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Synthesis and Antimicrobial Activity of New Substituted Anilinobenzimidazoles

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A series of benzimidazole derivatives carrying different heterocycles such as 1,2,3-thiadiazole, 1,3,4-thiadiazole, thiazolidine, 2,3-dihydro-thiazole, 1,3,4-oxadiazole, semicarbazone and substituted thiosemi-carbazones were synthesized. Also a series of 1-methylbenzimidazole carrying hydroxy ethyl-amide, substituted sulfonyl hydrazide and benzoyl hydrazide from aminobenzoyl group at position 2 of 1-methylbenzimidazole were synthesized. The antimicrobial evaluation of some of the new compounds was carried out.

Key words: 1,2,3-Thiadiazol, Oxadiazol, Semicarbazone, Substituted thiosemicarbazones, Sulfonyl hydrazide, Benzoyl hydrazide

INTRODUCTION

Benzimidazole is one of the most important heterocyclic rings which cover a versatile class of compounds possessing different pharma-cological activities. 2-[p-4-Aryl-3-cyano-2-oxo-1(H)-pyridin-6-yl)anilino]-benzimidazoles [I] were found to display in vitro antibacterial and antifungal activities (Omar *et al.*, 1995). On the other hand, anti-6-{[(hydroxyimino)phenyl]methyl}-1-[(1-methylethyl)sulphonyl]-benzimidazol-2-amin [II] is a potent inhibitor of rhinovirus multiplication (Wikel *et al.*, 1980). Also, 5,6-dichloro-2-(benzylthio)-1-β-D-ribofuranosyl benz-imidazole [III] was found highly active against human cytomegalovirus (Zou *et al.*, 1997). Its activity was more potent than that of acyclovir and foscarent (Mohamed, 2001).

Also various semicarbazides and thiosemicarbazides have been reported to show antibacterial and antifungal activities (Dewani *et al.*, 1973). On the other hand thiazole and 1,2,3-thiadiazole have been reported to show antibacterial and antifungal activities (Singh *et al.*, 1981 and Fahmy *et al.*, 2001). It was reported that (Eislager *et al.*, 1981), the amino side chain which incorporated to certain heterocyclic ring system increases the pharmacological activities such as antimicrobial, antimalarial and DNA intercalation.

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Moreover, sulphonyl hydrazides were reported as useful fungicides and bacteriocides (Fathalla and El-Bazza, 1991). It was also reported to affect Ehrlich ascites tumor cell and show antiaflatoxigenic property (Fathalla and El-Bazza, 1991; Fathalla and Rizk, 1993).

Furthermore, 1,3,4-oxa- or thiadiazoles were reported to display broad spectrum of biological activities such as antibacterial and antifungal (Ferandes *et al.*, 1986; Singh *et al.*, 1981). We report here the synthesis of some derivatives of anilinobenzimidazoles containing the above mentioned moieties for their antimicrobial activity evaluation.

MATERIALS AND METHODS

All melting points were uncorrected and were taken in open capillaries on a gallenkamp apparatus. Infrared spectra were determined in KBr on a Perkin Elmer Model-137 infracord. The ^{1}H-NMR spectra were measured in DMSO-d6 using Jeol EX-270 MHz spectrometer. The Mass spectra were recorded on GCMS-QP 1000 EX Sehimadzu gas chromatography US apparatus.

Synthesis of compounds

2-(*p*-Acetylanilino)-1-methyl benzimidazole (1b). A mixture of 2-chloro-1-methylbenzimidazole (1.66 g, 0.01 mole) and *p*-aminoacetophenone (1.30 g, 0.01 mole) in absolute ethanol (20 mL) containing few drops of concentrated hydrochloric acid was refluxed for 4 h. After cooled, the formed precipitate was filtered off, washed with cold

dilute ammonium hydroxide solution followed by water and recrystallized to give compound **1b** (Table I, II).

2-[*p*-(ω-Aminosemicarbazono-α-ethyl)anilino]-1-(H) or methyl benzimidazoles (2a,b). To a mixture of semicarbazide hydrochloride (1.11 g, 0.01 mole), crystalline sodium acetate (0.82 g, 0.01 mole) water (10 mL) was added compound **1a** or **1b** (0.01 mole) in ethanol (50 mL). The mixture was heated to reflux for 3 h on water bath The reaction mixture was cooled and the formed solid was filtered off, and recrystallization gave compounds **2a**, **b**, respectively. (Table I, II).

2-[*p*-(ω-Substituted thiosemicarbazono-α-ethyl)-anilino]-1-(H) or methyl benzimidazoles (3a-g). To a mixture of thiosemicarbazide or substituted thiosemicarbazide (0.01 mole) and compound **1a**, **b** (0.01 mole) in absolute ethanol (50 mL) was added few drops of hydrochloric acid. The reaction mixture was refluxed for 4-7 h and then concentrated. The formed precipitate was filtered off, washed with water and recrystallized to give compounds **3a-g**, respectively. (Table I, II).

2-[*p*-(1,2,3-Thiadiazol-4-yl)anilino]-1-(H) or methyl benzimidazoles (4a, b). Thionyl chloride (5 mL, 0.04 mole) was added slowly to semicarbazone or thiosemicarbazone (0.01 mole) and the reaction mixture was refluxed for 6 h After cooled, the reaction mixture was poured into ice-cold saturated sodium bicarbonate solution. The formed precipitate was filtered off, washed with water and the crude product was crystallized from the proper solvent to give the corresponding thiadiazolo compounds **4a**, **b**, respectively. (Table I, II).

