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Synthesis, Antimicrobial and Molluscicidal Activities of New Benzimidazole Derivatives

Z. M. Nofal, H. H. Fahmy, and H. S. Mohamed
Therapeutical Chemistry Dept., National Research Centre, Dokki, Cairo, Egypt

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A series of benzimidazole Schiff's bases, thiosemicarbazides were synthesized, azole ring systems as 1,3,4-triazole, 1,3,4-oxadiazole were prepared. 1-Methylbenzimidazole incorporated to substituted dithio-carbamate, thiophenol, diethylamine via acetamido group were synthesized. A series of pyrimidinobenzimidazoles, triazinobenz-imidazoles, and 2-(acetonylamino)-1-methyl-benzimidazole were prepared. The antimicrobial and molluscicidal activities of some newly prepared compounds were carried out.

Key words: Schiffs bases, Thiosemicarbazides 1,3,4-triazole, 1,3,4-oxadiazole, Pyrimidino-benzimidazoles, Triazinobenzimidazoles

INTRODUCTION

A wide range of benzimidazoles are important clinically as useful drugs (Mohamed, 2001). Some benzimidazole derivatives are proved to possess antibacterial (Wolly, 1944), antifungal (Fahmy *et al.*, 2001), antiviral activities (Wikel *et al*, 1980) furthermore, 1,2,4-triazoles are reported to display broad spectrum of biological activity such as antibacterial, antifungal, analgesic and antiinflammatory (Abbas *et al.*, 1991).

Moreover Schiff's bases possess anticancer activity in animal screening, since the -N=CH group is a structural modification of the azomethene linkage. (Kamel *et al.*, 2000; Kaslow *et al.*, 1945). Broad spectrum of biological acti-vities was associated with various thiosemicarbazide and their cyclized products such as oxadiazoles have been reported to possess antibacterial and antifungal activities (fahmy *et al.*, 2001).

Also, several carbamate derivatives and dithiocarbamates showed wide biological activities such as fungicides (Ishi and Ito, 1970) and bactericides (Miyazaki *et al.,* 1979). This activity of dithiocarbamate refers to their structural complementarity to active site of acetyl cholinesterase and their consequent action as substrates with very low turn over number (Sengupta and Avasthi, 1975), furthermore, several sulphur compounds showed a wide biological activity especially as antimicrobial agent (Joshi *et al.*, 1997).

On the other hand, the presence of basic Mannich side chain in the drug may overcome the water insolubility proplem through the hydrochloride formation which renders the drug more easily absorbed, and the biological effect will be increase (El-Masry *et al.*, 2000). Moreover, the pyrimidino[1,2-a]benzimidazoles are known to exhibit CNS depressant and antiinflammatory activity (Abdou, *et al.*, 1987), also it was found that some of triazinobenzimidazole compounds are known to possess biological activities (Dose and White, 1973).

All these premises encouraged the design and synthesis of a series of substituted benzimidazoles containing the above mentioned moieties in an attempt to combine antibacterial, antifungal, antiviral activities in one and the same molecule. The chemotherapy of bilharziasis has been met with toxicity problems, combating the disease through control of snails is still considered as main factor (Kamel *et al.*, 1996). Moreover, it was reported that a series of salicylanilides possess molluscicidal and anthelmintic activities (Nawwar G. A. M., 1994), also benzimidazole derivatives such as albendazole, cambendazole, fenbendazole, flubendazole and mebendazole possess anthelmintic activity (Coleman, *et al.,* 1993). This led us to study the molluscicidal activity of benzimidazole derivatives.

In the present work it was of interest to synthesize some substituted benzimidazole to be evaluated as antimicrobials

Correspondence to: Dr. H. H. Fahmy, Therapeutical Chemistry Dept., National Research Centre, Dokki, Cairo, Egypt
E-mail: hh_fahmy@yahoo.com

and molluscicides against *Biomphalaria alexandrina* snails, the intermediate host of *schistosoma mansoni*.

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-d_6 or $CDCl_3$ using Jeol EX-270 MHz spectrometer. The Mass spectra were recorded on GCMS-QP 1000 EX Sehimadzu gas chromatography US apparatus. Elemental microanalyses were performed on a VARIO ELEMENTAR at the Microanalytical Laboratory Services, National Research Centre, Cairo, Egypt.

Synthesis of compounds

2-[*p*-(2-Mercapto-1,3,4-triazol-5-yl)anilino]-1-methyl-benzimidazole (2)

A mixture of carbohydrazide **1** (2.81 g, 0.01 mole) and ammonium thiocyanate (3.80 g, 0.05 mole) was fused at 180°C for 1/2 h The solid mass was dissolved in hot water and acidified with concentrated hydrochloric acid. The isolated yellowish precipitate was crystallized to give **2** (Table I, II).

2-[*N*-(1-Methylbenzimidazol-2-yl)]aminobenzoic acid hydrazide Schiffs bases (3a-c)

General Method:

To a solution of carbohydrazide **(1)** (2.81 g, 0.01 mole) in glacial acetic acid (10 ml), the appropriate aromatic aldehydes (0.01 mole) were added. The reaction mixture was refluxed for 3-6 h, after cooling the separated solid was collected by filteration, washed with water, dried and recrystallized to give corresponding hydrazono derivatives **3a-c**, respectively (Table I, II).

4-Alkyl and/or aryl-1-{p-[N-(1-methyl-benzimidazol-2-yl)amino-benzoyl]}-thiosemicarbazides (4a-d)

General Method:

A mixture of carbohydrazide **(1)** (2.81 g, 0.01 mole) and the appropriate isothiocyanate (0.01 mole) in dry benzene (20 ml) was refluxed for 3 h The solid formed after cooling was filtered off and recrystallized to give the corresponding thiosemicarbazide derivatives **4a-d**, respectively (Table I, II).

2-[*p*-(2-Substitutedamino-1,3,4-oxadiazol-5-yl)anilino]-1-methyl-benzimidazoles (5a-c).

Method A:

To a boiling solution of **4a,b,d** (0.01 mole) in dioxane (30 ml), powdered yellow mercuric oxide (2.16 g, 0.01 mole) was added over a period of 30 min. The suspension mixture was refluxed with stirring for 8 h, filtered while hot and the obtained black precipitate was washed with boiling dioxane (5 ml). The filterate and washing solution were combined, then concentrated under reduced pressure. The product was precipitated by addition of water (20 ml), the precipitate was collected by filteration, washed with water, dried and recrystallized from ethanol to give the corresponding oxadiazole derivatives **5a-c**, respectively (Table I, II).

