SYNTHESES, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF SUBSTITUTED-THIAZOLO-1,3,4-THIADIAZOLES, 1,3,4-OXADIAZOLES AND 1,2,4- TRIAZOLES

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ABSTRACT: *Starting from readily available 2-substituted-4-methylthiazole-5-carboxylic acid hydrazide (1), The title compounds were prepared. The reaction of compound 1 (R=CH_3) with formic acid yielded 1-(formyl)-2-(2,4-dimethylthiazole-5-carboxyl) hydrazine (2). Refluxing the latter with phosphorous pentasulfide in xylene afforded compound 3, the reaction of compound 2 with phosphorous pentoxide afforded compound 4. Reaction of compound 1 with substituted isothiocyanates followed by cyclization of the intermediate 5 in basic medium gave 4-alkyl-5-(2- substituted-4-methyl-5-thiazolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6). Alkylation of compound 6 followed by subsequent oxidation of intermediate 7 gave compound 8. The reaction of acid chloride 11 with hydrazide 12 afforded compound 13 which was cyclized by P_2S_5 or P_2O_5 to compounds 14 or 15 respectively. Compounds 3 and 14 showed significant activities against E. Coli and Bacillus Subtilis.*

KEY WORDS: *Triazole, Thiazole, Antibacterial activity.*

INTRODUCTION

In view of the potential biological activity of members of the 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole [1-3], it was of interest to prepare the title compounds as possible effective drugs against tropical diseases [4].

RESULTS AND DISCUSSION

The syntheses of the title compounds was accomplished as shown in Scheme. Refluxing 2,4-dimethylthiazole-5- carboxylic acid hydrazide (**1**, R=CH_3) [5], with formic acid for 15 hours afforded 1-(formyl)-2-(2,4-dimethylthiazole-5-carboxyl)hydrazine (**2**). Refluxing 0.01 mol of compound **2** with 0.006 mol phosphorous pentasulfide yielded 2-(2,4-dimethylthiazolyl)-1,3,4-thiadiazole (**3**). The usual reaction for the formation of 1,3,4-oxadiazole, namely the reaction of