2-{*p*-[3-Acetyl-5-(N-substituted acetamido)-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl]anilino}-1-(H) or methyl benzimidazoles (5a-c). A mixture of compound **3a**, **d**, **f** (0.01 mole) and acetic anhydride (5 mL) was heated to reflux for 4 h The reaction mixture was concentrated under reduced pressure, cooled and then poured into ice-cold water. The formed precipitate was filtered off and recrystallization gave compounds **5a-c**, respectively. (Table I, II)

2-{*p*-[2-(3-Substituted-4-oxothiazolidin-2-ylidene)-hydrazonoethyl] anilino}-1-(H) or methyl benzimidazoles (6a-c). To a solution of substituted thiosemicarbazone **3a**, **d**, **g** (0.01 mole) and ethyl bromoacetate (1.1 mL, 0.01 mole) in absolute ethanol (20 mL) was heated to reflux for 6 h The reaction mixture was cooled and the precipitated product was filtered off and recrystallized to give compounds **6a-c**. (Table I, II).

2-{*p*-[2-(3-p-Chlorophenyl-4-phenyl-2,3-dihydrothiazol-2-ylidene)-hydrazonoethyl] anilino}-1-(H) or methyl benzimidazoles (7a, b). To a solution of compound **3c**, **f** (0.01 mole) in absolute ethanol (20 mL), the phenacyl bromide (0.01 mole) was added. The reaction mixture was heated to reflux for 8 h, concentrated under reduced pressure. After cooled, the formed precipitate was isolated by filtration and recrystallized to give compounds **7a**, **b**. (Table I, II).

***p*-[*N*-(1-Methylbenzimidazol-2-yl)amino]ethylbenzoate (8).** A mixture of 2-chloro-1-methyl benzimidazole (1.66 g, 0.01 mole) and 4-aminobenzoic acid ethyl ester (1.65 g, 0.01 mole) was refluxed in absolute ethanol (20 mL) containing few drops of concentrated hydrochloric acid for 5 h The reaction mixture was concentrated and the incoming white solid was isolated by filtration, dried under vaccum and recrystallized to give compound **8**. (Table I, II).

***p*-[*N*-(1-Methylbenzimidazol-2-yl)amino]benzoic acid hydrazide (9).** To a warm solution of 98% hydrazine hydrate (10 mL), benzoate **8** (2.95 g, 0.01 mole) was added portionwise. The reaction mixture was refluxed with stirring for 3 h after cooled the incoming solid was isolated by filtration and Recrystallized to give to compound **9**. (Table I, II).

2-{*p*-[*N*-(Substituted hydroxyethyl)carbamoyl]anilino}-1-methyl benzimidazoles (10a, b). A mixture of ester compound **8** (2.95 g, 0.01 mole) and the appropriate substituted hydroxyethylamine (0.08 mole), 2-hydroxyethylamine and bis-(2-hydroxyethyl)amine was heated under reflux for 6h at 130°C. The reaction mixture was evaporated under reduced pressure, the residue was solidified with acetone/petroleum ether (40-60) [1:3]. The formed precipitate was collected by filteration, washed with petroleum ether. Recrystallization gave compounds **10a**, **b**, respectively. (Table I, II).

N^1-{*p*-[*N*-(1-Methylbenzimidazol-2-yl)aminobenzoyl]}-N^2-substituted sulphonyl hydrazides (11a-c). To a solution of carbohydrazide (**9**) (2.81 g, 0.01 mole) in absolute ethanol (30 mL), the sulphonyl chlorides (0.01 mole), *p*-bromophenyl-sulphonyl chloride, *p*-tolylsulphonyl chloride and/or uracil-5-sulphonyl chloride was added. The reaction mixture was refluxed for 4-6 h and cooled. The formed solid was isolated by filtration, dried and recrystallized from the proper solvent to give **11a-c** (Table I, II).

N^1-{*p*-[*N*-(1-Methylbenzimidazol-2-yl)aminobenzoyl]}-N^2-substituted benzoyl hydrazides (12a, b). To a solution of carbohydrazide (**9**) (2.81 g, 0.01 mole) in dry pyridine (10 mL), the acid chloride (0.01 mole), benzoyl chloride and/or *p*-chlorobenzoyl chloride was added. The reaction mixture was heated to reflux for 4 h, cooled and poured into cruched ice. The formed solid was isolated by filtration, washed with water and petroleum ether and dried. The residue was recrystallized to give **12a**, **b** respectively. (Table I, II).

2-[*p*-(2-*p*-Chlorophenyl-1,3,4-oxadiazol-2-yl)anilino]-1-methyl-benzimidazole (13). Method A: A mixture of compound **12b** (4.19 g, 0.01 mole) and polyphosphoric acid (20 g), was heated in oil bath at 140-160°C for 4 h.

