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Synthesis and Antimicrobial Activity of Novel Tetrahydrobenzothienopyrimidines

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Due to the rapidly growing number of resistant strains of bacteria, the search for antibacterial agents with new modes of action will always remain an important and challenging task. Thus, the reaction of 2-substituted or unsubstituted-4-(4-acetylanilino)-5,6,7,8-terahydrobenzo[b] thieno[2,3-d]pyrimidine derivatives **1-3** with the hydrazine derivatives, semi and / or thiosemicarbazides, provided the corresponding hydrazones **4-6** and semi and/or thiosemicarbazones **7-9**. Claisen-Schmidt condensation of compounds **1** or **2** with the appropriate aldehyde yielded the chalcones **10**, **11** which, when treated with hydroxylamine hydrochloride gave rise to the isoxazoline-containing compounds **12**, **13**. Furthermore, reacting the respective chalcones **10**, **11** with different hydrazines, urea and/or thiourea, furnished compounds **14**, **15**, **16**, and **17** respectively. Representative compounds were tested for their antimicrobial activity against *Candida Albicans* and certain gram-positive and gram-negative bacteria. Their MICs were then determined. Compound **15e**, showed a broad spectrum of activity while most of the other compounds showed varying antimicrobial activity.

Key words: 2-Substituted or unsubstituted-4-[(4-substituted)anilino] tetrahydrobenzothienopyrimidine derivatives, Antimicrobial activity

INTRODUCTION

In an era of increasing bacterial resistance to classical antibacterial agents, it has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets *via* genomic, improving existing antibiotics and most importantly by identifying new antibacterial agents (Moneer, 2001; Bodnar *et al.*, 2002; McCain *et al.*, 2002) with novel structures and mode of action. This will always remain the primary goal.

Following in this vein, it was apparent that thienopyrimidines have received considerable attention because of their effective properties as antibacterial agents (Peter *et al.*, 1975; Knud Erik and Erik, 1981; Zekany and Makleit, 1987; El-Kerdawy *et al.*, 1993; Mahran *et al.*, 1998; Moneer *et al.*, 2002), antiviral agents (Kharizomenova *et al.*, 1981; Siegfried *et al.*, 1990), antifungal agents (Reddy *et al.*, 1985) and other activities (Mrs. Kulshreshtha *et al.*, 1981; Gangiee *et al.*, 2003). Encouraged by this, the thienopyrimidine was incorporated into a 3-membered structure, namely, the tetrahydrobenzothienopyrimidines, which was found to exhibit marked antibacterial activity (El-Enany and El-Shafie, 1989; Sabnis *et al.*, 1990; Aboulwafa *et al.*, 1992; Kapustina *et al.*, 1992; Moneer, 2001) and antifungal activity (Shoetsu *et al.*, 1989; Kapustina *et al.*, 1992; Aboulwafa *et al.*, 1992).

In this present work, interest was expressed in synthesizing some new tetrahydrobenzothieno[2,3-d]pyrimidines (**1-3**), which underwent further substitution reactions or which incorporated other heterocyclic ring structures, viz oxazole, pyrazole and pyrimidine ring systems, for evaluation as antimicrobial agents and against the medically important pathogenic fungi *Candida Albicans*.

MATERIALS AND METHODS

Melting points (°C, uncorrected) were recorded on an Electrothermal I A 9100 Digital Melting Point Apparatus. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Varian Gemini 200 MHz and a Jeol FX 90 Q 90 MHz Fourier Transform NMR spectrometer, using TMS as an internal standard (chemical shifts in ppm). IR spectra as KBr

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pellets were recorded on a Schimadzu 435 IR spectrophotometer. Elemental analyses were carried out at the Micro analytical Center, Cairo University. The TLC was performed in chloroform: benzene in the ratio 9: 1 using TLC aluminium sheets precoated with silica gel 60 F254-, layer thickness 0.2mm. The mass spectra were recorded on an HP Model MS 5988 at 70 eV.