Method B:

Equimolar quantities of carbohydrazide **1** (2.18 g, 0.01 mole) and the appropriate isocyanates (0.01 mole), namely methyl, phenyl and/or *p*-chlorophenylisocyanate in dioxane (30 ml) were heated under reflux for 3 h, then cooled. The separated crystalline product was filtered off and recrystallized from ethanol to give **5a-c**, respectively, as identified by m.p. and TLC in comparison with authentic sample from method A (Table I, II).

2-(Substituted thiocarbonylmercaptoacetylamino)-1-methyl benzimidazole (7a-d)

A solution of ammonium dithiocarbamic acid derivatives (0.01 mole) in dry acetone (20 ml) was added to a stirred solution of compound **6** (2.23 g, 0.01 mole) in dry acetone (20 ml). The stirring was continued for 30 min at room temperature then the reaction mixture was refluxed in water bath for 4 h. It was cooled and the formed precipitate was filtered off, then crystallized from the proper solvent to give the corresponding compounds **7a-d** (Table I, II).

S-[*N*-(1-Methylbenzimidazol-2-yl)aminoacetyl]thiophenol (8)

Compound **6** (2.23 g, 0.01 mole) was dissolved in dry benzene (30 ml), and thiophenol (1.65 g, 0.015 mole) and few drops of piperidine were added. The reaction mixture was refluxed on a water bath for 6 h, then concentrated. The separated solid was filtered off, washed with water, dried and crystallized to give compound **8** (Table I, II).

2-(Diethylaminoacetamido)-1-methylbenzimidazole (9)

Compound **6** (2.23 g, 0.01 mole) was dissolved in dry benzene (30 ml), and diethyl amine (1.2 ml, 0.012 mole) was added. The reaction mixture was refluxed on a water bath for 5 h The solvent was evaporated under reduced pressure, the solid that remained was crystallized to give compound **9** (Table I, II).

2-Amino-4-aryl-3-cyano-3,4-dihydro-pyrimidino[1,2-a]-benzimidazoles (14a,b)

2-Aminobenzimidazole (1.33 g, 0.01 mole) was dissolved in ethanol (10 ml), and α-cyanocinnamonitrile derivatives **13a,b** (0.01 mole) and few drops of piperidine were

added. The reaction mixture was warmed to 60°C with stirring for 5 min. After the reaction mixture left at room temperature the precipitated solid was filtered and crystallized from dimethylformamide to give pyrimidino [1, 2-a]benzimidazoles **(14a,b)** (Table I, II).

4-Aryl-3-cyano-2-oxo-3,4-dihydro-1*H*-pyrimidino[1,2-a]-benzimidazole (16a,b)

Table I. The physical and analytical data of the prepared compounds

Comp. No.	m.p. °C Solvent of recrystallization	Yield %	Formula (M. wt)	Analysis % Calcd/found		
				C	H	N
2	275 (DMF)	70	$C_{16}H_{14}N_6S$ (322.38)	59.60 59.39	4.38 4.42	26.07 25.85
3a	198 ($AcOH/H_2O$)	80	$C_{22}H_{19}N_5O$ (369.47)	71.52 71.30	5.18 5.25	18.96 18.71
3b	159 ($AcOH/H_2O$)	65	$C_{22}H_{18}FN_5O$ (387.46)	68.20 68.00	4.68 4.70	18.08 18.00
3c	120 ($AcOH/H_2O$)	75	$C_{23}H_{21}N_5O_2$ (399.44)	69.15 69.01	5.30 5.50	17.53 17.20
4a	192 (DMF/H_2O)	85	$C_{17}H_{18}N_6OS$ (354.42)	57.61 57.40	5.12 5.15	23.73 23.59
4b	185 (DMF/H_2O)	90	$C_{22}H_{20}N_6OS$ (416.49)	63.44 63.21	4.84 4.95	20.18 20.09
4c	160 (DMF/H_2O)	80	$C_{23}H_{22}N_6OS$ (430.52)	64.16 64.01	5.15 5.30	19.52 19.40
4d	218 (DMF/H_2O)	79	$C_{22}H_{19}ClN_6OS$ (450.98)	58.61 58.50	4.25 4.21	18.65 18.51
5a	130 (EtOH)	75	$C_{17}H_{16}N_6O$ (320.35)	63.73 63.50	5.04 5.23	26.24 26.30
5b	260 (EtOH)	70	$C_{22}H_{18}N_6O$ (382.41)	69.09 68.89	4.74 4.90	21.98 21.50
5c	180 (EtOH)	64	$C_{22}H_{17}ClN_6O$ (416.86)	63.38 63.17	4.08 4.12	20.16 20.07
7a	140 (MeOH)	55	$C_{15}H_{20}N_4OS_2$ (336.46)	53.54 53.33	5.99 6.10	16.65 16.59
7b	180 (EtOH)	66	$C_{16}H_{20}N_4OS_2$ (348.47)	55.14 55.00	5.79 5.81	16.08 16.00
7c	170 (EtOH)	67	$C_{15}H_{18}N_4O_2S_2$ (350.38)	51.40 51.29	5.18 5.25	15.99 15.70
7d	160 (MeOH)	71	$C_{16}H_{21}N_5OS_2$ (363.49)	52.87 52.75	5.82 5.99	19.27 19.00
8	150 ($CHCl_3$)	75	$C_{16}H_{15}N_3OS$ (297.36)	64.62 64.43	5.08 5.21	14.13 14.00
9	90 (Pet. Ether 60/80)	50	$C_{14}H_{20}N_4OS$ (260.33)	64.58 64.31	7.74 7.90	21.52 21.05
14a	270 (DMF)	85	$C_{17}H_{12}FN_5$ (305.31)	66.87 66.80	3.96 3.98	22.94 22.72
14b	205 (DMF)	90	$C_{18}H_{15}N_5$ (301.34)	71.73 71.70	5.01 5.03	23.24 23.19
16a	195 (EtOH)	75	$C_{17}H_{11}FN_4O$ (306.29)	66.66 66.50	3.62 3.68	18.29 18.15
16b	225 (EtOH)	80	$C_{18}H_{14}N_4O_2$ (318.31)	67.91 67.85	4.43 4.47	17.60 17.41
17	210 (EtOH)	98	$C_{12}H_{11}N_3O$ (213.23)	67.59 67.52	5.20 5.31	19.71 19.65
18	270 (EtOH)	90	$C_{11}H_{13}N_3O$ (203.23)	65.01 65.00	6.45 6.50	20.68 20.54
20a	250 (DMF)	90	$C_9H_8N_4O$ (188.18)	57.44 57.40	4.29 4.31	29.79 29.51
20b	290 (DMF)	95	$C_{10}H_{10}N_4O$ (202.21)	59.39 59.35	4.98 4.99	27.71 27.55