Table I. Physical and analytical data of the prepared compounds

Comp. No	M.P. (°C) Solvent for recryst.	Yield (%)	Mol. formula. Mol. wt	Analysis % (calc./found) C	H	N	S
1b	215 EtOH	85	$C_{16}H_{15}N_3O$ (265.30)	72.43 72.15	5.69 6.00	15.83 15.58	
2a	302 EtOH	69	$C_{16}H_{16}N_6O$ (308.38)	62.32 62.09	5.23 5.32	27.25 27.21	
2b	108 EtOH	72	$C_{17}H_{18}N_6O$ (322.36)	63.33 63.21	5.62 5.50	26.07 26.00	
3a	295 MeOH	80	$C_{16}H_{16}N_6S$ (324.40)	59.23 59.21	4.97 4.99	25.91 25.80	
3b	260 EtOH	75	$C_{17}H_{18}N_6S$ (338.47)	60.34 60.20	5.36 5.51	24.84 24.79	
3c	290 MeOH	78	$C_{22}H_{19}ClN_6S$ (434.93)	60.75 60.54	4.41 4.46	19.35 19.33	
3d	260 EtOH	85	$C_{17}H_{18}N_6S$ (338.42)	60.33 60.33	5.36 5.40	24.83 24.62	
3e	240 MeOH	82	$C_{18}H_{20}N_6S$ (352.45)	61.33 61.19	5.71 5.69	23.84 23.80	
3f	230 EtOH	75	$C_{23}H_{21}ClN_6S$ (448.96)	61.53 61.48	4.71 4.71	18.72 18.55	
3g	200 MeOH	55	$C_{23}H_{28}N_6S$ (420.56)	65.68 65.42	6.71 6.80	19.98 19.55	
4a	235 EtOH	59	$C_{15}H_{11}N_5S$ (293.34)	61.41 61.40	3.78 3.87	23.87 23.60	10.92 10.88
4b	218 MeOH	52	$C_{16}H_{13}N_5S$ (307.36)	62.50 62.48	4.26 4.31	22.78 22.61	10.43 10.30
5a	180 EtOH/H_2O	70	$C_{20}H_{20}N_6O_2S$ (408.47)	58.80 58.72	4.93 4.95	20.57 20.35	
5b	190 EtOH/H_2O	72	$C_{21}H_{22}N_6O_2S$ (422.50)	59.69 59.66	5.24 5.27	19.89 19.79	
5c	120 EtOH/H_2O	63	$C_{27}H_{25}ClN_6O_2S$ (533.03)	60.83 60.62	4.73 4.90	15.76 15.51	
6a	210 EtOH	69	$C_{18}H_{16}N_6OS$ (364.42)	59.32 59.25	4.43 4.45	23.06 23.00	
6b	180 EtOH	65	$C_{19}H_{18}N_6OS$ (378.44)	60.29 60.21	4.79 4.79	22.21 22.00	
6c	309 EtOH	55	$C_{25}H_{28}N_6OS$ (460.58)	65.18 65.08	6.12 6.21	18.25 18.10	
7a	250 EtOH/H_2O	63	$C_{30}H_{23}ClN_6S$ (535.04)	67.34 67.13	4.33 4.50	15.70 15.50	
7b	200 EtOH/H_2O	65	$C_{31}H_{25}ClN_6S$ (549.07)	67.81 67.61	4.59 4.65	15.31 15.15	
8	190 EtOH	85	$C_{17}H_{17}N_3O_2$ (295.33)	69.13 69.03	5.80 5.96	14.23 14.03	
9	221 EtOH/H_2O	95	$C_{15}H_{15}N_5O$ (281.31)	64.04 63.90	5.37 5.50	24.99 24.71	
10a	180 EtOH	65	$C_{17}H_{18}N_4O_2$ (310.34)	65.79 65.30	5.85 5.99	18.05 18.00	
10b	150 EtOH	62	$C_{19}H_{22}N_4O_3$ (354.40)	64.45 64.29	6.26 6.36	15.81 15.57	
11a	250 DMF/H_2O	80	$C_{21}H_{18}BrN_5O_3S$ (500.36)	50.41 50.20	3.63 3.90	13.99 13.70	6.41 6.35
11b	238 DMF	85	$C_{22}H_{21}N_5O_3S$ (435.49)	60.67 60.50	4.86 4.90	16.08 16.00	7.36 7.00
11c	320 DMF	67	$C_{19}H_{17}N_7O_5S$ (455.45)	50.10 49.90	3.76 3.81	21.53 21.20	7.04 6.82
12a	228 EtOH	75	$C_{22}H_{19}N_5O_2$ (385.41)	68.56 68.30	4.96 4.99	18.17 18.02	

Table I. Continued

Comp. No	M.P. (°C) Solvent for recryst.	Yield (%)	Mol. formula. Mol. wt	Analysis % (calc./found) C	H	N	S
12b	255 EtOH	69	$C_{22}H_{18}ClN_5O_2$ (419.88)	62.93 62.80	4.32 4.40	16.68 16.49	
13	310 MeOH	51	$C_{22}H_{16}ClN_5O$ (401.84)	65.75 65.60	4.01 4.21	17.43 17.08	
14	295 DMF/H_2O	55	$C_{22}H_{16}ClN_5S$ (417.90)	63.23 63.04	3.86 3.98	16.76 16.49	7.67 7.95

The reaction mixture was poured into cruched ice, and left for 1h The incoming solid was isolated by filtration, washed with water and then air dried. The residue was recrystallized to give **13** (Table I, II).

Method B: A mixture of **12b** (4.19 g, 0.01 mole) in the concentrated sulfuric acid (10 mL, 98%) was heated at