Synthesis of compounds

2-Substituted or unsubstituted4-(4-acetylanilino)-5,6,7,8-tetrahydro benzo[b] thieno [2,3-d]pyrimidine (1-3)

A quantity of *p*-aminoacetophenone (0.01 mol), was added portion wise to a hot solution of 4-chloro-2-methyl or benzyl or unsubstituted-5,6,7,8-tetrahydrobenzo[b] thieno [2,3-d] pyrimidine (0.01 mol) in absolute ethanol (30 mL), containing few drops of concentrated hydrochloric acid. The reaction mixture was then refluxed for approximately 6 h, the precipitate formed was filtered, washed with dilute ammonium hydroxide followed by water and then recrystallized from ethanol (Table I). **3**: ^{1}H-NMR (200 MHz, DMSO-d_6+D_2O) δ 1.75-1.83 (2m, 4H), 2.52 (s, 3H), 2.69-3.11 (2m, 4H), 4.15 (s, 2H), 7.25- 7.89 (m, 9H), 8.48 (s, 1H) ppm. **1**: MS (EI) *m/z* 323.15 $[M]^+$, calculated= 323.42.

2-Substituted or unsubstituted-4-[4-(1-hydrazono or substituted hydrazonoethyl) anilino]-5,6,7,8-tetrahydrobenzo [b] thieno [2,3-d] pyrimidine (4-6)

A solution of the corresponding *N*-substituted acetophenone **1-3** (0.0014 mol) in absolute ethanol (30 mL) and 1 mL glacial acetic acid was heated under reflux with the appropriate hydrazine derivative (0.0018 mol) for 8-10 h. The precipitate formed was collected, dried and recrystallized from the suitable solvent (Table II). **4a:** IR(KBr) 3450, 3350, 3200, 2950, 2850, 1610, 1550, 1480 cm^{-1}. **5c:** IR (KBr) 3400, 2900 (br.), 1610, 1600, 1550, 1500, 1330 cm^{-1}. **4a:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.9 (2m, 4H), 2.3 (s, 3H), 2.8-3.2 (2m, 4H), 6.25 (s, 2H), 7.3-8.1 (m, 1H & m, 4H), 8.32 (s, 1H) ppm. **4a:** MS (EI) *m/z* 337.2 $[M]^+$, calculated = 337.45. **5c:** MS (EI) *m/z* 517 $[M]^+$, calculated = 517.57.

Table I. Physical and analytical data of the prepared compounds **1** and **3**

Compd No.	R^1	Formula M. wt Cryst. solvent	Yield% mp °C	Analysis % Calcd	Found
1	H	$C_{18}H_{17}N_3OS$ 323.42 Ethanol	80 170-1	C 66.9 H 5.3 N 13.0	66.9 5.3 13.1
3	CH_2Ph	$C_{25}H_{23}N_3OS$ 413.54 Ethanol	77 222-3	C 72.6 H 5.6 N 10.2 S 7.8	72.6 5.9 10.0 7.8

Table II. Physical and analytical data of the prepared compounds **4a-c**, **5a-c**, and **6**

Compd No.	R	R^1	Formula M. wt Cryst. Solvent	Yield% mp °C	Analysis % Calcd	Found
4a	H	H	$C_{18}H_{19}N_5S$ 337.45 DMF/H_2O	62 211-2	C 64.1 H 5.7 N 20.8	64.4 5.2 20.3
4b	H	Ph	$C_{24}H_{23}N_5S$ 413.55 DMF/H_2O	52 163-5	C 69.7 H 5.6 N 16.9	69.3 5.5 16.6
4c	H	2,4-$(NO_2)_2$Ph	$C_{24}H_{21}N_7O_4S.H_2O$ 521.56 DMF/H_2O	68 273-5	C 55.3 H 4.4 N 18.8	55.2 4.0 19.1
5a	CH_3	H	$C_{19}H_{21}N_5S$ 351.48 DMF/Ethanol	96 323-5	C 64.9 H 6.0 N 19.9	65.3 5.6 19.5
5b	CH_3	Ph	$C_{25}H_{25}N_5S.H_2O$ 445.59 DMF/H_2O	98 124-6	C 67.4 H 6.1 N 15.7	67.1 5.8 15.9
5c	CH_3	2,4-$(NO_2)_2$Ph	$C_{25}H_{23}N_7O_4S$ 517.57 DMF	97 306-7	C 58.0 H 4.5 N 18.9	58.4 4.9 18.7
6	CH_2Ph	Ph	$C_{31}H_{29}N_5S.H_2O$ 521.69 DMF/H_2O	92 208-9	C 71.4 H 6.0 N 13.4	71.9 5.1 13.4

2-Substituted or unsubstituted-4-[4-(4-semi or thiosemi or methyl thio semicarbazonoethyl) anilino]-5,6,7,8-tetrahydrobenzo [b] thieno [2,3-d] pyrimidine (7-9)