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1021-9986/98/1/14 7/$/2.70

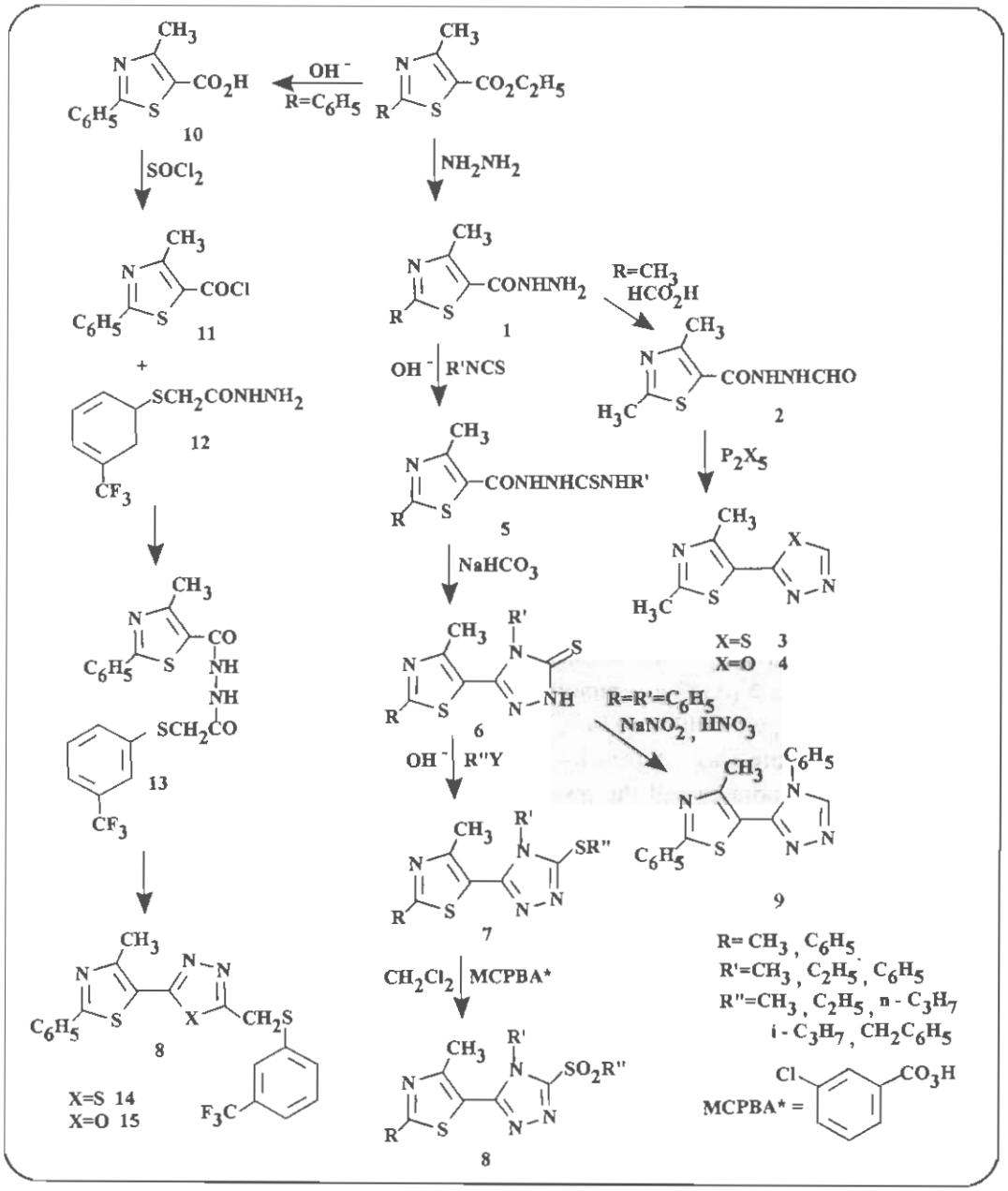
compound **2** with phosphorous pentoxide afforded 2-(2,4-dimethylthiazolyl)-1,3,4-oxadiazole (**4**) in 60% yield.

Reaction of compound **1** with alkyl (or phenyl) isothiocyanates in ethanol at room temperature [6] with aqueous sodium hydroxide gave compound **5**. Refluxing compound **5** with aqueous sodium bicarbonate afforded 4-alkyl (or phenyl)-5-(2-substituted-4-methyl-5-thiazolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**6**).

Reaction of compound **6** with alkyl halide afforded 3-(2-substituted-4-methyl-5-thiazolyl)-4-alkyl(or phenyl)-5-alkylthio-4H,1,2,4-triazole (**7**). Oxidation of compound **7** with *m*-chloroperbenzoic acid gave 3-(2-substituted-4-methyl-5-thiazolyl)-4-alkyl(or phenyl)-5-alkylsulfonyl-4H-1,2,4-triazole (**8**).

The physical constants of compounds **5, 6, 7** and **8** are summerized in Tables 1 to 4 .

Reaction of compound **11** [7] with hydrazide **12** [8], afforded compound **13**. The latter was readily cycli-



Scheme

zed with P_2S_5 to give substituted-1,3,4-thiadiazole **14**. Reaction of **13** with P_2S_5 yielded compound **15**. Reaction of compound **6a** (R=R'=Ph) with sodium nitrite in aqueous nitric acid at 5°C yielded 3-(2-phenyl-4-methyl-5-thiazolyl)-4-phenyl-4H-1,2,4-triazole (**9**)

EXPERIMENTAL

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The UV spectra were recorded using a Perkin-Elmer model 267 spectrograph (potassium bromide disks). The ^{1}H NMR spectra were recorded on a Bruker FT-80 or a Varian 400 Unity Plus spectrometers and chemical shifts (δ) are in *ppm* relative to internal tetramethyl silane. The mass spectra were run on a Finnigan model TSQ-70 spectrometer at 70 eV. All compounds gave satisfactory C, H, N analyses.

1-Formyl-2-(2,4-dimethylthiazolyl)hydrazine(2)

A solution of compound **1** (0.35 g, 0.005 mol) in formic acid (10 mL) was refluxed for 15 hours at 90°C. The solvent was evaporated and the residue was crystallized from methanol to give 0.75 g (80%) of compound **2**; mp 62°C.