Table II. Spectral data for prepared compounds

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6, 270 MHz, δ ppm), MS m/z (%)
1b	IR: 3490 (NH), 1720 (C=O) and 1668 (C=N). **^{1}H-NMR** (DMSO-d_6): 2.55 (s, 3H, $COCH_3$), 3.80 (s, 3H, CH_3-N), 7.1-8.00 (m, 8H, Ar-protons) and 9.40 (s, 1H, NH, exchangeable with D_2O). **MS**: M^+, 265 (100).
2a	IR: 3490, 3320 (NH_2, NH), 1664 (C=O) and 1620 (C=N). **^{1}H-NMR** (DMSO-d_6): 2.35 (s, 3H, C-CH_3), 6.70 (s, 2H, NH_2), 7.30-8.00 (m, 8H, Ar-protons) and at 8.30, 9.50, 11.70 (3s, 3H, 3NH) NH_2, NH exchangeable with D_2O. **MS**: M^+, 308 (3), 250 (100), 208 (76.06), 132 (8.5), 118 (6.85), 104 (14).
2b	**^{1}H-NMR** (DMSO-d_6): 2.30 (s, 3H, C-CH_3), 3.80 (s, 3H, N-CH_3), 6.60 (s, 2H, NH_2), 7.30-8.00 (m, 8H, Ar-protons) and 10.25, 11.45 (2s, 2H, 2NH), NH, NH_2 exchangeable with D_2O.
3a	IR: 3440, 3240 (NH_2, NH), 2920 (CH), 1660 (C=N) and 1080 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.30 (s, 3H, CH_3), 7.25-8.10 (m, 9H, 8Ar-protons and NH of benzimidazole), 8.35 (s, 2H, NH_2) and 10.30, 11.75 (2s, 2H, 2NH) NH_2, NH exchangeable with D_2O. **MS**: M^+, 324 (16), 307 (100).
3b	IR: 3197 (NH), 2980 (CH), 1660 (C=N) and 1052 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.30 (s, 3H, C-CH_3), 3.05 (s, 3H, N-CH_3), 7.30-8.15 (m, 8H, Ar-protons) and 8.55, 10.25, 11.75, 13.25 (4s, 4H, 4NH, exchangeable with D_2O).
3c	IR: 3382 (NH), 2960 (CH), 1654 (C=N), 1089 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.40 (s, 3H, C-CH_3), 7.30-8.20 (m, 12H, Ar-protons) 10.75, 11.65 (2s, 2H, 2NH) and 13.20 (brs, 2H, 2NH) NH exchangeable with D_2O. **MS**: 307 (1.36), 249 (7.62), 208 (3.64), 171 (10.21), 169 (33.65), 129 (32.6), 127 (100).
3d	IR: 3430, 3200 (NH_2, NH), 2960 (CH), 1640 (C=N) and 1090 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.40 (s, 3H, C-CH_3), 3.80 (s, 3H, N-CH_3), 7.30-8.40 (m, 10H, 8Ar-protons and NH_2) and 10.25, 11.45 (2s, 2H, 2NH).
3e	IR: 3340 (NH), 2813 (CH), 1640 (C=N) and 1099 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.30 (s, 3H, C-CH_3), 3.10 (s, 3H, NH-CH_3), 3.80 (s, 3H, N-CH_3), 7.30-8.00 (m, 8H, Ar-protons) and 8.40, 10.10, 11.30 (3s, 3H, 3NH, which exchangeable with D_2O).
3f	IR: 3350 (NH), 2927 (CH), 1659 (C=N) and 1197 (C=S).

Table II. Continued.

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6, 270 MHz, δ ppm), MS m/z (%)
3g	IR: 3360 (NH), 2958 (CH), 1646 (C=N) and 1114 (C=S). **^{1}H-NMR** (DMSO-d_6): 1.90-1.10 (m,10H, 5-CH_2 of cyclohexyl ring), 2.35 (s, 3H, C-CH_3), 3.80 (s, 3H, N-CH_3), 4.25 (m, 1H, CH of cyclohexyl ring), 7.30-8.05 (m, 9H, 8Ar-protons and NH) and 10.20, 11.10 (2s, 2H, 2NH), NH exchangeable with D_2O. MS: M^+, 420 (0.16), 321 (0.88), 263 (1.73), 222 (100), 141 (2.06), 98 (12.07), 83 (16.54).
4a	IR: 3080 (NH), 1650 (C=N) and 1600 (C=C). **^{1}H-NMR** (DMSO-d_6): 7.25-8.35 (m, 9H, 8Ar-protons and NH of benzimidazole), 9.55 (s, 1H, CH of thiadiazole ring) and 11.75 (s, 1H, NH of *p*-aminophenyl group) NH exchangeable with D_2O. **MS**: M^+, 293 (40), 176.2 (100).
4b	IR: 3070 (NH), 1645 (C=N) and 1600 (C=C). **^{1}H-NMR** (DMSO-d_6): 3.78 (s, 3H, N-CH_3), 7.25-8.35 (m, 8H, Ar-protons), 9.55 (s, 1H, CH of thiadiazole ring) and 11.70 (s, 1H, NH of *p*-aminophenyl group, which exchangeable with D_2O).
5a	IR: 3367 (NH), 2930 (CH), 1690, 1675 (two C=O) and 1620 (C=N). **^{1}H-NMR** (DMSO-d_6): 2.00, 2.20, 2.30 (3s, 9H, 2CO-CH_3 and C-CH_3), 7.10-7.55 (m, 9H, 8Ar-protons and NH of benzimidazole) and 11.65, 12.50 (2s, 2H, 2NH) NH exchangeable with D_2O). **MS**: M^+, 408 (12.10), 307 (100).
5b	IR: 3440 (NH), 2935 (CH), 1700, 1675 (two C=O) and 1620 (C=N). **^{1}H-NMR** (DMSO-d_6): 2.05, 2.15, 2.25 (3s, 9H, 2CO-CH_3 and C-CH_3), 3.70 (s, 3H, N-CH_3), 7.20-7.70 (m, 8H, Ar-protons) and 11.60, 12.25 (2s, 2H, 2NH which exchangeable with D_2O). **MS**: M^+, 422 (48.10), 321 (100).
5c	IR: 3350 (NH), 2930 (CH), 1690, 1676 (two C=O) and 1600 (C=C). **MS**: M^+, 532, 534 (7.02), (2.81) and 364 (100).
6a	**^{1}H-NMR** (DMSO-d_6): 2.4 (s, 3H, C-CH_3), 3.85 (s, 2H, CH_2 of thiazolidinone ring, 7.25-8.00 (m, 8H, Ar-protons) and 11.25, 12.00, 13.00 (3s, 3H, 3NH, which exchangeable with D_2O). **MS**: M^+, 364 (100).
6b	IR: 3260 (NH), 2895 (CH), 1720 (C=O of thiazolidinone ring) and 1560 (C=N). MS: M^+, 378 (40), 117 (100).
6c	IR: 3400 (NH), 2927 (CH), 1718 (C=O) and 1640 (C=N). **^{1}H-NMR** (DMSO-d_6): 1.15-1.80 (m, 10H, cyclohexyl protons), 2.40 (s, 3H, C-CH_3), 3.75 (s, 3H, N-CH_3), 3.90 (s, 2H, CH_2 of thiazolidinone), 4.25-4.45 (m, 1H, CH of cyclohexyl), 7.25-8.00 (m, 8H, Ar-protons) and 10.40 (s, 1H, NH, which exchangeable with D_2O). **MS**: M^+, 460 (94), 378 (100).
7a	IR: 3330 (NH), 2970 (CH), 1660 (C=N) and 744 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 2.20 (s, 3H, CH_3), 6.65 (s, 1H, CH of thiazoline ring), 7.15-7.95 (m, 17H, Ar-protons) and 11.30, 13.10 (2s, 2H, 2NH which exchangeable with D_2O). **MS**: M^+, 534, 536 (95.05), (42.47), 234 (100).
7b	IR: 3400 (NH), 1640 (C=N) and 696 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 2.25 (s, 3H, C-CH_3), 3.80 (s, 3H, N-CH_3), 6.65 (s, 1H, CH of thiazoline ring), 7.15-8.15 (m, 17H, Ar-protons) and 10.85 (s, 1H, NH which exchangeable with D_2O). MS: M^+, 548, 550 (52), (18), 265 (100).
8	IR: 3338 (NH), 1719 (C=O) and 1245 (C-O). **^{1}H-NMR** (DMSO-d_6): 1.30 (t, 3H, CH_2-CH_3), 3.75 (s, 3H, N-CH_3), 4.25 (q, 2H, CH_2-CH_3), 7.10-8.10 (m, 8H, Ar-protons) and 9.40 (s, 1H, NH which exchangeable with D_2O). MS: M^+, 295 (100).
9	IR: 3284, 3200 (NH_2, NH) and 1640 (C=O). **^{1}H-NMR** (DMSO-d_6): 3.70 (s, 3H, N-CH_3), 4.49 (brs, 2H, NH_2), 7.05-7.95 (m, 8H, Ar-protons) and 9.20, 9.62 (2s, 2H, 2NH) NH_2, NH exchangeable with D_2O. MS: M^+, 281 (33), 250 (100).