A mixture of compounds **1**, **2**, or **3** (0.01 mol), of the suitable semi or thiosemi carbazide (0.013 mol) in absolute ethanol (15 mL), was heated under reflux for 8-13 h. The reaction mixture was then concentrated, cooled and poured over crushed-ice. The precipitate formed was collected, washed with water, left to dry and crystallized from the appropriate solvent. (Table III) **8a:** IR (KBr) 3475,3450, 3200, 2950,2850, 1710, 1600, 1590, 1520 cm^{-1}. **8b:** IR (KBr) 3425, 3275, 3150, 2925, 2850, 1600, 1560, 1500, 1190 cm^{-1}. **8c:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.8-2.1 (2m, 4H), 2.2 (s, 3H), 2.52 (s, 3H), 2.77-3.1 (2m, 4H), 3.6 (s, 3H), 7.4-7.9 (m, 4H), 8.2 (s, 1H), 10.1 (s, 1H) ppm. **9**: ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.8-2.1(2m, 4H), 2.2 (s, 3H), 2.52 (s, 3H), 2.77-3.1 (2m, 4H), 3.6 (s, 3H), 4.07 (s, 2H), 7.4-7.9 (m, 4H), 8.2 (s, 1H), 10.1 (s, 1H) ppm. **8c:** MS (EI) *m/z* 424.25 $[M]^+$, 337 $[M-87.25]^+$.

2-Methyl or unsubstituted-4-[4(2-hydroxy or 4-bromo or 4-nitrocinnamoyl) anilino]-5,6,7,8-tetrahydrobenzo [b] thieno [2,3-d] pyrimidine (10, 11)

Alcoholic potassium hydroxide (10 %, 1.5 mL) was added, to a hot solution of compounds **1** or **2** (0.0015 moL) in

Table III. Physical and analytical data of the prepared compounds **7**, **8a-c**, and **9**

Compd No.	R	R^2	X	Formula M. wt Cryst. solvent	Yield % m.p °C	Analysis % Calcd	Analysis % Found
7	H	H	S	$C_{19}H_{20}N_6S_2$ 396.54 DMF/H_2O	52 199-201	C 57.6 H 5.1 N 21.2 S 16.2	57.6 5.0 21.2 16.2
8a	CH_3	H	O	$C_{20}H_{22}N_6OS$ 394.50 DMF/H_2O	89 142-4	C 60.9 H 5.6 N 21.3	60.7 5.2 21.0
8b	CH_3	H	S	$C_{20}H_{22}N_6S_2$ 410.57 DMF/H_2O	98 246-8	C 58.5 H 5.4 N 20.5	58.2 5.3 20.3
8c	CH_3	CH_3	S	$C_{21}H_{24}N_6S_2$ 424.59 DMF/H_2O	96.6 270-1	C 59.4 H 5.7 N 19.8	59.1 5.4 20.1
9	CH_2Ph	CH_3	S	$C_{27}H_{28}N_6S_2$ 500.69 DMF/Ethanol	83 260-2	C 64.8 H 5.6 N 16.8	64.6 5.5 16.4

absolute ethanol (25 mL), followed by an equimolar amount of the desired aldehyde. The reaction mixture was refluxed for 8 h, concentrated, poured over crushed-ice and the precipitate formed was filtered, washed with water, left to dry and finally crystallized from the suitable solvent (see Table IV). **10:** IR (KBr) 3500,3450, 2950, 2900, 1675, 1605, 1560, 1510 cm^{-1}. **11a:** IR (KBr) 3450, 3400, 2900, 2800, 1660, 1590, 1550 cm^{-1}. **10:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.9 (m, 4H), 2.8-3.2 (m, 4H), 3.64 (s, 1H), 7.6 (d, 1H), 7.8-8.4 (m, 9H &1H), 8.48(d, 1H), 8.6 (s, 1H) ppm.