Table II. Spectral data for prepared compounds

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6 or $CDCl_3$, 270 MHz, δ), MS m/z (%)
2	IR: 3225 (NH), 3069 (CH), 2571 (SH), 1645 (C=N), 1181 (C=S). **MS**: M^+ 322 (100).
3a	IR: 3420, 3255 (NH), 1650 (C=O), 1600 (C=C). **MS**: M^+ 369 (19.58), 250 (100).
3b	IR: 3336, 3280 (NH), 1650 (C=O), 1600 (C=C), 1060 (C-F). **^{1}H-NMR (DMSO-d_6):** 3.75 (s, 3H, N-CH_3), 7.10-8.10 (m, 12H, Ar-protons), 8.45 (s, 1H, -N=CH), 9.30, 11.70 (2s, 2H, 2NH, exchangeable with D_2O). **MS**: M^+ 387 (17.22), 250 (100).
3c	IR: 3280 (NH), 1646 (C=O), 1600 (C=C), 1249 (C-O-C). **^{1}H-NMR (DMSO-d_6):** 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, N-CH_3), 7.00-8.00 (m, 12H, Ar-protons), 8.65 (s, 1H, -N=CH), 9.35, 11.60 (2s, 2H, 2NH, exchangeable with D_2O). MS: M^+ +1 400 (32), 250 (100).
4a	IR: 3307, 3200 (NH), 2940 (CH), 1650 (C=O), 1190 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.90 (s, 3H, NH-$\underline{CH_3}$), 3.80 (s, 3H, N-CH_3), 7.05-8.00 (m, 8H, Ar-protons), 8.10, 9.20, 9.30, 10.15 (4s, 4H, 4NH, exchangeable with D_2O).
4b	IR: 3275, 3160 (NH), 3050 (CH), 1650 (C=O), 1190 (C=S). **MS**: 281 (9.66), 250 (33.61), 222 (8.37), 135 (100), 77 (89)
4c	IR: 3310, 3212 (NH), 2980 (CH), 1657 (C=O), 1180 (C=S). **^{1}H-NMR** (DMSO-d_6): 2.15 (s, 3H, Ar-$\underline{CH_3}$), 3.70 (s, 3H, N-CH_3), 7.10-8.00 (m, 12H, Ar-protons), 9.35, 9.50, 9.55, 10.35 (4s, 4H, 4NH, exchangeable with D_2O). MS: M^+ 430.30 (0.90), 281 (9.01), 250 (23.20), 222 (58.34), 149 (100), 106 (81.86), 91 (79.74), 77 (31.10).
4d	IR: 3318, 3200 (NH), 2940 (CH), 1659 (C=O), 1185 (C=S), 740 (C-Cl).
5a	IR: 3390, 3226 (NH), 3045 (CH), 1640 (C=N). **^{1}H-NMR** (DMSO-d_6): 2.90 (s, 3H, NH-$\underline{CH_3}$), 3.75 (s, 3H, N-CH_3), 7.10-8.15 (m, 9H, 8Ar-protons and NH), 9.35 (s, 1H, NH exchangeable with D_2O). MS: M^+ 320 (100).
5b	IR: 3400, 3246 (NH), 3049 (CH), 1600 (C=C). **^{1}H-NMR** (DMSO-d_6): 3.75 (s, 3H, N-CH_3), 7.00-8.10 (m, 13H, Ar-protons), 9.35, 10.55 (2s, 2H, 2NH, exchangeable with D_2O). MS: M^+ 382 (100).
5c	IR: 3400, 3250 (NH), 3050 (CH), 1630 (C=N), 740 (C-Cl). **^{1}H-NMR** (DMSO-d_6): 3.75 (s, 3H, N-CH_3), 7.05-8.10 (m, 12H, Ar-protons), 9.35, 10.80 (2s, 2H, 2NH, exchangeable with D_2O). MS: M^+ 416 (100), M^++2 418 (37.5).
7a	IR: 3261 (NH), 2953 (CH), 1630 (C=O), 1180 (C=S). **^{1}H-NMR** (DMSO-d_6): 1.15, 1.25 (2t, 6H, $2CH_3$ of diethyl groups), 3.60 (s, 3H, N-CH_3), 3.80, 3.90 (2q, 4H, $2CH_2$ of diethyl groups), 4.25 (s, 2H, CH_2-S), 7.15-7.50 (m, 4H, Ar-protons) 12.49 (s, 1H, NH, exchangeable with D_2O). MS: M^+ 336 (33.93), 220 (100).
7b	IR: 3150 (NH), 2938 (CH), 1660 (C=O), 1160 (C=S). MS: M^+ 348 (4.38), M^++1 349 (14.8), 147 (100).
7c	IR: 3257 (NH), 2960 (CH), 1680 (C=O), 1175 (C=S). ^{1}H-NMR (DMSO-d_6): 3.60 (s, 3H, N-CH_3), 3.70 (t, 4H, $N(CH_2)_2$ of morpholine ring), 4.10 (t, 4H, $O(CH_2)_2$ of morpholine ring), 4.25 (s, 2H, CH_2-S), 7.12-7.52 (m, 4H, Ar-protons), 12.50 (s, 1H, NH, exchangeable with D_2O).
7d	IR: 3220 (NH), 2960 (CH), 1662 (C=O), 1180 (C=S).
8	IR: 3235 (NH), 3050 (CH), 1680 (C=O). **^{1}H-NMR** (DMSO-d_6): 3.50 (s, 3H, N-CH_3), 3.90 (s, 2H, CH_2-S), 7.10-7.50 (m, 9H, Ar-protons), 12.50 (s, 1H, NH). MS: M^+ 297 (9.47), 174 (100).
9	IR: 3403 (NH), 2970 (CH), 1685 (C=O). **^{1}H-NMR** ($CDCl_3$): 1.40 (t, 6H, $2CH_3$ of diethyl groups), 3.20 (q, 4H, $2CH_2$ of diethyl groups), 3.70 (s, 3H, N-CH_3), 3.80 (s, 2H, CO-CH_2-N), 7.15-7.50 (m, 4H, Ar-protons),10.60 (s, 1H, NH, exchangeable with D_2O).
14a	IR: 3446, 3320 (NH_2), 3050 (CH), 2186 (C≡N), 1680, 1640 (C=N). **^{1}H-NMR** (DMSO-d_6): 5.20, 7.60 (d, 2H, two methine protons of pyrimidine ring), 6.85 (s, 2H, NH_2), 7.00-7.40 (m, 8H, Ar-protons), 8.60 (s, 2H, 2NH, due to tautomerization, NH_2 exchangeable with D_2O).
14b	IR: 3440, 3324 (NH_2), 3045 (CH), 2177 (C≡N), 1680, 1630 (C=N). ^{1}H-NMR (DMSO-d_6): 2.25 (s, 3H, CH_3), 5.20, 7.65 (d, 2H, two methine protons of pyrimidine ring), 6.85 (s, 2H, NH_2 exchangeable with D_2O), 7.05-7.45 (m, 8H, Ar-protons). **MS:** M^+ 301 (80.18), 234 (100)

