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Synthesis and Antimicrobial Activity of Some New 1-Alkyl-2-alkylthio-1,2,4-triazolobenzimidazole Derivatives

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Some new derivatives of 1,2,4-triazolo[2,3-a]benzimidazoles were synthesized through the reaction of 1,2-diaminobenzimidazole with carbon disulfide. The resulting 1,2,4-triazolo-[2,3-a]benzimidazole-2-thione intermediate was reacted with one equivalent of alkyl halides to give the corresponding 2-*alkylthio* derivatives, which were further alkylated through the reaction with another one equivalent of different alkyl halides to afford the target compounds; 1-alkyl-2-alkylthio-1,2,4-traizolo[2,3-a]benzimidazoles. On the other hand, the 1,2-disubstituted derivatives with two identical alkyl substituents were prepared by the reaction of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione with two equivalents of the alkyl halides. The structures of the new compounds were assigned by spectral and elemental methods of analyses. The synthesized compounds were tested for their antibacterial and antifungal activities. Most of the tested compounds proved comparable results with those of **ampicillin** and **fluconazole** reference drugs. The study indicated that, the antibacterial as well as the antifungal activities of the test compounds were improved with increase in the bulkiness of the introduced alkyl groups. Also, some active antibacterial compounds were tested for their antimycobacterial activity. All the test compounds showed equipotent antitubercular activity as that of **INH** as a reference drug.

Key words: 1,2-Diaminobenzimidazole, Hydroxylamine-*O*-sulfonic acid, 1,2,4-triazolo[2,3-a]benzimidazoles, Antimicrobial

INTRODUCTION

The emergence of resistant strain of bacteria especially M. tuberculosis to major classes of antimicrobial agents is recognized as a serious health concern particularly, the emergence of multidrug resistant strains of Gram-positive bacterial pathogens is a problem of ever increasing significance (He *et al.*, 2004). Organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermids* (MRSE), vancomycin-resistant enterococci (VRE), and penicillin and cephalosporin-resistant streptococci are continously challenging chemists, physicians and patients (He *et al.*, 2004). Consequently, the search for chemotherapeutic agents constitutes a real challenge facing microbiologists, pharmacologists as well as medicinal chemists.

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Currently, benzimidazole derivatives are an object of sustained interest due to the vast range of their potential activities as analgesics (Stecher *et al.*, 1989a) (e.g. bezitramide), antihistaminics e.g. astemazole (European pharmacopoeia, 1996a) and clemazole (Stecher *et al.*, 1989b), anthelmintics (United state pharmacopoeia, 2002a) (e.g. thiabendazole, flubendazole, mebendazole, and albendazole) and antiulcer agents (e.g. lansoprazole (Stecher *et al.*, 1989c) and omeprazole (European pharmacopea, 1996b). Moreover, compounds containing triazole ring were found to have antifungal (Stecher *et al.*, 1989d, 1989e) (e.g. fluconazole and itraconazole), antiviral (United state pharmacopoeia, 2002b) (e.g. ribavarin), antineoplastic (Stecher *et al.*, 1989f, 1989g) (e.g. anastrazole and letrazole) and sedative-hypnotics e.g. alprazolam (United state pharmacopoeia, 2002c) and estazolam (Stecher *et al.*, 1989h). On the other hand, it has been observed that 1,2,4-triazole moiety has a great versatility in fusing to various ring systems and the *N*-bridged heterocycles derived from them are associated with different phar-

macological activities (Savini *et al.*, 2001). Taking the above pursuing facts into account, the present work aims at the synthesis of 1,2,4-triazolo[2,3-a]benzimidazoles to be tested *in vitro* against Gram positive, Gram negative bacteria and fungi along with evaluation of some selected compounds against M. tuberculosis.

MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, model SMP1, UK], and were uncorrected. Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merck) were used for thin layer chromatography. A developing solvent system of chloroform/methanol (9:1) was used and the spots were detected by ultraviolet lamp (Spectroline, model CM-10, U.S.A.).

IR spectra (KBr discs) were recorded on a shimadzu IR-470 spectrometer, Japan. Faculty of Pharmacy, Assiut University, Assiut. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz), U.S.A. Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using $CDCl_3$ unless otherwise stated as a solvent. Elemental analyses were performed at the Unit of Microanalyses, Assiut University, Assiut, Egypt.

The starting material 1,2-diaminobenzimidazole; compound **1** was prepared according to a reported procedure through the reaction of *o*-phenylenediamine with cyanogen bromide (Hartmann *et al.*, 1943), followed by amination using hydroxylamine-*O*-sulfonic acid (Zeiger *et al.*, 1977).