2-Methyl or unsubstituted-4-[4-(5-aryl-4, 5-dihydro-isoxazol-3-yl) anilino]-5,6,7,8-tetrahydrobenzo [b] thieno [2,3-d] pyrimidine (12, 13)

A mixture of compounds **10** or **11** (0.0007 mol), hydroxylamine hydrochloride (0.0009 mol) and sodium hydroxide (0.09 g) in absolute ethanol (25 mL) was heated under reflux for 13-16 h. The highly colored solution was concentrated and poured over crushed-ice. The precipitate was collected, washed with water, dried and recrystallized from the appropriate solvent (See Table V). **13a:** IR (KBr) 3500, 3200, 2950, 2850, 1610, 1560, 1510 cm^{-1}. **13a:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.85 (2m, 4H), 2.51 (s, 3H), 2.8-3.2 (2m, 4H), 3.65 (s, 1H), 3.75 (s, 2H), 7.3-8.1 (m, 9H &1H), 8.5 (s, 1H) ppm. **13a:** MS (EI) *m/z* 457.4 $[M^+ +1]^+$, calculated = 456.57.

Table IV. Physical and analyatical data of the prepared compounds **10** and **11a**

Compd No.	R	R^3	Formula M. wt Cryst. solvent	Yield % mp °C	Analysis % Calcd	Analysis % Found
10	H	2-(OH)Ph	$C_{25}H_{21}N_3O_2S.H_2O$ 445.54 Ethanol	79 170-1	C 67.4 H 5.2 N 9.4	67.6 4.8 9.6
11a	CH_3	2-(OH)Ph	$C_{26}H_{23}N_3O_2S.H_2O$ 459.57 DMF/Ethanol/H_2O	90.5 181-2	C 68.0 H 5.5 N 9.1	67.5 5.1 9.6

2-Methyl or unsubstituted-4-[4-(5-aryl-4,5-dihydro-pyrazol-3-yl) anilino]-5,6,7,8-tetrahydrobenzo [b] thieno [2,3-d] pyrimidine (14, 15)

Equimolar amounts of compounds **10** or **11** (0.001 mol), and the appropriate hydrazine derivative were refluxed in a mixture of absolute ethanol (25 mL) and dimethyl-

Table V. Physical and analytical data of the prepared compounds **12** and **13a,b**

Compd No.	R	R^3	Formula M. wt Cryst.solvent	Yield % mp °C	Analysis % Calcd	Analysis % Found
12	H	2-(OH)Ph	$C_{25}H_{22}N_4O_2S$ 442.54 Ethanol	97 230-2	C 67.9 H 5.0 N 12.7	67.7 5.4 12.7
13a	CH_3	2-(OH)Ph	$C_{26}H_{24}N_4O_2S$ 456.57 DMF/H_2O	93.5 243-5	C 68.4 H 5.3 N 12.3	68.0 5.8 12.1
13b	CH_3	4-(NO_2)Ph	$C_{26}H_{23}N_5O_3S$ 485.57 DMF/H_2O	93.5 205-7	C 64.3 H 4.8 N 14.4 S 6.6	64.3 4.7 14.2 6.6

formamide (5 mL) for 13-20 h. The resulting solution was concentrated then poured over crushed-ice. The solid produced was filtered, dried and recrystallized from the suitable solvent. (See Table VI). **15c:** IR (KBr) 3400, 2900,1590, 1550, 1500,1330 cm^{-1}. **15f:** IR (KBr) 3300, 2900,1600, 1580,1540, 530 cm^{-1}. **14:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.85 (2m, 4H), 2.85 (d, 2H), 2.9-3.2 (2m, 4H), 3.6 (s, 1H), 7.2-8.2 (m, 11H), 8.3 (s, 1H) ppm. **15a:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.85 (2m, 4H), 2.51 (s, 3H), 2.85(d, 2H), 2.9- 3.2 (2m, 4H), 3.6(s, 1H), 7.2-8.2(m, 10H), 8.3 (s, 1H) ppm. **15e:** MS (EI) *m/z* 649.95[M]$^+$, 337.15(M-313). **15f:** MS (EI) *m/z* 518[M]$^+$, calculated= 517.57.

2-Methyl or unsubstituted-4-[4-(6-aryl-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl) anilino]-5,6,7,8-tetrahydro-benzo [b] thieno [2,3-d] pyrimidine (16, 17)

A mixture of compounds **10** or **11** (0.0007 mol) and the desired urea derivative (0.0008 mol) in the presence of catalytic amount of either conc. hydrochloric acid or sodium hydroxide (depending on the urea), in absolute ethanol (50 mL) was heated under reflux for 12-15 h. The resulting solution was concentrated and poured over crushed-ice to precipitate the required compounds. The precipitate obtained was collected, dried and recrystallized from the appropriate solvent. (Table VII). **17a:** IR (KBr) 3475, 3200(br), 2950, 2850, 1680,1600, 1580, 1500 cm^{-1}. **17a:** ^{1}H-NMR (90 MHz, DMSO-d_6+D_2O) δ 1.6-1.85 (2m, 4H), 2.51 (s, 3H), 2.8-3.2 (2m, 4H), 3.6 (s, 1H), 3.7 (s, 2H), 7.4-8.1 (m, 10H), 8.4 (s, 1H) ppm. **17d:** MS (EI) *m/z* 499.7 [M]$^+$.