Table II. Continued

Comp. No.	IR (KBr cm^{-1}), ^{1}H-NMR (DMSO-d_6 or $CDCl_3$, 270 MHz, δ), MS m/z (%)
16a	IR: 3240 (NH), 3050 (CH), 2170 (C≡N), 1660 (C=O). **^{1}H-NMR** (DMSO-d_6): 4.25 (br.s, 1H, NH), 6.20, 7.70 (d, 2H, two methine protons of pyrimidine ring), 7.00-7.50 (m, 8H, Ar-protons). MS: M^+ 306 (100).
16b	IR: 3130 (NH), 3050 (CH), 2165 (C≡N), 1650 (C=O). **^{1}H-NMR** (DMSO-d_6): 3.70 (s, 3H, OCH_3), 4.20 (br s, 1H, NH), 5.85, 7.55 (d, 2H, two methine protons of pyrimidine ring), 7.00-7.50 (m, 8H, Ar-protons). MS: M^+ 318 (60), 186 (100).
17	IR: 3080, 2950 (CH), 1680 (C=O), 1600 (C=C). **^{1}H-NMR** (DMSO-d_6): 2.30 (s, 3H, CH_3 of pyrimidinone ring), 3.75 (s, 3H, N-CH_3), 5.95 (s, 1H, CH of pyrimidinone ring), 7.35-8.50 (m, 4H, Ar-protons). MS: M^+ 213 (100).
18	IR: 3360 (NH), 2940 (CH), 1740 (C=O). **^{1}H-NMR** (DMSO-d_6): 2.25 (s, 3H, CO-CH_3), 3.70 (s, 3H, N-CH_3), 5.35 (s, 2H, CH_2-CO), 7.30-7.55 (m, 4H, Ar-protons), 9.05 (s, 1H, NH, exchangeable with D_2O). MS: M^+ 203 (63.50), 160 (100).
20a	IR: 3300, 3160 (2NH), 1660 (C=O, of triazine ring). **^{1}H-NMR** (DMSO-d_6): 4.70 (s, 2H, CH_2 of triazine ring), 7.15-7.40 (m, 4H, Ar-protons), 8.85, 9.43 (2s, 2H, 2NH, exchangeable with D_2O). MS: M^+ 188 (100).
20b	**^{1}H-NMR** (DMSO-d_6): 3.30 (s, 3H, N-CH_3), 4.40 (s, 2H, CH_2 of triazine ring), 7.00-7.20 (m, 4H, Ar-protons), 10.15 (s, 1H, NH, exchangeable with D_2O). MS: M^+ 202 (100).

To a solution of 2-aminobenzimidazole (1.33 g, 0.01 mole) in ethanol (10 ml); ethyl arylidine cyano acetate derivatives (0.01 mole) **(15a,b)** and few drops of piperidine were added. The reaction mixture was heated under reflux for 1 h, then cooled. The separated solid was filtered, washed with ether and crystallized to give compounds **(16a,b)** (Table I, II).

2-Oxo-4,10-dimethylpyrimidino[1,2-b]benzimidazole (17)

A mixture of 2-amino-1-methylbenzimidazole (1.47 g, 0.01 mole) and ethyl acetoacetate (10 ml) was heated under reflux at 130-140°C for 4 h. After cooling the separated solid material was filtered off and crystallized to give compound **(17)** (Table I, II).

2-[Acetonylamino]-1-methyl benzimidazole (18)

To a solution of 2-amino-1-methylbenzimidazole (1.47 g, 0.01 mole) in ethanol (20 ml), chloroacetone (0.65 ml, 0.01 mole) was added. The reaction mixture was stirred at room temperature for 3 h The separated solid was filtered and crystallized to give compound **18** (Table I, II)

3-Oxo-10-(1*H*) or methyl-2,3-dihydro-1,2,4-triazino[3,2-b]-benzimidazole (20a,b)

A mixture of *N*-(1-(H) or methyl-benzimidazole-2-yl)glycine ethyl ester **(19a,b)** (0.01 mole) and hydrazine hydrate 98% (2 ml) in absolute ethanol was refluxed for 2 h. After cooling the formed precipitate was filtered, washed with water and ether and crystallized to give compounds **20a,b** (Table I, II).

BIOLOGICAL PART

Antimicrobial activity

Materials

media:

Neutral agar for bacteria and yeast:

It consists of beef extract (1 g/litre), yeast extract (2 g/litre), pepton (5 g/litre), sodium chloride (5 g/litre) and agar (15 g/litre).