Table II. Continued

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6, 270 MHz, δ ppm), MS m/z (%)
10a	IR: 3440 (OH), 3295 (NH), 2945 (CH) and 1630 (C=O). **^{1}H-NMR** (DMSO-d_6): 3.35 (t, 2H, CH_2-NH), 3.50 (t, 2H, CH_2OH), 3.80 (s, 3H, N-CH_3), 4.75 (s, 1H, OH), 7.05-8.00 (m, 9H, 8Ar-protons and NH) and 9.25 (s, 1H, NH) NH, OH exchangeable with D_2O. MS: M^+, 310.1 (88.61), M^++1, 311.1 (100).
10b	IR: 3400 (br, OH, NH), 2960 (CH) and 1646 (C=O). **^{1}H-NMR** (DMSO-d_6): 3.05 (t, 4H, $2CH_2$-N), 3.65 (t, 4H, $2CH_2$OH), 3.85 (s, 3H, N-CH_3), 5.25 (brs, 2H, 2OH), 7.10-8.05 (m, 8H, Ar-protons) and 8.75 (brs, 1H, NH), NH, OH exchangeable with D_2O. MS: M^+, 354 (0.35), 250 (100), 222 (11.88), 208 (14.5), 146 (7.63), 131 (9.7).
11a	IR: 3360, 3200, 3100 (3NH), 1650 (C=O), 1600 (C=C), 1350, 1166 (SO_2). MS: 266 (4.14), 250 (100), 237 (13.40), 235 (13.96), 222 (32.66), 221 (32.66), 219 (13.1), 157 (15.37), 155 (14.99).
11b	IR: 3360, 3320, 3160 (3NH), 1665 (C=O), 1600 (C=C), 1330, 1160 (SO_2). **^{1}H-NMR** (DMSO-d_6): 2.35 (s, 3H, CH_3-Ph), 3.75 (s, 3H, N-CH_3), 7.10-7.95 (m, 12H, Ar-protons), 9.25, 9.75, 10.40 (3s, 3H, 3NH, which exchangeable with D_2O). MS: M^+, 435 (1.27), 266 (4.75), 250 (88.01), 251 (100), 222 (19.88), 155 (19.33), 91 (19.95).
11c	IR: 3421, 3220, 3160 (5NH), 1640, 1660, 1680 (3C=O), 1600 (C=C), 1330, 1160 (SO_2). MS: 266 (9.33), 250 (100), 111 (20.12), 83 (16.34).
12a	IR: 3374, 3240 (3NH), 3045 (CH), 1686, 1645 (2C=O). **^{1}H-NMR** (DMSO-d_6): 3.75 (s, 3H, N-CH_3), 7.10-8.00 (m, 13H, Ar-protons) and 9.35, 10.30, 10.50 (3s, 3H, 3NH, which exchangeable with D_2O). MS: M^+, 385 (11.34), 250 (100).
12b	IR: br. Band centered at 3255 (3NH), 1640, 1625 (2C=O), 736 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 3.75 (s, 3H, N-CH_3), 7.05-7.90 (m, 12H, Ar-protons) and 9.25, 9.80, 10.50 (3s, 3H, 3NH, which exchangeable with D_2O).
13	IR: 3446 (NH), 3054 (CH), 1680, 1645 (C=N) and 760 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 3.80 (s, 3H, N-CH_3), 7.00-8.10 (m, 12H, Ar-protons) and 10.6 (s, 1H, NH, which exchangeable with D_2O). MS: M^+, 403 (9.50), 401 (29.90), 141 (32.5), 139 (100).
14	IR: 3413 (NH), 3054 (CH), 1600 (C=C), 740 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 3.80 (s, 3H, N-CH_3), 7.00-7.90 (m, 12H, Ar-protons) and 10.50 (s, 1H, NH, which exchangeable with D_2O).