Microbiological screening

Structure-activity relationship (SAR)

The antimicrobial activity of twenty representative new compounds was investigated against a variety of micro-organisms, including the gram-positive bacteria *Bacillus Subtilis* and *Staphylococcus Aureus*, the gram-negative

Table VI. Physical and analytical data of the prepared compounds **14** and **15a-h**

Compd No.	R	R^1	R^3	Formula M. wt Cryst. solvent	Yield % mp °C	Analysis %	
						Calcd	Found
14	H	H	2-(OH)Ph	$C_{25}H_{23}N_5OS$ 441.57 Ethanol	92 256-8	C 68.0 H 5.3 N 15.9	68.3 5.5 15.5
15a	CH_3	H	2-(OH)Ph	$C_{26}H_{25}N_5OS$ 455.58 Ethanol/H_2O	98 136-8	C 68.6 H 5.5 N 15.4	68.8 5.3 15.5
15b	CH_3	Ph	2-(OH)Ph	$C_{32}H_{29}N_5OS.H_2O$ 549.70 Ethanol/H_2O	87.5 165-7	C 69.9 H 5.7 N 12.7	69.6 5.5 13.3
15c	CH_3	H	4-(NO_2)Ph	$C_{26}H_{24}N_6O_2S$ 484.58 DMF/H_2O	82 176-8	C 64.5 H 5.0 N 17.3	64.5 4.7 17.3
15d	CH_3	Ph	4-(NO_2)Ph	$C_{32}H_{28}N_6O_2S$ 560.68 DMF/H_2O	85.5 178-80	C 68.6 H 5.0 N 15.0	69.1 4.9 15.1
15e	CH_3	2,4-(NO_2)$_2$Ph	4-(NO_2)Ph	$C_{32}H_{26}N_8O_6S$ 650.68 DMF/H_2O	94 141-3	C 59.1 H 4.0 N 17.2 S 4.9	59.0 4.5 17.2 4.9
15f	CH_3	H	4-(Br)Ph	$C_{26}H_{23}BrN_5S$ 517.57 DMF/Ethanol	68 210-2	C 60.3 H 4.5 N 13.5	59.2 4.4 13.5
15g	CH_3	Ph	4-(Br)Ph	$C_{32}H_{27}BrN_5S$ 593.67 DMF/Ethanol	68 180-2	C 64.7 H 4.6 N 11.8	64.0 4.9 11.4
15h	CH_3	2,4-(NO_2)$_2$Ph	4-(Br)Ph	$C_{32}H_{25}BrN_7O_4S$ 683.66 DMF/Ethanol	70 190-2	C 56.2 H 3.7 N 14.3	56.0 4.0 13.9

Table VII. Physical and analytical data of the prepared compounds **16** and **17a-d**

Compd No.	R	X	R^3	Formula M. wt Cryst. solvent	Yield % mp °C	Analysis %	
						Calcd	Found
16	H	O	2-(OH)Ph	$C_{26}H_{22}N_5O_2S$ 468.56 Ethanol	70 159-60	C 66.7 H 4.7 N 14.95	66.4 4.9 14.7
17a	CH_3	O	2-(OH)Ph	$C_{27}H_{25}N_5O_2S$ 483.59 Ethanol/H_2O	85 173-5	C 67.1 H 5.2 N 14.5	67.5 5.6 14.2
17b	CH_3	O	4-(NO_2)Ph	$C_{27}H_{24}N_6O_3S$ 512.59 DMF/H_2O	75 208-10	C 63.3 H 4.7 N 16.4 S 6.3	63.3 4.7 16.3 6.3
17c	CH_3	O	4-(Br)Ph	$C_{27}H_{24}BrN_5OS$ 546 DMF/H_2O	72 239-40	C 59.3 H 4.9 N 12.8	59.5 4.4 12.5
17d	CH_3	S	2-(OH)Ph	$C_{27}H_{25}N_5OS_2$ 499.66 Ethanol/H_2O	77 184-5	C 64.9 H 5.0 N 14.0 S 12.8	64.6 5.0 14.0 12.8

bacteria *Escherichia coli* as well as the unicellular fungi *Candida Albicans* (yeast). The minimum inhibitory concentration (MIC) was determined by the Paper Disc Diffusion method (Bauer *et al.*, 1966). The agar plate disc-diffusion method (Collins and Lyne, 1976) was used to assess the activity of the chosen compounds.