IR(KBr): ν_{max} (cm^{-1}), 3325, 3255 (NH), 3050 (aromatic), 1695, 1660(C=O).

Anal. Calcd. for $C_7H_9N_3O_2S$: C, 42.21; H, 4.52; N, 21.10. Found: C, 42.15; H, 4.56; N, 21.21.

2-(2,4-Dimethyl-5-thiazolyl)-1,3,4-thiadiazole(3)

To a solution of compound **2** (0.187 g, 1 mmol) in xylene (20 mL) phosphorous pentasulfide (0.14 g, 0.6 mmol) was added. The mixture was refluxed for 45 minutes. The solvent was evaporated and the residue was crystallized from methanol to give 0.1 g (55%) of compound **3**; mp 176-180°C.

UV(CH_3OH); λ_{max} (nm), 258($\log \varepsilon$ = 3.10); ^{1}H NMR ($CDCl_3$): δ(*ppm*), 7.93 (s, 1H, H_5 thiadiazole), 2.84(s, 3H, CH_3), 2.0(s, 3H, CH_3).

Anal. Calcd. for $C_7H_7N_3S_2$: C, 42.21; H, 4.52; N, 21.10. Found: C, 42.29; H, 4.70; N, 20.94.

2-(2,4-Dimethyl-5-thiazolyl)-1,3,4-oxadiazole(4)

To a solution of compound **2** (0.187 g, 1 mmol) in xylene (20 mL) was added slowly phosphorous pentoxide (0.142 g, 1 mmol). The mixture was refluxed for 1 hour. The solvent was evaporated. To the residue, water (2 mL) was added and extracted with chloroform. The solvent was evaporated and the residue was crystallized from ethanol to give 0.1 g (60%) of **4**; mp 192-196°C.

UV($CHCl_3$): λ_{max} (nm), 245($\log \varepsilon$ = 2.69); ^{1}H NMR ($CDCl_3$): δ(*ppm*), 8.41(s, 1H, H_5 oxadiazole), 2.77(s, 3H, CH_3), 2.74 (s, 3H, CH_3).

Anal. Calcd. for $C_7H_7N_3OS$: C, 46.40; H, 3.86; N, 23.20. Found: C, 46.23; H, 3.62; N, 23.15.

1-(2-Substituted-4-methylthiazole-5-carboxyl)-4-alkyl (or phenyl)-thiosemicarbazide (5)

To a solution of compound **1** (1 mmol) in ethanol (10 mL), alkyl (or phenyl) isothiocyanate (1 mmol) and sodium hydroxide (1 mmol, as a 2N solution) was added. The mixture was stirred for 24 hours and filtered. The filtrate was acidified with hydrochloric acid. The precipitate was filtered and crystallized from ethanol-water to give compound **5**.

The physical constants of compounds **5a** to **5e** are summarized in Table 1.

Table 1: The physical constants of compounds 5a to 5e.

Compound	R	R'	Vol. of alkyl(or phenyl) isothiocyanate needed(mL)	mp(°C)[a]	Yield (%)
5a	CH_3	C_2H_5	1.5	>265(dec.)	85
5b	C_6H_5	C_2H_5	1.5	205-208	85
5c	C_6H_5	CH_3	1.3	221-223	92
5d	CH_3	C_6H_5	2.5	159-160	83
5e	C_6H_5	C_6H_5	2.5	178-181	85

a : All compounds were crystallized from ethanol-water.

5-(2-Substituted-4-methyl-5-thiazolyl)-4-alkyl(or phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6)

A stirring mixture of compound **5** (1 mmol) and sodium bicarbonate (15 mmol) in water (10 mL) was refluxed for 12 to 16 hours. After cooling, the solution was acidified with hydrochloric acid and the precipitate was filtered. The crystals were crystallized from ethanol to give compound **6.**

5-(4-Methyl-2-phenyl-5-thiazolyl)-4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione(6d,R=C_6H_5 , R'=CH_3)

This compound was prepared according to the above procedure in 85% yield; mp 330 (decomp.); ^{1}H NMR($CDCl_3$): δ(*ppm*), 8.1(s, 1H), 7.9(m, 2H, aromatic), 7.45(m, 3H, aromatic), 3.56(s, 3H, CH_3), 2.55 (s, 3H, CH_3).