120°C for 3h The reaction mixture was poured into cruched ice with stirring. The incoming solid was isolated by filtration, washed with water to remove acid and air dried, to give **13** (55% yield), identical to authentic sample from method A. (m.p. and TLC comparison).

2-[*p*-(2-*p*-Chlorophenyl-1,3,4-thiadiazol-5-yl)anilino]-1-methyl-benzimidazole (14). A mixture of compound **12b** (4.19 g, 0.01 mole) and phosphorus pentasulfide (0.03 mole) fused in oil bath at 140-160°C for 3 h. The reaction mixture was cooled and neutralized with sodium hydroxide to remove the excess of phosphorus pentasulfide. The formed solid was isolated by filtration, washed with water and the residue was recrystallized to give **14** (Table I, II).

Antimicrobial Activity

Some of the newly synthesized compounds have been tested for their antimicrobial activity against one strain of Gram positive, Gram negative bacteria, Fungi and Yeast. We used gentamycine and ampicelline as references.

MATERIALS AND METHOD

1-Media

a- Neutral agar for bacteria and yeast: It consists of beef extract (1 gm/l), yeast extract (2 g/l), pepton (5 gm/l), sodium chloride (5 gm/l) and agar (15 gm/l).

b- Dox. for fungi: It consists of sodium nitrate (2.0 gm/l); glucose (15.0 gm/litre); KH_2PO_4 (1.0 gm/litre); Magnesium sulfate. $7H_2O$ (0.5 gm/l), Potassium chloride (0.5 gm/l); Ferrous sulfate. $7H_2O$ (0.007 gm/l) and agar (30.0 gm/l).

Table III. Preliminary screening test for some newly synthezied compounds

Comp. No.	*Bacillus subtilis*	*Escherichia Coli*	*Aspergillus niger*	*Candida albicans*
Gentamycine	+++	+++	-	-
Ampicelline	+++	+++	-	-
2b	+++	+	++	-
3a	++	+	-	-
3b	++++	+	-	-
3c	-	-	-	-
3d	-	+++	+	-
3e	-	-	+	-
3f	+	-	-	-
3g	-	-	+	+
4a	+++	-	+	+
4b	++++	++++	-	++
5a	++	-	+	-
5b	-	-	-	-
6a	+++	++	-	-
6b	+++	-	-	-
6c	-	+	-	-
7a	-	-	-	+
7b	-	-	-	++
8	++++	+++	+	-
9	++++	++++	-	-
10a	-	-	-	-
10b	-	+	-	+
11a	++	-	-	-
11b	-	-	-	-
11c	++++	++++	++++	++++
14	-	+++	-	-

Highly active	++++	(Inhibition zone ~30 mm)
Active	+++	(Inhibition zone 18-20 mm)
Moderatly active	++	(Inhibition zone 10-15 mm)
Slightly active	+	(Inhibition zone 7-10 mm)
Inactive:	-	(Inhibition zone < 7 mm)

Method

The antimicrobial screening of newly prepared compounds was performed according to the disk diffusion method (Fahmy *et al.*, 2001).

Whatman No.1 filter paper disk of 5 mm diameter were sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregenated with different compounds (500 μg/disk). The suitable media (neutral agar for bacteria, Dox for Fungi) were surface inoculated uniformly from the broth culture of the tested microorganisms. The imperg-nated disks were placed on the medium suitably spaced apart and the plates were incubated at 5°C for 1 h to permit good diffusion and then transferred to an incubator at 37°C for 24 h (bacteria) at 28°C for 72 h (yeast and fungi). Then the inhibition zones caused by the various compounds on the microorganisms were examned. The results of the preliminary screening test listed in Table III.

Results of antimicrobial activity

Based on Table III, it was found that most of the tested compounds showed activity against *B. subtilis* and *E. coli*.