Sample preparation

Sterilized filter paper discs (6 mm in diameter) were wetted with 10 µL each of a solution of the tested compound (10 mg/mL of the compound in DMF). The discs were then allowed to dry and placed on the surface of agar plates seeded with the test organism. Nutrient agar was used for bacterial plating and sabourauds dextrose agar for fungi.

Medium inoculation and cultivation condition

Each plate contained 15ml of the agar medium, previously seeded with 0.2 mL of an 18 h old broth culture of each organism. The inoculated plates were incubated at 37°C for 48 h with the test discs in place and the inhibition zones were measured in mm. Discs impregnated with DMF were used as controls. The antibacterial reference tetracycline discs and the antifungal reference nystatin discs were tested concurrently as standards.

Results of structure-activity relationship (SAR) of the newly prepared compounds towards different groups of microorganisms

Most of the prepared compounds were tested for their antimicrobial activity against the following various types of bacteria: 2 gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and one gram-negative bacteria (*Escherichia coli*). The antifungal activity was tested using the pathogenic yeast strain *Candida albicans*. Preliminary testing was carried out by measuring the inhibition zone on the agar plates in mm. Compounds with promising activity (inhibition zone > 10 mm) were subjected to minimum inhibitory concentration (MIC) determination. From the obtained results (Tables VIII and IX), it could be concluded that among the twenty tested compounds; thirteen compounds exhibited antimicrobial activity. It has been observed that the compounds tested fall into three main categories. First, the enones (compounds **10** and **11a**). Second, the hydrazino derivatives (compounds **4c**, **5a**, **b** and **5c**, **8a** and **8c**, **9**) and their cyclised counterparts (compounds **15a**, **15b**, **15d**, **15e**, **15f**, and **15g**, **17a**, **15b**, and **15c**). The third group is isosteric with the cyclised hydrazino derivatives (compounds **13a** and **13b**).

Within the enone series, substitution with a methyl group at position 2 of the tetrahydrobenzothienopyrimidine ring **11a**, decreased the activity against gram-positive bacteria to half in comparison to the unsubstituted analogue **10** and removed the activity against the *Candida albicans*.

The terminal amino group of the hydrazino when unsubstituted together with the presence of a methyl group at position 2 proved to be broad spectrum (active against gram-positive, gram-negative bacteria and *Candida albicans*). Further substitution of this hydrazino by reacting with different reagants or by being subjected to cyclisation

Table VIII. Antimicrobial activity of selected, newly prepared compounds, on different microorganisms

Compd. No.	G+ve Bacteria		G-ve Bacteria	Yeast
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*
	(mm) Diameter of inhibition zone			
4c	-	24	-	-
5a	23	19	-	14
5b	26	10	-	-
5c	-	10.5	-	-
8a	-	-	-	-
8c	-	-	-	13
9	-	-	-	-
10	25	22	-	15
11a	10	10	-	-
13a	-	-	-	-
13b	26	10	-	10.5
15a	-	-	-	-
15b	-	-	-	-
15d	26	10	10	-
15e	23	10	10.5	10
15f	-	-	-	-
15g	-	25	-	13.5
17a	23.5	10	-	-
17b	26.5	10	-	-
17c	-	25.5	-	13
Tetracyclin	28	26	20	-
Nystatine	-	-	-	24

Table IX. Minimum inhibitory concentration of the tested compounds against selected microorganisms MIC (μ_g/mL)

Compd No.	G +ve Bacteria		G ve Bacteria	Yeast
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*
4c	-	115	-	-
5a	125	115	-	155
5b	105	155	-	-
5c	-	205	-	-
8c	-	-	-	210
11a	250	215	-	-
13b	110	110	-	110
15d	215	250	245	-
15e	155	165	240	155
15g	-	105	-	105
17a	125	150	-	-
17b	110	145	-	-
17c	-	105	-	170
Tetracycline	40	2	200	-
Nystatine	-	-	-	6