Bacterial cultures were obtained from Botany Department, Faculty of Science, Assuit University. Fungal cultures were obtained from Assuit University Mycological Center (AUMC), Assuit University. The T.B. strain was obtained from the Chest Hospital, Assiut Governorate.

Chemistry

Synthesis of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione (2)

To a stirred solution of 1,2 diaminobenzimidazole (10.00 g, 0.052 mole) in dimethylformamide (100 mL), carbon disulfide (30 mL, 0.47 mole) was added. The reaction mixture was refluxed for 16 h and the formed precipitate was filtered, washed with methanol and dried. The product is insoluble in organic solvents, therefore it is purified by dissolving in 5% KOH. The alkaline solution was cooled in an ice bath then rendered acidic by addition of conc. HCl with stirring. The precipitate was filtered, washed with distilled water and dried. The process of purification was repeated till constant melting point (Table I).

Synthesis of 2-alkylthio-1,2,4-traizolo[2,3-a]benzimidazoles (3a-d)

To a suspension of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione; compound **2** (9.5 g, 0.05 mole) and potassium carbonate (6.9 g ,0.05 mole) in dry acetone (100 mL), the appropriate alkyl halide (0.05 mole) was added. The reaction mixture was stirred for 10-12 h at the ambient temperature. Acetone was evaporated; the residue was treated with water and then extracted with chloroform (3×30 mL). The chloroform extract was washed with water and dried over anhydrous magnesium sulfate. Chloroform was evaporated and the residue was crystallized from the appropriate solvent. Yields, m.p., elemental analysis, and ^{1}H-NMR spectral data are listed in Tables I & II.

Synthesis of 1-alkyl-2-alkylthio-1,2,4-traizolo[2,3-a] benimidazoles (4-7;$_{a-d}$)

Method A:

To a suspension of the appropriate 2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazoles; compounds **3a-d** (0.005 mole) and potassium carbonate (0.69 g, 0.005 mole) in dry acetone (15 mL), the required alkyl halide (0.005 mole) was added. The reaction mixture was stirred for 15-20 h at the ambient temperature and then processed as mentioned above. Yields, m.p., and other data are listed in Table I.

Method B:

This method was used for preparation of 1-alkyl-2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazoles with two identical alkyl substituents (R = R^1); compounds **4a, 5b, 6c,** and **7d**. These compounds were prepared as mentioned under method **A** except (0.01 mole) of the appropriate alkyl halide and (0.70 g, 0.005 mole) of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione were used. Yields are slightly higher than method **A** and m.p. for one and the same compound prepared by the two methods are identical and mixed m.p. showed no depression. Some representative compounds, also, showed one and the same ^{1}H-NMR spectrum for one and the same compound prepared by both methods. Therefore method **B** was adopted thereafter for synthesis of such compounds.

Antimicrobial screening

Antibacterial activity

Organisms and culture conditions:

Six bacterial species represent both Gram positive and Gram negative strains were used to test the antibacterial activities of the target compounds: *Bacillus cereus, Staphylococcus aureus and Micrococcus roseus* as representatives for the Gram positive strains, while the Gram negative strains were represented by *Serratia rodenii, Pseudomonas aeruginosa* and *Escherichia coli.*

Materials and method (William H, 1977):

Cell suspension of bacterial strains was prepared from 48 h old cultures grown on nutrient agar (NA) in sterilized water. One mL of the cell suspension was added to petri dishes of 9 cm diameter, then 15 mL of NA was poured into the plates. Plates were shaken gently to homogenize the inoculums.

Sterile 5 mm filter paper disks (Whatman) were impregnated with solutions of the tested compound and ampicillin (100 μM/mL in DMSO). In addition, other disks were impregnated with the solvent (DMSO) as a control. The impregnated disks were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 35 ± 2°C for 24-48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and results are given in Table III.

Antifungal activity.

Organisms and culture conditions:

Six pathogenic, phytopathogenic, or food poisoning fungal species were used in the present study: *Trichophyton rubrum, Candida albicans, Microsporum canis, Aspergillus flavus, Fusarium oxysporum,* and *Penicillium expansum.*

Materials and method (William H, 1977):

Spore suspension in sterile water was prepared from 2-5 days old culture of the test fungi growing on Potato dextrose agar (PDA) or Sabouraud Agar (SA) media. The final spore concentration was 5×10^4 spores/mL. About 15 mL of the growth medium was introduced onto sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of the spore suspension. Plates were shaken gently to homogenize the inoculums. The antifungal activity of the tested compounds was performed by the standard agar disk diffusion method as follows (Pai *et al.*, 1955):

Sterile 5 mm filter paper disks (Whatman) were impregnated with solutions of the tested compound and fluconzole (100 μM/mL in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as a control. The impregnated disks were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28 ± 2°C for 7 days. The radii of inhibition zones (in mm) were measured at successive intervals during the incubation period. Triplicate sets were applied for each treatment and the results are given in Table III.