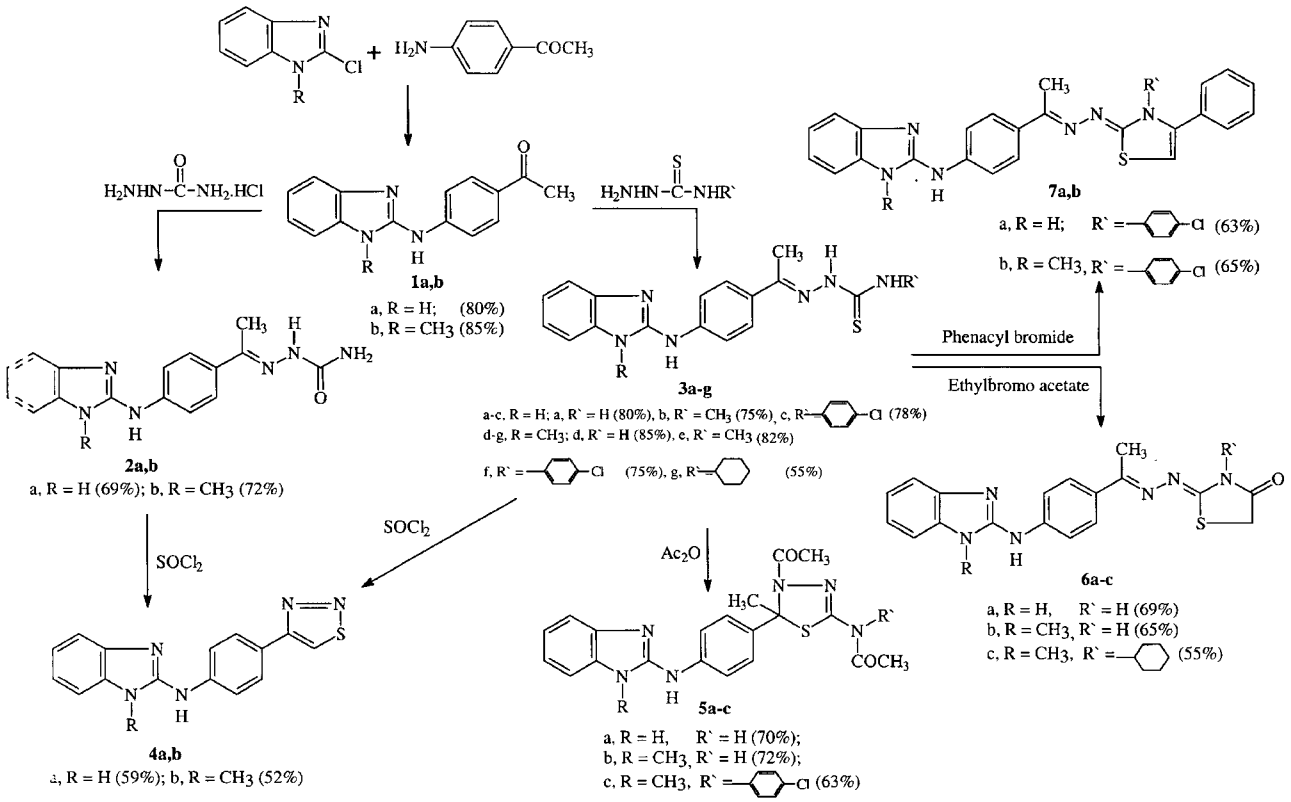
Compounds **4b**, **9**, **11c** were found to be highly active against *B. subtilis* and *E. coli*, while compounds **3b**, **8** were found highly active against *B. subtilis*. While compounds **4a**, **6a**, **b** were found active against *B. subtilis*. Compounds **3d**, **8**, **14** were found active against *E. coli*. Compounds **3a**, **5a**, **11a** and compound **6a** were found moderatly active against *B. subtilis*, and against *E. coli*, respectively. Compound **3f** and compounds **2b**, **3a**, **3b**, **6c**, **10b** were found slightly active against *B. subtilis* and against *E. coli*, respectively.

Most of the tested compounds showed no activity against *A. niger* except **11c** (highly active), compound **2b** (moderatly active) and compounds **3d**, **3e**, **3g**, **4a**, **5a**, **8** (slightly active).

Also, most of the tested compounds showed no activity against *C. albicans* except compound **11c** (highly active), compounds **4b**, **7b** (moderatly active), and compounds **3g**, **4a**, **7a**, **10b** (slightly active).

Due to the high activity of compound **11c**, we tested different concentrations of it, we found the MIC was 63 μg for *B. subtilis*, 55 μg for *E. coli* and 50 μg for *C. albicans*.

Generally, most of the tested compounds show activity against G +ve, G ve bacteria, (comparable to references). Compounds **3b**, **4b**, **8**, **9**, **11c** show higher activity than those of the references. Also, sulphonyl hydrazide derivative **11c** shows highly active against all of the tested microorganisms G +ve, G -ve bacteria, fungi and yeast, and it is found the best compound among these analogues.



Scheme 1. Synthetic Scheme for benzimidazole derivatives 1

CONCLUSION

Some general features can be drawn by the antimicrobial data.

1- Among the thiosemicarbazide of the new prepared benzimidazole derivatives (**3a**, **3b**, **3d**, **3f**, **3e**, **3g**) methylthiosemicarbazide **3b**. It was found the best compound among these analogues.

2- Structure activity relationship revealed that the presence of *p*-aminoethylbenzoate or *p*-aminobenzoic acid hydrazide at C_2 of benzimidazole substaintially increased the antimicrobial activity.

3- Among the compounds (**4a**, **b**, **5a**, **6a-c**, **7a**, **b** and **14**) bearing a heterocyclic pentatomic nucleus 1,2,3-thiadiazole derivative **4b**.

4- Uracil-5-sulphonyl hydrazide derivative (**11c**) was found the most active compound its activity may be due to the presence of uracil moiety.