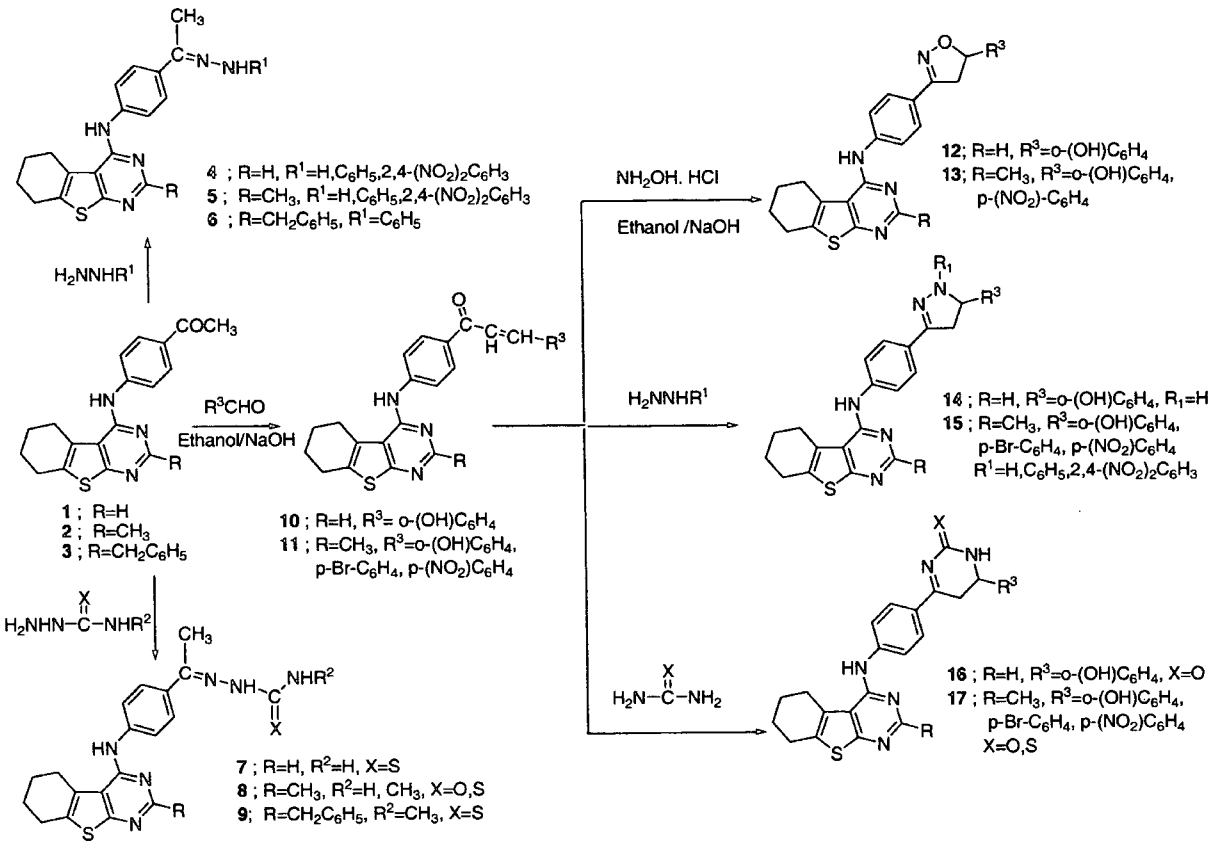
was affected in order to see the relevance of these changes on the activity. Thus, when substitution was affected with different aryl substituents; the activity decreased especially with phenyl and dinitrophenyl **5b** and **c**, to the extent of acting against only one of the gram-positive strains of the bacteria (*Bacillus subtillis*) instead of both of the two strains **5c**. Subsequently, the introduction of a carbamoyl moiety at the terminal amino group of the hydrazino led to the absence of the antibacterial and antifungal activities **8a**. The substituted thiocarbamoyl derivative **8c** showed activity against *Candida albicans* only. On the other hand, changing the 2-methyl group into a benzyl in addition to the substituted thiocarbamoyl group; gave an inactive compound. Incorporation of the hydrazino group into a ring system resulted in the formulation of dihydropyrazol **15** and oxotetrahydropyrimidine **17** was achieved. When the N^1 of the dihydropyrazol ring was unsubstituted **15a** or had a phenyl ring **15b** in addition to the ortho-hydroxyphenyl group present; inactive compounds were obtained. A nitrophenyl ring was then incorporated instead of the *o*-hydroxyphenyl to study the relevance of this change on the activity of these compounds. The results were good as the compounds having this group (**15d** and **15e**) showed activity against both gram-positive and gram-negative bacteria. The presence of a phenyl group at N^1 and a dinitrophenyl group gave these compounds a broad spectrum of activity **15e**. The bromophenyl group produced an inactive compound when N^1 was unsubstituted **15f**.