The physical constants of compounds **6a** to **6e** are summarized in Table 2.

3-(2-Substituted-4-methyl-5-thiazolyl)-5-alkylthio-4-alkyl (or phenyl)-4H-1,2,4-triazole (7)

To a stirring solution of compound **6** (1 mmol) in sodium hydroxide (2 mmol) and ethanol (0.5 mL), alkyl halide (1 mmol) was added dropwise. After the addition was complete, the mixture was stirred at room temperature from 1 to 2 hours. It was diluted with water (5 mL). The precipitate was filtered and crystallized from ethyl acetate to give compound **7.**

The physical constants of compounds **7a** to **7w** are summerized in Table 3.

Table 2 : The physical constants of compounds 6a to 6e.

Compound	R	R'	mp(°C)[a]	Yield(%)
6a	CH_3	C_2H_5	228-230	85
6b	CH_3	C_6H_5	202-204	82
6c	C_6H_5	C_6H_5	80-82	30
6d	C_6H_5	CH_3	>330(dec.)	85
6e	C_6H_5	C_2H_5	214-216	83

a : All compounds were crystallized from ethanol.

3-(4- Methyl-2-phenyl-5- thiazolyl)-4-methyl-5-methylthio-4H-1,2,4-triazole (7k, R=C_6H_5 , R', R"= CH_3)

This compound was prepared according to the above procedure in 85% yield; mp 137-140°C.
^{1}H NMR($CDCl_3$): δ(*ppm*), 7.93(m, 2H, aromatic), 7.46 (m, 3H, aromatic), 3.33(s, 3H, CH_3), 2.81(s, 3H, CH_3), 2.55(s, 3H, CH_3); MS: m/z(%), 302(M^+ , 48), 286(40), 269(40), 230(60), 175(88), 134(100), 102(75), 77(55).

Table 3 : The physical constants of compounds 7a to 7w.

Compound	R	R'	R"	mp(°C)[a]	Yield(%)
7a	CH_3	C_2H_5	CH_3	77-80	85
7b	CH_3	C_2H_5	C_2H_5	60-63	75
7c	CH_3	C_2H_5	n-C_3H_7	68(dec.)	70
7d	CH_3	C_2H_5	i-C_3H_7	182-184	50
7e	CH_3	C_2H_5	$CH_2C_6H_5$	66-69	83
7f	CH_3	C_6H_5	CH_3	153-155	70
7g	CH_3	C_6H_5	C_2H_5	137-141	68
7h	CH_3	C_6H_5	n-C_3H_7	98-100	65
7i	CH_3	C_6H_5	i-C_3H_7	113-115	60
7j	CH_3	C_6H_5	$CH_2C_6H_5$	104-106	75
7k	C_6H_5	CH_3	CH_3	137-140	85
7l	C_6H_5	C_2H_5	CH_3	103-106	60
7m	C_6H_5	C_2H_5	C_2H_5	80-84	55
7n	C_6H_5	C_2H_5	n-C_3H_7	174-176	60
7o	C_6H_5	C_2H_5	i-C_3H_7	170-172	55

Table 3 : continued

Compound	R	R′	R″	mp(°C)[a]	Yield(%)
7p	C_6H_5	C_2H_5	$CH_2C_6H_5$	88-90	65
7q	C_6H_5	C_6H_5	CH_3	223-226	67
7r	C_6H_5	C_6H_5	C_2H_5	87-90	60
7s	C_6H_5	C_6H_5	n-C_3H_7	170-173	55
7t	C_6H_5	C_6H_5	i-C_3H_7	162-166	50
7u	C_6H_5	C_6H_5	$CH_2C_6H_5$	172-175	70
7v	C_6H_5	C_6H_5	CH_2=$CHCH_2$	160-162	90
7w	C_6H_5	C_6H_5	-CH_2CH_2-	140-143	60

a: All compounds were crystallized from ethyl acetate

4-Alkyl (or phenyl)-5-alkylsulfonyl-3-(2-substituted-4-methyl-5-thiazolyl)-4H-1,2,4-triazole (8)

To a stirring solution of compound **7** (1 mmol) in dichloromethane (10 mL) at 0°C, *m*-chloroperbenzoic acid (3 mmol) was added. The mixture was stirred at room temperature overnight. It was washed with sodium bicarbonate solution (3×5 mL), dried (sodium sulfate), filtered and evaporated. The residue was crystallized from ethyl acetate to give compound **8** (yields 70 to 85%).