Dox. for fungi:

It consists of sodium nitrate (2.0 g/litre); glucose (15.0 g/litre), KH_2PO_4 (1.0 g/litre), magnesium sulphate $7H_2O$ (0.5 g/litre), potassium chloride (0.5 g/litre), ferrous sulphate $7H_2O$ (0.007 g/litre) and agar (30.0 g/litre).

Method

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

Whatman No.1 filter paper disk of 5mm 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 impregnated 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 for bacteria, at 28°C for 72 h for yeast and fungi. Then examined for the inhibition zones caused by

Table III. Preliminary screening test for some newly synthezied compounds

Comp. No.	*Bacillus subtilis*	*Escherichia Coli*	*Aspergillus niger*	*Candida albicans*
Gentamycin	+++	+++	-	-
Ampicillin	+++	+++	-	-
2	+	+	-	-
3a	-	-	-	-
3b	-	-	+	-
5a	-	-	-	++
5b	-	+	-	+
5c	-	++++	-	++

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)

the various compounds on the microorganisms. The results of the preliminary screening test listed in Table III.

Results of antimicrobial activity

From the data obtained Table III it was found that most of tested compounds showed no activity against *B. subtilis* except compound **2** showed slight activity. Compound **5c** was found to be highly active against *E. coli*, while compounds **2, 5b** were found to be slightly active.

It was found that most of the tested compounds showed no activity against *A. niger* except compound **3b** was found to be slightly active.

Compound **5a, 5c** were found to be moderatly active against *C. albicans* while compound **5b** was found to be slightly active.

Generally compound **5c** possess activity against *E. coli* higher than that of the references (Gentamycin and Ampicillin).

Conclusion

Some general features can be drawn by the antimicrobial data:

1. Among the compounds (2, 5a,b,c) bearing a heterocyclic pentatomic nucleus the triazole derivative **2** was found to be slightly active against *B. subtilis* on the other hand, the oxadiazole derivative **5c** was found to be the most active compound against *E. coli*, its activity may be due to the presence of the *p*-chlorophenyl at C-2 of oxadiazole. While the triazole and oxadiazole derivatives **2** and **5b** were found to be slightly active against *E. coli*. Moreover oxadiazole derivatives **5a, 5c** showed moderate activity against *C. albicans* while compound 5b was found to be slightly active.

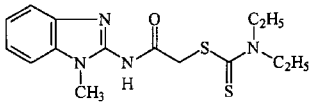
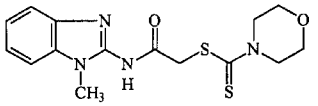
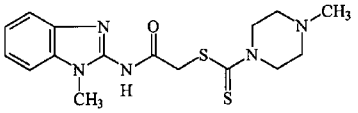
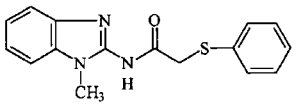
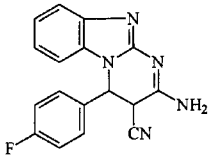
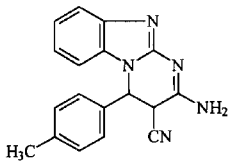
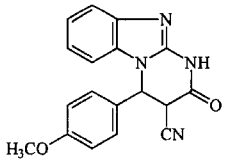
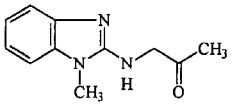
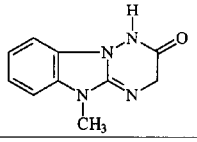
2. Schiffs base **3b** showed slight activity against A. *niger* its activity may be due to the presence of *p*-fluorophenyl Schiff's base side chain.

3. Interestingly the oxadiazole derivative **5c** proved to be more active than that of the references (Gentamycin and Ampicillin) against *E. coli.*

Molluscicidal activity of some newly synthesized benzimidazole derivatives

Some of the newly synthesized compounds have been tested for their molluscicdal activity against Biomphalaria

Table IV. Mortality counts of snails by effect of tested compounds

Comp. No	Formula Structure	% of Mortality
7a		70%
7c		100%
7d		100%
8		100%
14a		80%
14b		100%
16b		90%
18		100%
20b		100%

alexandrina snails, specific host of Schistosoma mansoni.

Materials

Snails :

Biomphalaria alexandrina snails were collected from irrigation canals, Giza Governorate. They were maintained as stock culture in a well-areated aquarium containing dechlorinated tap water with pieces of lettuce at temperature of 25-27°C.

Chemical agents:

Chemical compounds which synthesized in the present work and their structural formula were illustrated.

Methods

Firstly, preliminary screening of molluscicidal activity for all the tested compounds at standard concentration (100 ppm) were performed for evaluating the more active compounds against Biomphalaria alexandrina snails by the following method:

1. 100 ml (100 ppm) of each chemical agents were prepared by dissolving 1 mg in drop of tween 80, then dilute with dechlorinated water (100 ml).

2. Ten groups of ten mature snails each (8-10 mm-diameter, 2 month old) were immersed in a suitable beaker containing 100 ml of concentration (100 ppm) of the chemical agent. One group was employed beside a control group (water only) containing tween 80 and water. They were maintained under the same experimental conditions.

The exposure period was 24 h, the snails were then washed with dechlorinated water and transferred to a new beaker for recovery period of additional 24 h, Mortality counts were recorded and listed in Table IV.