The phenyl group instead lent it activity against only one strain of gram-positive bacteria and the *Candida albicans*. Compounds bearing the oxotetrahydropyrimidine ring **17** retained their activity comparative to their open-chain **5** and the dihydropyrazole **15** derivatives. The compound bearing the nitrophenyl moiety **17b**, showed activity against gram-positive bacteria (if not against gram-negative or yeast) as its dihydropyrazole analoges **15d** and **15e** and its open chain analoge **5c** was even less active. Whilst the compound with the bromophenyl ring **17c** retained the same activity as compound 15 g; that containing the orthohydroxyphenyl moiety **17a** showed activity against gram-positive bacteria, when both analoges containing the dihydropyrazole ring were completely devoid of activity.

The third group comprising the isostere dihydroisoxazole (**13**) showed activity against gram-positive bacteria and *Candida albicans* when a nitrophenyl moiety was included in the structure.

RESULTS AND DISCUSSION

The starting compound 2-methyl-4-(4-acetylamino)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine (**2**) was



Scheme 1. Synthesis of novel tetrahydrobenzothienopyrimidines

prepared as reported (Moneer, 2001). Likewise, the other two new intermediates (**1** and **3**) (Table I) were similarly synthesized utilizing the 4-chloro or 4-chloro-2-benzyl-5, 6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidines with *p*-amino acetophenone. These three intermediates were the initiators for the preparation of the work presented. Reaction of any of these with various hydrazines (Table II), or semi/ thiosemicarbazides (Eisa *et al.*, 1990; Zeid *et al.*, 1996) (Table III), furnished their new counterparts, the 2-substituted or unsubstituted-4-[4-(1-hydrazono or substituted hydrazonoethyl)anilino]-5,6,7,8-tetrahydrobenzo[b]thieno [2,3-d] pyrimidines (**4**-**6**) and 2-substituted or unsubstituted-4-[4-(4-semi or thiosemi or methylthiosemicarbazonoethyl) anilino]-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidines (**7**-**9**) respectively.

Application of Claisen-Schmidt reaction conditions to the starting materials **1** and **2** with the suitable aldehyde in ethanolic potassium hydroxide (Moneer, 2001), resulted in the corresponding chalcones; methyl or unsubstituted 4-[4-(2-hydroxy or 4-bromo or 4-nitro cinnamoyl)anilino]-5,6,7,8-tetrahydro benzo [b] thieno[2,3-d] pyrimidines (**10**, **11**); being obtained (Table IV). These chalcones were the building blocks for the preparation of the 2-methyl or unsubstituted-4-[4-(5-aryl-4,5-dihydroisoxazol-3-yl)anilino] 5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d] pyrimidines **12**, **13** through their reaction with hydroxylamine hydrochloride (Omar *et al.*, 1996; Yousef *et al.*, 1996) in the presence of catalytic amount of sodium hydroxide (Table V). Additionally, interaction between the respective chalcones and hydrazine hydrate (Abou-Ouf *et al.*, 1979; Omar *et al.*, 1996), phenyl hydrazine and 2, 4-dinitrophenyl hydrazine in absolute ethanol provided the 2-methyl or unsubstituted-4-[4-(5-aryl-4,5-dihydropyrazol-3-yl) anilino] 5,6,7,8-tetrahydrobenzo [b]thieno[2,3-d]-pyrimidine derivatives **14**, **15** (Table VI). Finally, the synthesis of 2-methyl or unsubstituted-4-{4-[6-aryl-2-(oxo or thioxo)-1,2,5,6-tetrahydropyrimidin-4-yl]anilino} 5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d] pyrimidine compounds (**16** and **17**) was accomplished *via* the treatment of compounds **10** and **11** with urea in ethanolic hydrochloric acid (Omar *et al.*, 1996; Yousef *et al.*, 1996) or thiourea in ethanolic sodium hydroxide (Omar *et al.*, 1996) (Table VII).

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