The physical constants of compounds **8a** to **8w** are summerized in Table 4.

3-(4-Methyl-2- phenyl-5-thiazolyl)-4- methyl-5-methylsulfonyl-4H-1,2,4-triazole (8k, R=C_6H_5 , R′ , R″= CH_3)

This compound was prepared according to the above procedure in 72% yield; mp 144-146°C.

^{1}H NMR($CDCl_3$): δ(*ppm*), 7.94(m, 2H, aromatic), 7.48 (m, 3H, aromatic), 3.93(s, 3H, CH_3–SO_2), 3.59(s, 3H, N–CH_3), 2.55(s, 3H, CH_3); MS: m/z(%), 334 (M^+, 36), 286(30), 269(50), 230(70), 134(40), 102(45), 77(25).

1-(4-Methyl-2- phenylthiazole-5- carboxyl)-2-[α-(3-trifluoromethyl phenylthio) acetyl] hydrazine (13)

A mixture of compound **11** (1.9 g, 8 mmol) and hydrazide **12** (2 g, 8 mmol) [8] in absolute ethanol (15 mL) was stirred overnight. The precipitate was filtered to give 2.9 g (85%) of **13**; mp 180-183°C.

^{1}H NMR($CDCl_3$): δ(*ppm*), 7.74(m, 9H, aromatic), 3.85 (s, 2H, CH_2), 2.68(s, 3H, CH_3).

Anal. Calcd. for $C_{20}H_{16}F_3N_3O_2S_2$: C, 53.21; H, 3.55; N, 9.31.

Found: C, 53.45; H, 3.36; N, 9.11.

2-(4-Methyl-2-phenyl-5-thiazolyl)-5-(3-trifluoromethylphenylthiomethyl)-1,3,4-thiadiazole (14)

This compound was prepared similar to compound **3**, in 40% yield; mp 100-103°C (CH_3OH), UV(CH_3OH): λ_{max}(nm), 246(log ε= 2.8). ^{1}H NMR ($CDCl_3$): δ(*ppm*), 7.86(m, 6H, aromatic), 7.51(m, 3H, aromatic), 4.56(s, 2H, CH_2), 2.68(s, 3H, CH_3); MS: m/z(%), 450(M^++1 , 37), 418(40), 396(55), 348(42), 220(100), 77(30).

Anal. Calcd. for $C_{20}H_{14}F_3N_3S_3$: C, 53.45; H, 3.11; N, 9.35.

Found: C, 53.55; H, 3.14; N, 9.54.

2-(4-Methyl-2-phenyl-5-thiazolyl)-5-(3-trifluoromethylphenylthiomethyl)-1,3,4-oxadiazole (15)

This compound was prepared similar to compount **4** in 35% yield; mp 110-112°C.

UV(CH_3OH): λ_{max}(nm), 241(log ε=2.63); ^{1}H NMR ($CDCl_3$): δ(*ppm*), 7.85(m, 6H, aromatic), 7.48(m, 3H, aromatic), 4.65(s, 2H, CH_2), 2.69(s, 3H, CH_3); MS: m/z(%), 433(M^+, 28), 418(30), 348(50), 220(100), 148(72), 77(60).

Anal. Calcd. for $C_{20}H_{14}F_3N_3OS_2$: C, 55.42; H, 3.16; N, 9.69.

Found: C, 55.35; H, 3.31; N, 9.71.

3-(4-Methyl-2-phenyl)-4-phenyl-4H-1,2,4-triazole (9)

To a stirring mixture of $NaNO_2$ (0.004 g, 6

Table 4 : The physical constants of compounds 8a to 8w.