RESULTS AND DISCUSSION

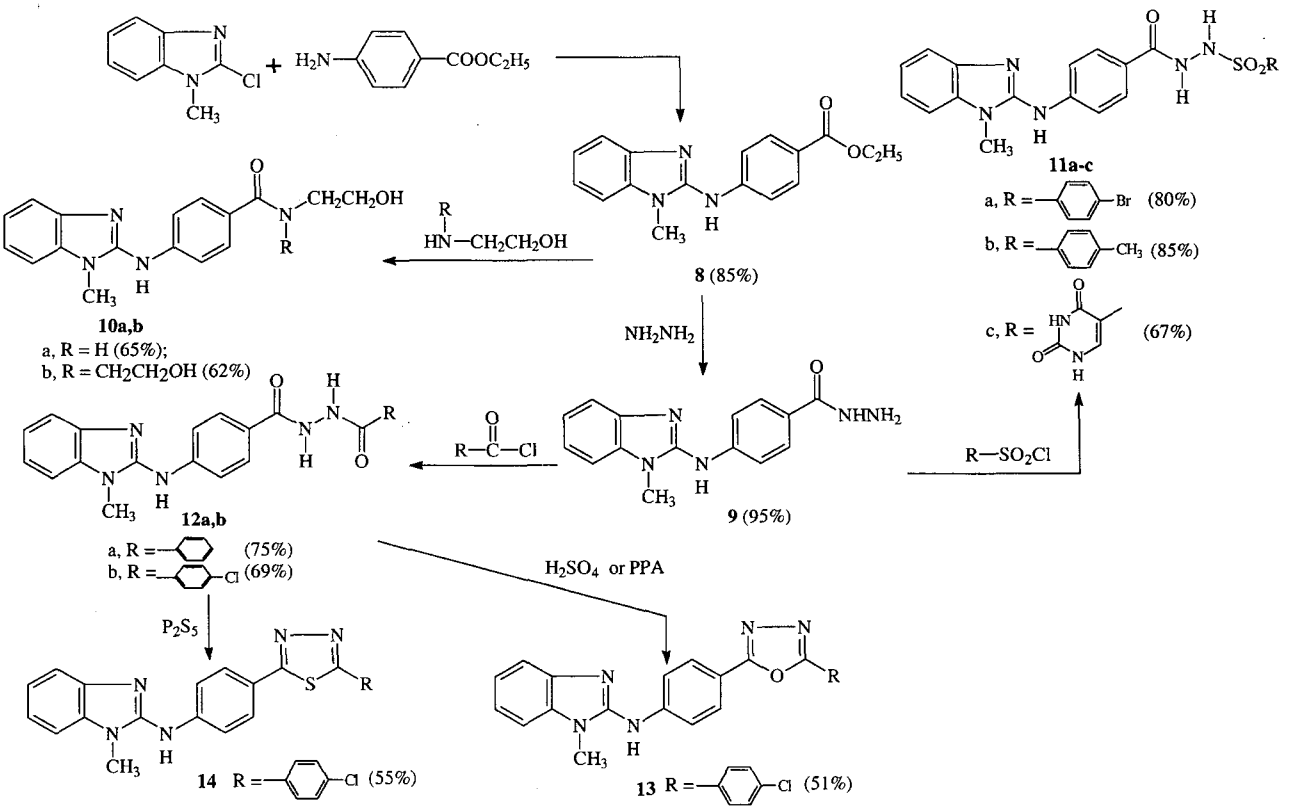
2-Chlorobenzimidazole and 2-chloro-1-methylbenzimidazole (Harrison *et al.*, 1963) were reacted with *p*-aminoacetophenone according to the reported method (Omar *et al.* 1995) to give 2-(*p*-acetylanilino)-benzimidazole (**1a**) and 2-(p-acetylanilino)-1-methylbenzimidazole (**1b**), respectively (Scheme 1).

Reaction of compounds **1a**, **b** with semicarbazide hydrochloride and sodium acetate in ethanol (Mandour *et al.*, 1995) gave semicarbazones **2a**, **b** (Scheme 1).

On the other hand, the reaction of compounds **1a**, **b** with thiosemi-carbazide or substituted thiosemicarbazides (Mandour *et al.*, 1995) in absolute ethanol with few drops of hydrochloric acid afforded thiosemi-carbazones and substituted thiosemicarbazones **3a-g**, respectively (Scheme 1).

Furthermore, the semicarbazones **2a**, **b** were subjected to oxidative cyclization using thionyl chloride (Hozien, 1993) to give 4-substituted 1,2,3-thiadiazoles **4a**, **b** (Scheme 1). It should be mentioned that thiosemicarbazones **3a**, **d** subjected to oxidative cyclization using thionyl chloride, led to the formation of the same compounds **4a**, **b**. Compounds **4a**, **b** obtained by either oxidative cyclization from semicarbazones or thiosemicarbazones using thionyl chloride showed the same melting points and the same R_f values. Melting points of the mixture of above showed no depression.

On the other hand, thiadiazoline derivatives **5a-c** were prepared by cyclization of the substituted thiosemicarbazone derivatives **3a**, **d**, **f** by boiling in acetic anhydride (Mohamed, 2001). While cyclo condensation of compounds **3a**, **d**, **g** using ethyl bromoacetate in boiling ethanol (Mohamed,



Scheme 2. Synthetic Scheme for benzimidazole derivatives **11**

2001) afforded 4-thiazolidinone derivatives **6a-c** (Shceme 1).

Also, cyclization of compound **3c, f** with equimolar amounts of phenacyl bromide in absolute ethanol (Bilinski *et al.*, 1988) afforded the corresponding thiazoline derivatives **7a, b** (Scheme 1).

Reaction of 2-chloro-1-methylbenzimidazole with p-aminobenzoic acid ethyl ester in absolute ethanol with few drops of concentrated hydrochloric acid afforded the ester compound 8 (Scheme 2). This compounds was transformed to the hydrazide 9 by treatment with hydrazine hydrate (Scheme 2). On the other hand, the reaction of ester compound 8 with hydroxy ethyl amines, namely 2-hydroxyethyl amine and/or bis-(2-hydroxyethyl)amine, afforded compound **10a, b** (Scheme 2).

Moreover, the hydrazide 9 was reacted with different sulfonyl chlorides (Fathalla *et al.*, 1995) to give the sulfonyl hydrazides **11a-c** (Scheme 2).

The reaction of the hydrazide 9 with acid chlorides (Fahmy and El-Eraky 2001) gave the corresponding benzoyl hydrazide derivatives **12a, b**, respectively (Scheme 2).

Cyclo condensation of compound **12b** with sulfuric acid or polyphosphoric acid (Fathalla *et al.*, 1995) gave the corresponding oxadiazole derivative **13**. Cyclization of **12b** with phosphorus pentasulfide gave the corresponding thiadiazole derivative **14** (Scheme 2).

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