Antitubercular activity

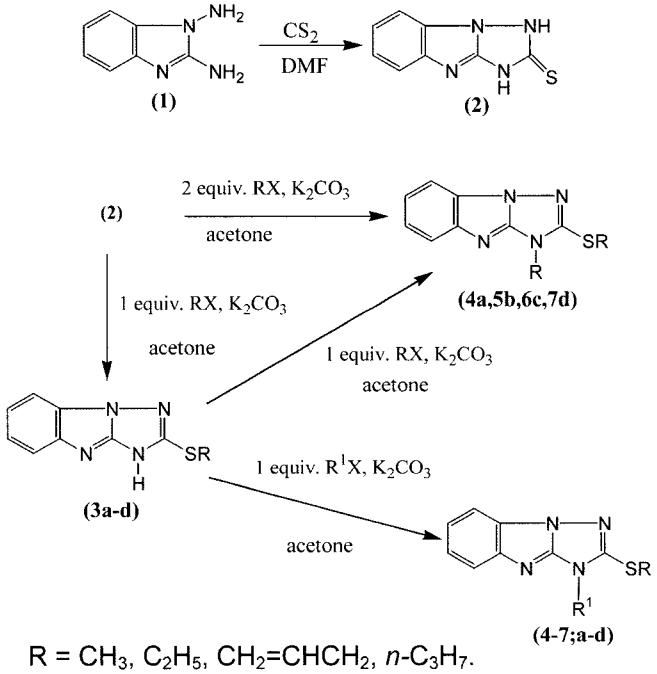
The anti-TB activity of the tested compounds were carried out using Rist and Grosset proportion method (Canetti *et al.*, 1983). The synthesized compounds **4c**, **5b**, **5c**, **6b**, and **6c** and INH were solubilized in dimethyl sulfoxide at a concentration of 100 μMol/mL. The appropriate amounts of the tested compound were diluted with Lowenstein-jensen media to give concentration of 10 μMol of the growth media. The media containing different compounds were inspissated at 70°C for 1 h in hot air oven for three successive days. The sterilized media were then inoculated by 10-3 and 10-5 dilutions of the reference strain [Bovine T.B., reference strain]. The growth and inhibitory activity of the tested compounds and **INH** were evaluated after incubation at 37°C for eight weeks. Each batch of the tests including a control experiment using the standard strain of Bovine T.B. in a media free from drugs.

RESULTS AND DISCUSSION

Chemistry

The starting material 1,2-diaminobenzimidazole; compound **1** was prepared according to a reported procedure through the reaction of *o*-phenylenediamine with cyanogen bromide (Hartmann *et al.*, 1943), followed by amination using hydroxylamine-*o*-sulfonic acid (Zeiger *et al.*, 1977). The structure of compound **1** was confirmed by matching its physical and spectral data with the reported (Zeiger *et al.*, 1977). ^{1}H-NMR spectrum of compound 1 showed two broad singlets at δ 5.48 and 6.08 equivalents to 2H corresponding to the two amino groups besides a multiplet at δ 7.30-8.10 due to the four aromatic protons as reported (Zeiger *et al.*, 1977).

The key intermediate 1,2,4-triazolo[2,3-a]benzimidazole-2-thione; compound **2** was prepared by the reaction of



R = CH_3, C_2H_5, CH_2=$CHCH_2$, *n*-C_3H_7.

R^1 = R = CH_3, C_2H_5, C_3H_5, *n*-C_3H_7.

