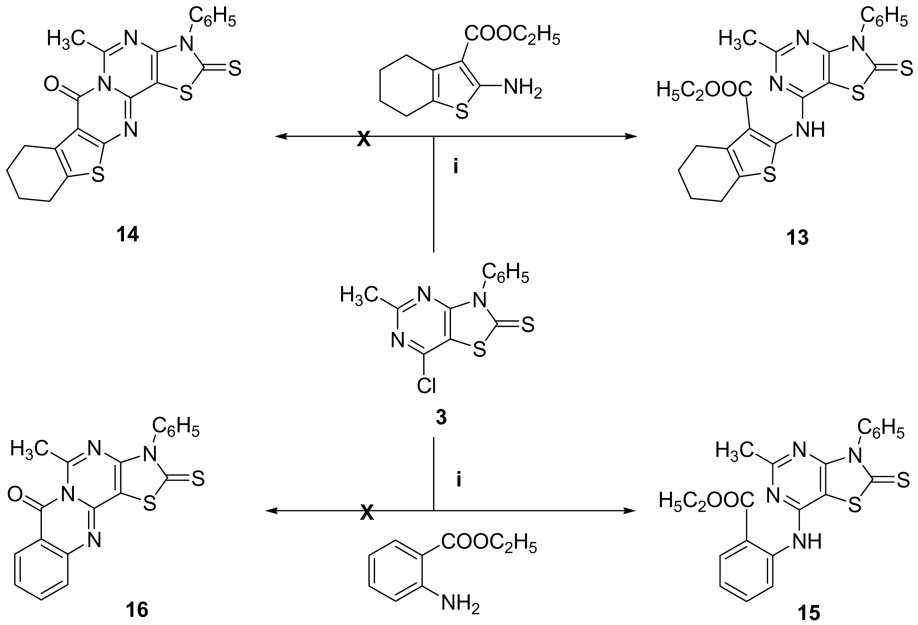
Methodology for *in vitro* antimicrobial screening

Inhibition zone measurement

The antibacterial and antifungal testing was studied using the cup diffusion technique. The products, as 1 mg/mL solutions in dimethylformamide (DMF), were evaluated *in vitro* for antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8735), and *Pseudomonas aeruginosa* (ATCC 9027), and for antifungal activity against *Candida albicans* (ATCC 10231). Sterile nutrient agar was inoculated with the test organisms (each 100 mL of the medium received 1 mL of 24 h-broth culture), and then seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar, and each cup received 0.1 mL of the test compound solution. The plates were then incubated at 37°C for 24 h. The activities were estimated as zones of inhibition in mm diameter (Table II). A 5 μg/mL solution of ampicillin and a solution containing 0.01% of clotrimazole were used as reference standards. DMF did not show any inhibition zones.

Minimal inhibitory concentration (MIC) measurement

Using the two-fold serial dilution method (Scott *et al.* 1989), the test organisms were grown in suitable broth for 24 h for bacteria and 48 h for fungi at 37°C. Two-fold serial dilutions of the test compound solutions were prepared using the suitable broth to obtain concentrations between 500 and 15.62 μg/mL, with the concentration of DMF not



Reaction conditions: i: Heat in oil bath, 150-160°C, 30min., 76-78%.

Scheme 3. Synthesis of 7-[3-(Ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b]thieno-2-yl]amino and 7-[2-(Ethoxycarbonyl)anilino]-5-methyl-3-phenyl-thiazolo[4,5-d]pyrimidine- 2(3*H*)-thiones (13 and 15)

exceeding 2.5%. The tubes were then inoculated with the test organisms (each 5 mL received 0.1 mL of the above inoculum) and were incubated at 37°C for 48 h. The tubes were then observed for the presence or absence of microbial growth. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC). The MIC values of the prepared compounds are listed in Table III.

Minimal bacteriostatic concentration (MBC) measurement

A loopful from the tube not showing visible growth (MIC) was spread over a quarter of a Muller-Hinton agar plate. After an overnight incubation (18 h), the plates were examined for growth. The tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates were judged to contain the MBC of that compound for the respective test organism (Table III).

RESULTS OF ANTIMICROBIAL ACTIVITY

The data (Tables II and III) revealed that compounds **4b**, **5a-b**, **6a-b**, **7**, **10**, **11**, **13**, and **15** possess antimicrobial activity against *E. coli*, with IZ = 18-20 mm and MIC 62.5 μg/mL, or nearly one-sixth the activity of ampicillin. The thiazolopyrimidines **4c**-**d** and **5b** showed activity against *P. aeruginosa*, with IZ = 18-20 mm and MIC 62.5 μg/mL, or about one-third the activity of ampicillin. Compounds **4b**, **7**, **8**, **9**, and **11** showed the most antifungal activity against *C. albicans,* with IZ = 20-25 mm and MIC 31.25 μg/mL, with only one-sixth the activity of clotrimazole. The maximum antifungal activity was observed with compound **9** (IZ = 25 mm), which has the long and flexible 1-phenyl-3-oxopropyl moiety. Conversion of this moiety into a rather rigid pyrazoline ring decreased the antifungal activity of compounds **10** and **12** (IZ = 19, 18, respectively). Introduction of an acetyl function in position 1 of the pyrazoline ring imparted slightly higher antifungal activity for compound **11** (IZ = 22).

Thus, some of the compounds were more active against *C. albicans* than the gram-negative bacteria, *E. coli* and *P. aeruginosa*, but inactive against the gram-positive bacteria, *S. aureus.*

Table II. Inhibition zones (IZ) in mm diameter

Compound No.	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
4b	-	20	17	21
4c	-	16	18	19
4d	-	17	19	-
4e	-	17	-	16
5a	-	18	-	19
5b	-	19	19	20
6a	-	18	-	18
6b	-	20	16	18
7	-	19	-	20
8	-	17	-	22
9	-	17	17	25
10	-	19	-	19
11	-	18	-	22
12	-	17	-	18
13	-	20	15	18
15	-	19	16	19
Ampicillin	25	28	32	-
Clotrimazole	-	-	-	35

(-) no inhibiton zone

Table III. MIC and MBC in μg/mL of the most active compounds

Compound No.	*S. aureus*		*E. coli*		*P. aeruginosa*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4b	-	-	62.5	125	-	-	31.25	31.25
4c	-	-	-	-	62.5	62.5	-	-
4d	-	-	-	-	62.5	62.5	-	-
5a	-	-	62.5	250	-	-	-	-
5b	-	-	62.5	250	62.5	62.5	-	-
6a	-	-	62.5	250	-	-	-	-
6b	-	-	62.5	125	-	-	-	-
7	-	-	62.5	250	-	-	31.25	62.5
8	-	-	-	-	-	-	31.25	62.5
9	-	-	-	-	-	-	31.25	125
10	-	-	62.5	125	-	-	-	-
11	-	-	62.5	250	-	-	31.25	62.5
13	-	-	62.5	125	-	-	-	-
15	-	-	62.5	125	-	-	-	-
Ampicillin	5	-	10	-	25	-	-	-
Clotrimazole	-	-	-	-	-	-	5	-

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