Compound	R	R′	R″	mp(°C)[a]
8a	CH_3	C_2H_5	CH_3	88-90
8b	CH_3	C_2H_5	C_2H_5	144-146
8c	CH_3	C_2H_5	n-C_3H_7	oil
8d	CH_3	C_2H_5	i-C_3H_7	oil
8e	CH_3	C_2H_5	$CH_2C_6H_5$	oil
8f	CH_3	C_6H_5	CH_3	85-88
8g	CH_3	C_6H_5	C_2H_5	140-142
8h	CH_3	C_6H_5	n-C_3H_7	164-166
8i	CH_3	C_6H_5	i-C_3H_7	170-171
8j	CH_3	C_6H_5	$CH_2C_6H_5$	72-74
8k	C_6H_5	CH_3	CH_3	144-146
8l	C_6H_5	C_2H_5	CH_3	90-92
8m	C_6H_5	C_2H_5	C_2H_5	84-86
8n	C_6H_5	C_2H_5	n-C_3H_7	67-70
8o	C_6H_5	C_2H_5	i-C_3H_7	165-168
8p	C_6H_5	C_2H_5	$CH_2C_6H_5$	224-226
8q	C_6H_5	C_6H_5	CH_3	217-219
8r	C_6H_5	C_6H_5	C_2H_5	280(dec.)
8s	C_6H_5	C_6H_5	n-C_3H_7	124-127
8t	C_6H_5	C_6H_5	i-C_3H_7	193-195
8u	C_6H_5	C_6H_5	$CH_2C_6H_5$	193-195
8v	C_6H_5	C_6H_5	$CH{=}CHCH_2$	131-134
8w	C_6H_5	C_6H_5	$-CH_2-CH_2-$	100-102

a: All compounds were crystallized from ethyl acetate.

mmol), water (2 mL) and fuming nitric acid (0.25 mL) at 5°C compound **6c** was added portionwise during 20 minutes. After addition was complete, stirring was continued for 45 minutes. It was diluted with water (3 mL), made alkaline (pH=10) by a sodium hydroxide solution (as 0.1 N). The mixture was saturated with sodium chloride and extracted with a mixture of chloroform-2-propanol (3:1) several times. Organic layer was dried over sodium sulfate and filtered. The solvent was evaporated and the residue was crystallized from ethanol to give 0.38 g, (60%) of **9**; mp 89-91°C.

MS: m/z(%), 318(M^+,30), 244(20), 217(30), 214(40), 203(100), 189(14), 135(17), 104(25), 96(30), 71(16).

Anal. Calcd. for $C_{18}H_{14}N_4S$: C, 67.92; H, 4.40; N, 17.61.

Found: C, 68.03; H, 4.49; N, 17.48.

Antibacterial and antifungal assay

Compounds **3, 5a, 5d, 5e, 6a, 6b, 6c, 7a, 7f, 7k, 7g, 8a, 8f, 8k, 8g** and **14** were tested against *Bacillus subtilis* (PTCC 6633), *Staphylococcus aureus* (PTCC 6538), *Escherichia coli, Klebsiella pneumoniae* (PTCC 10031), *Staphylococcus epidermis* (PTCC 12228), *Pseudomonas aeruginosa* (PTCC 27853), *Candidia albicans* (PTCC 10231) and *Aspergillus niger* (PTCC 16404).

The compounds were dissolved in acetone and diluted to 1 mg/1 mL concentration. To a standard paper disk of 6 mm diameter, the latter was added untill the desired amount of compounds was absorbed by the disk (30, 60 and 90 μg/disk). The disks were placed on inoculated assay medium surface.

Gentamycine was used for comparison in bacterial

media and miconazole for comparison in fungal media. Blank disks inoculated with acetone were used too. Growth inhibition diameters were measured in mm. Compounds **3** and **14** showed significant activity against *Bacillus subtilis* and *Escherishia coli* in comparison with standard (17 mm for standard 14 mm for compound **14** and 12 mm for compound **3**). None of the compounds showed significant antifungal activity.

Received, 14th October 1996 ; Accepted, 12th May 1997

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