Scheme 1. Preparation of compounds **2**, **3a-d**, and **4-7a-d**

1,2-diaminobenzimidazole with carbon disulfide in dimethylformamide, (Scheme 1). Structure of compound **2** was confirmed from its IR, ^{1}HNMR, MS as well as elemental analysis. **IR** spectrum of compound **2** showed a broad band at 34×10 cm^{-1} (NH) stretching, while as ^{1}H-NMR revealed a broad singlet at δ 12.00-13.00 ppm equivalent to 2 NH protons which are exchangeable with deuterium oxide and a multiplet at δ 7.33-8.13 corresponding to the four aromatic protons. MS spectrum of compound **2** revealed the molecular ion peak M$^+$ at (*m/z* 190;

Table I. The physicochemical constants of compounds **2**, **3a-d**, **4-7**; **a-d**

Compd No.	R	R^1	Yield %	M.p°C crys.solvent	Mol. formula (M. Wt.)	Microanalyses Calculated/Found			
						C%	H%	N%	S%
2	H	H	80	318-322*	$C_8H_6N_4S$ (190.23)	50.51 50.02	3.18 3.05	29.45 29.27	16.86 16.53
3a	CH_3	H	68	143-145 Ethanol	$C_9H_8N_4S$ (204.25)	52.92 52.57	3.95 3.95	27.43 26.95	15.70 15.14
3b	C_2H_5	H	71	123-124 Acetone	$C_{10}H_{10}N_4S$ (218.28)	55.02 54.54	4.62 4.64	25.67 25.49	14.69 14.67
3c	allyl	H	68	101-102 Acetone	$C_{11}H_{10}N_4S$ (230.29)	57.37 57.25	4.38 4.28	24.33 24.19	13.92 13.31
3d	*n*-C_3H_7	H	72	158-159 MeOH/H_2O	$C_{11}H_{12}N_4S$ (232.31)	56.87 56.38	5.21 5.41	24.12 23.99	13.80 13.68
4a	CH_3	CH_3	75	150-152 EtOH/H_2O	$C_{10}H_{10}N_4S$ (218.28)	55.02 54.71	4.62 4.46	25.67 25.30	14.69 14.61
4b	CH_3	C_2H_5	71	176-177 Pet. ether	$C_{11}H_{12}N_4S$ (232.31)	56.87 56.44	5.21 5.79	24.12 24.46	13.80 13.61
4c	CH_3	allyl	77	118-120 Pet. ether	$C_{12}H_{12}N_4S$ (244.32)	58.99 59.54	4.95 4.54	22.93 23.05	–
4d	CH_3	*n*-C_3H_7	72	158-159 MeOH/H_2O	$C_{12}H_{14}N_4S$ (246.33)	58.51 58.47	5.73 5.65	22.74 22.74	–
5a	C_2H_5	CH_3	68	132-134 EtOH/H_2O	$C_{11}H_{12}N_4S$ (232.31)	56.87 56.68	5.21 5.57	24.12 24.09	13.80 13.79
5b	C_2H_5	C_2H_5	72	179-181 Pet. ether	$C_{12}H_{14}N_4S$ (246.33)	58.51 58.63	5.73 5.38	22.74 22.62	13.02 12.91
5c	C_2H_5	allyl	65	157-158 Pet. ether	$C_{13}H_{14}N_4S$ (258.34)	60.44 59.98	5.46 5.90	21.69 21.82	12.41 12.37
5d	C_2H_5	*n*-C_3H_7	60	180-182 Ethanol	$C_{13}H_{16}N_4S$ (260.36)	59.97 59.56	6.19 5.90	21.52 21.58	–
6a	allyl	CH_3	71	110-112 Ethanol	$C_{12}H_{12}N_4S$ (244.32)	58.99 59.44	4.95 4.62	22.93 22.64	13.12 13.01
6b	allyl	C_2H_5	70	120-122 Pet. ether	°$C_{13}H_{14}N_4S$ (258.34)	60.44 59.97	5.46 5.28	21.69 22.04	12.41 12.28
6c	allyl	allyl	70	Liquid**	$C_{14}H_{14}N_4S$ (270.35)	62.20 61.25	5.22 5.14	20.72 20.40	–
6d	allyl	*n*-C_3H_7	70	152-153 Ethanol	$C_{14}H_{16}N_4S$ (272.37)	61.74 61.36	5.92 5.46	20.57 20.41	–
7a	*n*-C_3H_7	CH_3	68	132-133 Ethanol	$C_{12}H_{14}N_4S$ (246.33)	58.51 58.36	5.73 5.82	22.74 22.15	13.02 12.65
7b	*n*-C_3H_7	C_2H_5	71	179-181 EtOH/H_2O	$C_{13}H_{16}N_4S$ (260.36)	59.97 59.90	6.19 5.75	21.52 21.46	–
7c	*n*-C_3H_7	allyl	63	157-158 Pet.ether	$C_{14}H_{16}N_4S$ (272.37)	61.74 61.73	5.92 5.28	20.57 20.24	–
7d	*n*-C_3H_7	*n*-C_3H_7	72	180-182 Pet. ether	$C_{14}H_{18}N_4S$ (274.39)	61.28 60.77	6.61 6.40	20.42 20.24	–

*Compound **2** was purified by dissolving in 5% KOH followed by acidification and washing with water.
Compound **6c was purified by column chromatography (chloroform/methanol, 9:1).

intensity 100%), corresponding to the molecular weight of this compound. Also, the spectrum showed prominent peaks at (*m/z* 132; intensity 66.2%) and (*m/z* 90; intensity 50.7%).