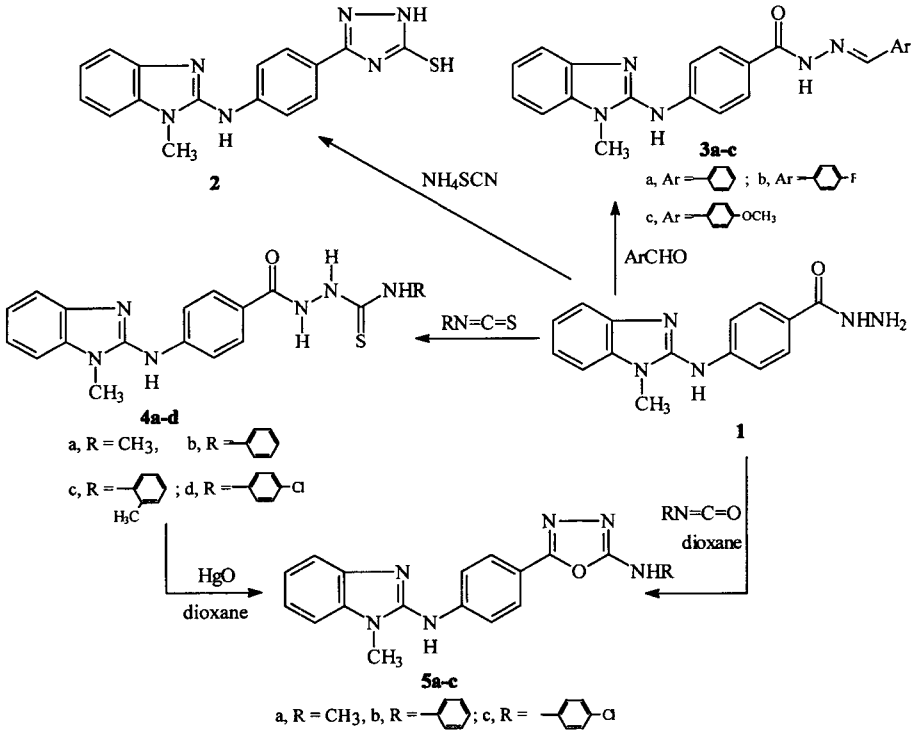
Table IV showed that compounds 7c, 7d, 8, 14b, 18, 20b recorded 100% death for Biomphalaria alexandrina snails, so we have selected some of these compounds and prepared serial concentrations to study the activity of these compounds by determination LC_{50} and LC_{90}.

Determination of LC_{50} and LC_{90}:

Stock solution at 100 ppm of each compound was prepared by dissolving 100 mg in few drops of tween 80, then diluted with dechlorinated water (1 litre). Series of dilution that would permit the computation of LC_{50} values were prepared. And repeat the previous steps. LC_{50} values were estimated according to the stanard method

Table V. Molluscicidal activity of newly synthesized compounds against Biomphalaria snails

Comp. No.	LC50 (ppm)	LC90 (ppm)
7d	20	40
8	35	80
14b	45	70
16b	50	80



Scheme 1. Synthetic scheme for benzimidazole derivatives I

WHO (Memoranda, 1965).

Results of molluscicidal activity

From Table V, it was found that compounds 7d, 8 showed moderate mollscicidal activity against Biomphalaria alexanderina snails, in comparison to Bayluscide (0.2 ppm) which is still the molluscicidal of choice.

DISCUSSIONS

Fusion of the carbohydrazide **1** (Mohamed, 2001) with ammonium thiocyanate (Zayed *et al.*, 1981) gave the corresponding 1,2,4-triazole-5-thiol derivative **2** (Scheme 1).

On the other hand, condensation of compound **1** with aromatic aldehydes (El-Masry *et al*, 2000) in acetic acid afforded the corresponding Schiffs bases **3a-c** (Scheme 1).

Also, reaction of the carbohydrazide **1** with different substituted alkyl- and/or arylisothiocyanate (Fahmy *et al.*, 2001) gave the corresponding thiosemicarbazide derivatives **4a-d** (Scheme 1).

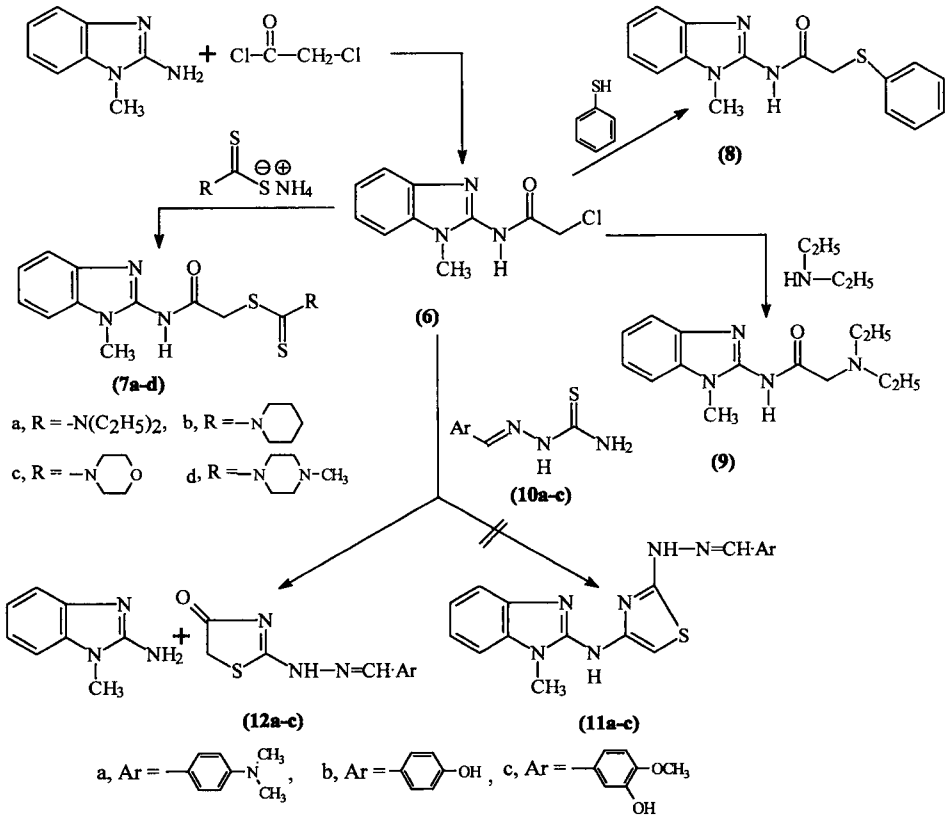
Moreover, 2-[*p*-(2-substituted amino-1,3,4-oxadiazol-5-yl)anilino]-1-methyl benzimidazoles **(5a-c)** were prepared by two methods. The first one involved cyclodesulphurization of the substituted thiosemicarbazides **4a,b,d** using freshly prepared yellow mercuric oxide in boiling dioxane (Aboul Wafa and Berto, 1992). The second method involved a one pot synthesis of the target compounds **5a-c** through the reaction of carbohydrazide **1** with substituted alkyl or aryl isocyanate in boiling dioxan, (Mohamed, 2001). This reaction involved addition and in situ cyclization of semicarbazide benzimidazoles (Scheme 1). All the analytical and spectral data (TLC, m.p., IR, ^{1}H-NMR and Mass) of compounds **5a-c** prepared by the two method were identical.