The target compounds; 2-alkylthio-1,2,4-traizolo[2,3-a] benzimidazole (R^1 = H, 3a-d) were synthesized by the reaction of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione; compound **2** with one equivalent of alkyl halides (Scheme 1). The reaction afforded the *S*-alkyl derivatives as it is reported that *S*-alkylation supersedes the *N*-alkylation as the cause of the difference in nucleophilicity (Smith, 1981; Gülerman *et al.*, 2001). Structures of compounds **3a-d** were confirmed by IR & ^{1}H-NMR spectrometry as well as elemental analyses. The IR spectra of compounds **3a-d** showed a broad band at 3410-3420 cm^{-1} (NH) stretching, while their ^{1}H-NMR spectra approved the presence of a broad singlet at δ 12.00-13.00 ppm corresponding to NH which is exchangeable with deuterium oxide. Moreover the *S*-alkyl groups give a pattern in the ^{1}H-NMR spectra in accordance with the expected structures of such compounds (Table II).

On the other hand, 1-alkyl-2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazoles; compounds **4-7;$_{a-d}$** were prepared either through reaction of compounds **3a-d** with another

Table II. ^{1}H-NMR data of compounds **2**, **3a-d**, and **4-7a-d**

Comp. No.	R	R^1	^{1}H-NMR ($CDCl_3$, δ ppm)
2*	H	H	7.33-8.16 (4H; m, $C_6\underline{H}_4$) and 12.00 (2H; hump, N<u>H</u>).
3a*	CH_3	H	2.75 (3H; s, $SC\underline{H}_3$), 5.58 (1H; hump, N<u>H</u>), and 7.26-8.01 (4H; m, $C_6\underline{H}_4$).
3b	C_2H_5	H	1.45 (3H; t, $SCH_2C\underline{H}_3$), 2.90-3.58 (2H; q, $SC\underline{H}_2CH_3$), 7.20-8.00 (4H; m, $C_6\underline{H}_4$), and 12.00 (1H; hump, N<u>H</u>).
3c	allyl	H	3.87 (2H; d, $SC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.00-5.58 (2H; m, $SCH_2CH{=}C\underline{H}_2$), 5.83-6.63 (1H; m, $SCH_2C\underline{H}{=}CH_2$), 7.25-8.00 (4H; m, $C_6\underline{H}_4$), and 12.50 (1H; hump, N<u>H</u>).
3d	*n*-C_3H_7	H	1.07 (3H; t, $SCH_2CH_2C\underline{H}_3$), 1.60-2.21 (2H; m, $SCH_2C\underline{H}_2CH_3$), 3.26 (2H; t, $SC\underline{H}_2CH_2CH_3$), 7.26-8.03 (4H; m, $C_6\underline{H}_4$), and 12.50 (1H; hump, N<u>H</u>).
4a	CH_3	CH_3	2.78 (3H; s, $SC\underline{H}_3$), 3.95 (3H; s, $NC\underline{H}_3$), and 7.40-8.06 (4H; m, $C_6\underline{H}_4$)
4b	CH_3	C_2H_5	1.56 (3H; t, $NCH_2C\underline{H}_3$), 2.76 (3H; s, $SC\underline{H}_3$), 4.10-4.63 (2H; q, $NC\underline{H}_2CH_3$), and 7.26-8.05 (4H; m, $C_6\underline{H}_4$)
4c	CH_3	allyl	2.83 (3H; s, $SC\underline{H}_3$), 5.00 (2H; d, $NC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.18-5.65 (2H; m, $NCH_2CH{=}C\underline{H}_2$), 5.76-6.60 (1H; m, $NCH_2C\underline{H}{=}CH_2$), and 7.30-8.18 (4H; m, $C_6\underline{H}_4$).
4d	CH_3	*n*-C_3H_7	1.00 (3H; t, $NCH_2CH_2C\underline{H}_3$), 1.71-2.25 (2H; m, $NCH_2C\underline{H}_2CH_3$), 2.80 (3H; s, $SC\underline{H}_3$), 4.23 (2H; t, $NC\underline{H}_2CH_2$), and 7.20-8.03 (4H; m, $C_6\underline{H}_4$).
5a	C_2H_5	CH_3	1.50 (3H; t, $SCH_2C\underline{H}_3$), 3.05-3.50 (2H; q, $SC\underline{H}_2CH_3$), 3.90 (3H; s, $NC\underline{H}_3$), and 7.33-8.11 (4H; m, $C_6\underline{H}_4$).
5b	C_2H_5	C_2H_5	1.43-1.60 (6H; m, $SCH_2C\underline{H}_3$ & $NCH_2C\underline{H}_3$), 3.03-3.60 (2H; q, $SC\underline{H}_2CH_3$), 4.08-4.61 (2H; q, $NC\underline{H}_2CH_3$), and 7.28-8.06 (4H; m, $C_6\underline{H}_4$).