While acylation of 2-amino-1-methylbenzimidazole with chloro-acetylchloride in dry benzene afforded 2-chloro-acetamido-1-methyl-benzimidazole **(6)** in high yield according to the literature method (Mohamed, 2001) (Scheme 2). It serves as useful starting material for synthesis of dithiocarbamate derivatives.

Thus, compound **6** was reacted with ammonium salts of substituted dithiocarbamate (Vogel, 1978) to give the corresponding 2-[substituted thiocarbonylmercaptoacetylamino]-1-methylbenzimidazoles **(7a-d).**

On the other hand, compound **6** was reacted with thiophenol in dry benzene and few drops of piperidine to give compound **8** (Scheme 2). While the reaction of compound **6** with diethylamine in dry benzene afforded the Mannich bases **9** (Scheme 2).

Attempted cyclocondensation of 2-chloroacetamido-1-



Scheme 2. Synthetic scheme for benzimidazole derivatives II

methyl benzimidazole **(6)** with arylidene thiosemicarbazones **(10a-c)** in absolute ethanol or in dioxan at different degrees of temperature to give the desired cyclic compounds **11a-c** was failed and instead, 2-amino-1-methyl benz-imidazole and 2-hydrazonothiazolone **(12a-c)** were obtained (Scheme 2).

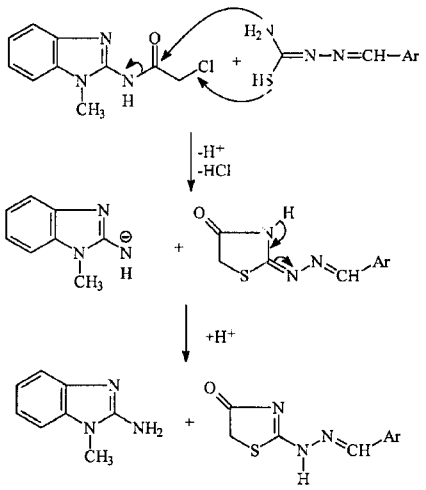
The structure of compounds **12a-c** were confirmed by elemental analysis, IR, ^{1}H-NMR, mass spectra and m.p. which were recorded in literatures (Kenji *et al.*, 1955).

For **12a**: m.p. 235°C in *Lit.* 242°C, **12b**: m.p. 320°C in *Lit.* 319°C, **12c**: m.p. 267°C decomp. in *Lit.* 268°C decomp.

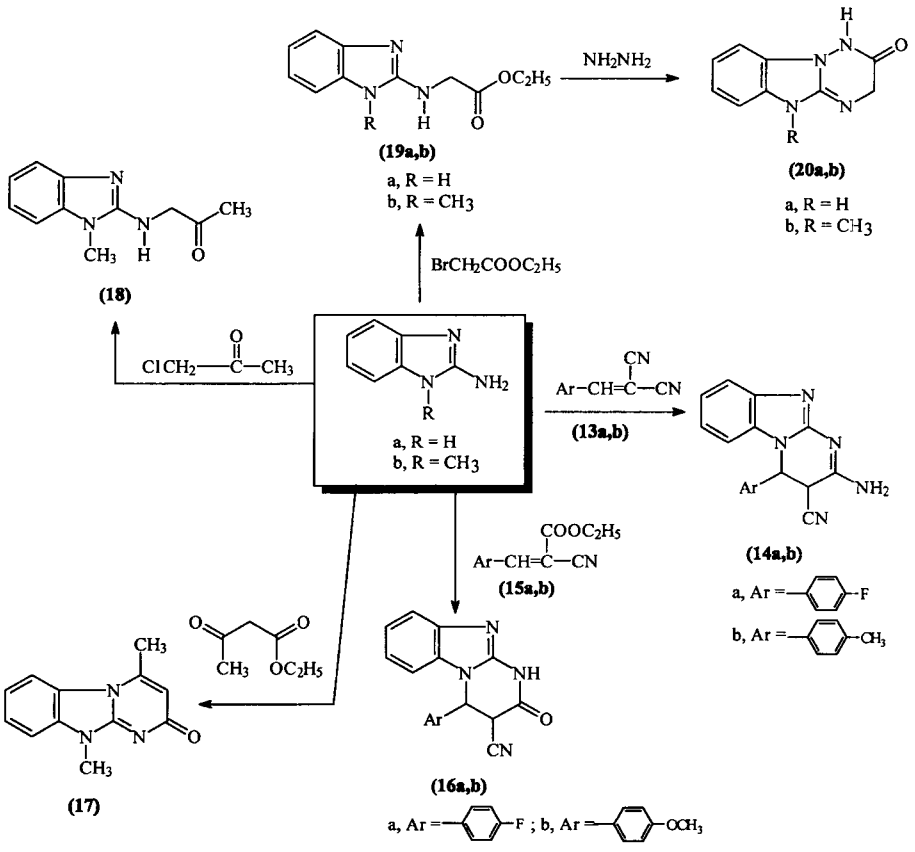
The mechanism of the reaction may be occurred as shown in scheme 3.

2-aminobenzimidazole was allowed to react with α-cyano-cinnamonitrile derivatives **13a,b** in ethanol and few drops of piperidine as catalyst (Abdou *et al.*, 1987; Zaki and Fathalla, 1997) to give substituted pyrimidino [1,2-a]benzimidazoles (**14a,b)** (Scheme 3). While cyclo-condensation of 2-aminobenzimidazole with ethyl arylidine cyanoacetate (Zaki and Fathalla, 1997) (**15a,b**) in ethanol in the presence of few drops of piperidine afforded pyrimidino[1,2-a]benzimidazole derivatives **16a,b** (Scheme 4).

On the other hand, cyclocondensation of 2-amino-1-methyl benzimidazole with ethylacetoacetate (Kandeel *et al.*, 1996) afforded pyrimidinobenzimidazole **17** (Scheme 4), also 2-amino-1-methylbenz-imidazole was reacted with chloroacetone (Bielak and Bilinski, 1990) in absolute ethanol to give 2-(acetonylamino)-1-methylbenzimidazole



Scheme 3. Plausible mechanism of undesired reactin with compound **6** and **10 a~c**



Scheme 4. Synthetic scheme for benzimidazole derivatives III

18 (Scheme 4).