5c	C_2H_5	allyl	1.46 (3H; t, $SCH_2C\underline{H}_3$), 3.03-3.55(2H; q, $SC\underline{H}_2CH_3$), 4.86 (2H; d, $NC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.06-5.63 (2H; m, $NCH_2CH{=}C\underline{H}_2$), 5.76-6.50 (1H; m, $NCH_2C\underline{H}{=}CH_2$), and 7.26-8.10 (4H; m, $C_6\underline{H}_4$).
5d	C_2H_5	*n*-C_3H_7	0.95 (3H; t, $NCH_2CH_2C\underline{H}_3$), 1.46 (3H; t, $SCH_2C\underline{H}_3$), 1.73-2.43 (2H; m, $NCH_2C\underline{H}_2CH_3$), 3.03-3.56 (2H; q, $SC\underline{H}_2CH_3$), 4.23 (2H; t, $NC\underline{H}_2CH_2CH_3$), and 7.25-8.10 (4H; m, $C_6\underline{H}_4$).
6a	allyl	CH_3	3.83 (3H; s, $NC\underline{H}_3$), 3.93 (2H; d, $SC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.00-5.68 (2H; m, $SCH_2CH{=}C\underline{H}_2$), 5.77-6.47 (1H; m, $SCH_2C\underline{H}{=}CH_2$), and 7.10-8.10 (4H; m, $C_6\underline{H}_4$).
6b	allyl	C_2H_5	1.54 (3H; t, $NCH_2C\underline{H}_3$), 3.90 (2H; d, $SC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 4.05-4.64 (2H; q, $NC\underline{H}_2CH_3$), 4.98-5.57 (2H; m, $SCH_2CH{=}C\underline{H}_2$), 5.75-6.64 (1H; m, $SCH_2C\underline{H}{=}CH_2$), and 7.12-8.12 (4H; m, $C_6\underline{H}_4$).
6c	allyl	allyl	3.95 (2H; d, $SC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 4.92 (2H; d, $NC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.05-5.64 (4H; m, $SCH_2CH{=}C\underline{H}_2$ & $NCH_2CH{=}C\underline{H}_2$), 5.77-6.54 (2H; m, $SCH_2C\underline{H}{=}CH_2$ & $NCH_2C\underline{H}{=}CH_2$), 7.28-7.65 (3H; m, $C_6\underline{H}_4$), and 7.77-8.10 (1H; m, $C_6\underline{H}_4$).
6d	allyl	*n*-C_3H_7	1.05 (3H; t, $NCH_2CH_2C\underline{H}_3$), 1.73-2.43 (2H; m, $NCH_2C\underline{H}_2CH_3$), 3.92 (2H; d, $SC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 4.23 (2H; t, $NC\underline{H}_2CH_2CH_3$), 5.00-5.58 (2H; m, $SCH_2CH{=}C\underline{H}_2$), 5.85-6.65 (1H; m, $SCH_2C\underline{H}{=}CH_2$), and 7.20-8.00 (4H; m, $C_6\underline{H}_4$).
7a	*n*-C_3H_7	CH_3	1.08 (3H; t, $SCH_2CH_2C\underline{H}_3$), 1.48-2.24 (2H; m, $SCH_2C\underline{H}_2CH_3$), 3.28 (2H; t, $SC\underline{H}_2CH_2CH_3$), 3.90 (3H; s, $NC\underline{H}_3$), and 7.17-8.12 (4H; m, $C_6\underline{H}_4$).
7b	*n*-C_3H_7	C_2H_5	1.12 (3H; t, $SCH_2CH_2C\underline{H}_3$), 1.44-2.32 (5H; m, $NCH_2C\underline{H}_3$ & $SCH_2C\underline{H}_2CH_3$), 3.32 (2H; t, $SC\underline{H}_2CH_2CH_3$), 4.12-4.67 (2H; q, $NC\underline{H}_2CH_3$), and 7.28-8.22 (4H; m, $C_6\underline{H}_4$).
7c	*n*-C_3H_7	allyl	1.14 (3H; t, $SCH_2CH_2C\underline{H}_3$), 1.54-2.28 (2H; m, $SCH_2C\underline{H}_2CH_3$), 3.34 (2H; t, $SC\underline{H}_2CH_2CH_3$), 4.95 (2H; d, $NC\underline{H}_2CH{=}CH_2$, J = 8.50 Hz), 5.15-5.74 (2H; m, $NCH_2CH{=}C\underline{H}_2$), 5.80-6.54 (1H; m, $NCH_2C\underline{H}{=}CH_2$), and 7.15-8.17 (4H; m, $C_6\underline{H}_4$).
7d	*n*-C_3H_7	*n*-C_3H_7	1.06 (6H; t, $SCH_2CH_2C\underline{H}_3$ & $NCH_2CH_2C\underline{H}_3$), 1.68-2.40 (4H; m, $SCH_2C\underline{H}_2CH_3$ & $NCH_2C\underline{H}_2CH_3$), 3.25 (2H; t, $SC\underline{H}_2CH_2CH_3$), 4.2 (2H; t, $NC\underline{H}_2CH_2CH_3$), and 7.25-8.05 (4H; m, $C_6\underline{H}_4$).

* Compounds **2** and **3a** were measured in DMSO-d_6

equivalent of the different alkyl halides or by the reaction of compound **2** with two equivalents of the alkyl halides; in case of compounds **4a**, **5b**, **6c**, and **7d** (Scheme 1). Table I shows the physicochemical constants of the newly synthesized compounds **2**, **3-7**; **a-d**.

The structures of compounds **4-7**; **a-d** were confirmed by IR, ^{1}H-NMR spectrometry along with elemental analyses. The disappearance of the absorption band at 3410-3420 cm^{-1} (NH) in the IR spectra as well as the broad signal in the ^{1}H-NMR spectra are taken as proofs for introduction of the second alkyl groups. Moreover, compounds **4-7**; **a-d** gave patterns in the ^{1}H-NMR spectra in accordance with their expected structures (Table II).

Antimicrobial activities

Antibacterial activity

The newly synthesized compounds **2**, **3b**, **4-7**;**a-c** were tested for their antibacterial activity *in vitro* against *Bacillus cereus, Staphylococcus areus,* and *Micrococcus roseus* as representatives of Gram positive strains and *Serratia rodenii, Pseudomonas aeruginosa,* and *Escherichia coli* as representativs of Gram negative ones, using a reported method as described in the experimental part (William H, 1977). The results are cited in Table 3 and expressed as inhibition zones in mm.

Results of the antibacterial activity indicate that compound **2** (R = R^1 = H) is only active against *Staphylococcus areus* and *Serratia rodenii.* Where substitution with alkyl groups, (**3b** and **4a**; R = R^1 = CH_3 or R = CH_2CH_3, R^1 = H) afforded compounds that are completely inactive. On the other hand, substitution with higher alkyl groups or, in general, the disubstituted derivatives (**4b-d**, **5-7**; **a-d**; R = CH_3, C_2H_5, C_3H_5, *n*-C_3H_7, R^1 = C_2H_5, C_3H_5, *n*-C_3H_7) showed improved antibacterial activity.

Antifungal activity

Compounds **2**, **3b**, **4-7**;**a-c** were tested for their antifungal activity *in vitro* using the standard agar disk diffusion method (William H, 1977) against *Trichophyton rubrum* (Castellani) Sabouraud, *Candida albicans* (Robin) Berkhout, *Microsporum canis* Gruby, *Aspergillus flavus* Thom, *Fusarium oxysporum* Schlechtendal and *Penicillium expansum* Link. The results of the antifungal activity are given in table 3 and expressed as inhibition zones in mm.

Again, the results of the antifungal activity indicate that compound **2** (R = R^1 = H) is only active against *Candida albicans* and *Fusarium oxysporum.* Substitution with lower alkyl groups (**3b** and **4a**; R = R^1 = CH_3 or R = CH_2CH_3, R^1 = H) shows a slight increase in the antifungal activity. On the other hand, the disubstituted derivatives (**4b-d**, **5-7**; **a-d**; R = CH_3, C_2H_5, C_3H_5, *n*-C_3H_7, R^1 = C_2H_5, C_3H_5, *n*-C_3H_7) exhibited improved antifungal activity.

The results in table 3 indicate that compounds **4c**, **5b**, **5c**, **6b**, and **6c** are the most active derivatives as

Table III. Anti-microbial activity of the compounds **2**, **3b**, **4-7**; **a-c**

Organism / Compds.	Zones of inhibition (mm.) against: Bactertia G +ve: Bacillus Cereus	Staph. areus	Micrococcus lotus	Bactertia G -ve: Esch. coli	Serratia rodenii	Pseud. aerginosa	Fungi: Candida albicans	Trichoph. rubrum	Microsporium canis	Asperg. flavus	Penicillium chrys	Fusar. Oxysp.
Ampicillin	15	14	15	16	15	16	NT	NT	NT	NT	NT	NT
Fluconazole	NT	NT	NT	NT	NT	NT	20	18	10	0*	0	0
2	0	7	0	0	8	0	8	0	0	0	0	10
3b	0	0	0	0	0	0	10	0	0	0	0	14
4a	0	0	0	0	0	6	0	0	0	0	8	0
4b	6	6	12	0	0	8	12	13	12	8	9	8
4c	15	8	6	0	6	8	8	10	10	9	8	6
5a	10	8	7	10	0	0	8	0	8	6	9	11
5b	0	9	14	0	8	0	8	0	8	6	9	16
5c	7	8	12	7	6	0	8	10	7	7	10	18
6a	6	0	0	8	0	0	12	7	8	0	0	8
6b	10	9	6	8	0	0	15	12	10	8	10	10
6c	15	12	0	0	7	0	10	8	8	10	12	10
7a	6	12	6	0	0	0	0	0	0	9	6	0
7b	8	15	8	12	6	0	0	15	12	10	10	10
7c	8	7	8	0	0	0	15	8	10	6	6	8

*N.T = Not tested. 0 = No inhibition.

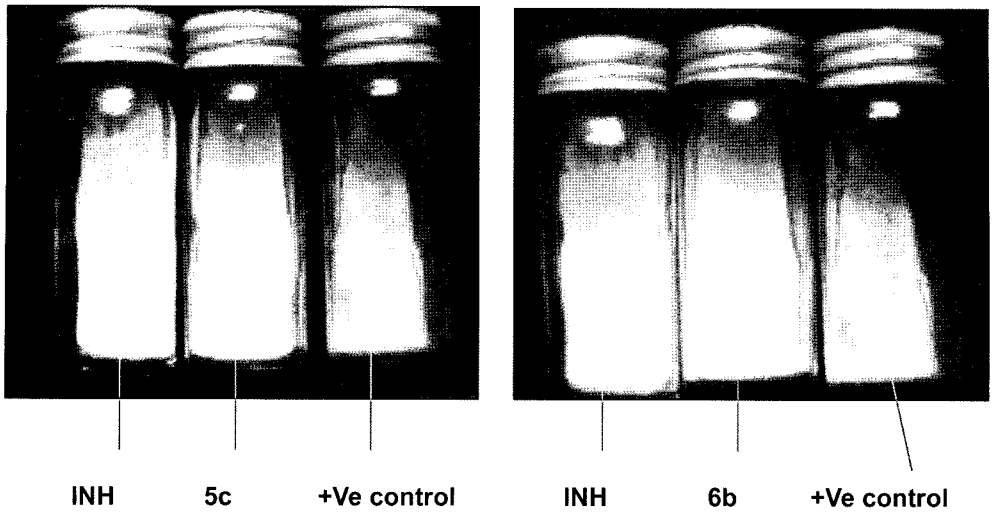


Fig. 1. Representative photos for the antitubercular activity

antibacterial and antifungal among the tested compounds. Accordingly; they were subjected for further exploration to evaluate their anti-tubercular activity.

Antitubercular activity

Five of the newly synthesized compounds **4c**, **5b**, **5c**, **6b**, and **6c** were selected and tested for their anti-tubercular activity using Rist and Grosset proportion method (Canetti *et al.,* 1983) in a concentration of 10 μMol in comparison to **INH** as a reference drug. All the tested compounds showed equipotent activity in comparison to **INH.** Observation of growth for bacilli of the microorganisms was observed for a period of six weeks, the control showed growth of bacilli after six weeks, while the test compounds as well as the reference drug gave no growth till a period of eight weeks, Fig. 1.

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