Reaction of 2-aminobenzimidazole and 2-amino-1-methylbenz-imidazole with ethyl bromoacetate afforded *N*-(1*H*-benzimidazol-2-yl) glycine ethyl ester and *N*-(1-methylbenzimidazol-2-yl) glycine ethyl ester **(19a,b)** respectively (Sindelar *et al.,* 1989). Cyclocondensation of compound **19a,b** with hydrazine hydrate in ethanol gave the triazinobenz-imidazole derivatives **20a,b** (Scheme 4).

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REFERENCES

Abbas, S. E., Abou-Yossef, H. E., El-Taliaw, G. M., and Hassan, A. B., Synthesis of certain 1,2,4-triazoles derived from diclofenac as potential antiinflammatory agents. *Egypt. J. Pharm. Sci.*, 32, 503-514 (1991).

Abdou, O. A., Bahia, Y. R., and Suzan, I. A., Reaction with 2-aminobenzimidazole: Synthesis of several new pyrimido[1,2-a]benzimidazole derivatives. *Arch. Pharm. (weinheim)*, 320, 642-646 (1987).

Aboul Wafa, O. M. and Berto, F. A, G., Benzo[b]thiophenes Part I: Synthesis and antimicrobial activity of benzo[b]thionyl-1,3,4-oxadiazole, 1,2,4-triazoline, and thiazoline derivatives. *ibid.*, 325, 123-127 (1992).

Bielak, E. and Bilinski, S., Reactions of 1-(x-benzoyl)-4-R-thiosemi-carbazide with chloroacetone and omega-bromoacetophenone. III-4-phenyl- and 4-(p-tolyl)thiosemicarbazide of O-nitrobenzoic acid. *Ann. Univ. Mariae Curie Sklodowska*, 45, 131-139 (1990).

Coleman, D. L., Wood, N. L., and Darling, W. M., Martindale the Extra-pharmacopoei Edited by James E. F. Reynolds Published by Direction of the Council of Royal Pharmaceutical Society of Great Britain 13th edition, 37-55 (1993).

Dewani, M. B., Pathak, V. S. and Amine, U. M., Synthesis of some 1-acyl-4-substituted thiosemicarbazides. *Indian J. Chem.*, 11, 1078-1079 (1973).

Dose, E. A. and White, E. R., U.S. Patent, 3, 725, 406 (1973), Fungicidal benzimidazole derivatives. *Chem. Abst.*, 79, 62595 (1973).

El-Masry, A. H., Fahmy, H. H. and Abdelwahed, S. H. A., Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecules*, 5, 1429-1438 (2000).

Fahmy, H. H., El-Masry, A. and Abdelwahed, S. H. A., Synthesis and preliminary antimicrobial screening of new benzimidazole heterocycles. *Arch. Pharm. Res.*, 24(1), 27-34 (2001).

Ishi, T. and Ito, T., Japan Pat. 7034, 804 (Cl. CO 7C, Aolm) 1970, Fungicidal S-(benzyl)-N,N-dialkyldithiocarbamates. *Chem. Abst.*, 74, 87665v (1971).

Joshi, S., Matkar, S., Khosla, N. and Bhandari, V., synthesis and biological screening of N^4-phthalimidomethyl sulphonamides. *J. Indian Chem. Soc.*, 74, 156-157 (1997).

Kamel, M. M., Fahmy, H. H. and Abdou, W. A. M., Synthesis of some substituted tetralins of possible molluscicidal activity. *Egypt. J. Chem.* 39(6), 573-580 (1996).

Kamel, M. M., Nofal, Z. M., Fahmy, H. H. Refai, M. and Haiba, M. E., New quinolines and thiazolo quinolines with possible antitumor activity. *Proc. Pakistan Acad. Sci.*, 37(1), 41-55 (2000).

Kandeel, E. M., Hammouda, M. and Metwally, M. A., Aminoazoles in heterocyclic synthesis. Synthesis of new pyrimidobenzimidazoles and pyrazolonopyridines as well as triazolo-, tetrazolo- and pyrazolo-pyrimidines of pharmaceutical interest. *Boll. Chim. Farm.*, 135(4), 232-235 (1996).

Kaslow, C. E. and Stayner, R. D., Ozonolysis of styryl derivatives of nitrogen heterocycles. *J. Amer Chem. Soc.*, 67, 1716-1717 (1945).

Kenji, K., Nagashima, H., Ninoi, N. and Handa, T., (Gifu Coll Pharm.). Synthesis and antibacterial activity of 2-salicylidene hydrazono-4-thiazolidone and its related compounds. *J. Pharm. Soc. Japan,* 75, 438-441 (1955).

Memoranda, That took place on 17-21 November 1964 in Geneva, Switzerland, Molluscicide Screening and evaluation, *Bull WHO*, 33, 567 (1965).

Miyazaki, Y.; Uchiyama, Y. and Noguchi, T., Dithiocarbamate derivatives. Japan Pat. 7111, 172, (Cl CO 7C, Aolr) 1979; *Chem. Abst.*, 75, 5535m (1979).

Mohamed, H. S., Ph. D. (Science). Synthesis of Some new heterocyclic compounds containing benzimidazole of expected biological activity. *Thesis, Faculty of Science, Cairo University*, (2001).

Nawwar, G. A. M., Salicylamides containing amino acid or pyran moieties with molluscicidal activity. *Arch. Pharm. (Weinheim)*, 327, 201-205 (1994).

Omar, M. T., Fahmy, H. H. and Mohamed, H. S., Synthesis of some novel benzimidazole derivatives as antimicrobial agents. *Egypt. J. Pharm. Sci.*, 37, 609-620 (1996).

Sengupta, A. K. and Avasthi, K. J., Studies on potential pesticides: Part IV. Synthesis of several new dithiocarbamates. *J. Indian Chem. Soc.*, 433 (1975).

Sindelar, K., Metysova, J. and Protiva, M., 2-amino-substituted derivatives of benzimidazoles from 2-isothiocyanato carboxylates. *Collect. Czech. Chem. Commun.,* 54(1), 229-234

(1989).

Vogel, A. I., "Text book of practical organic chemistry" 4th edition, Longman Group UK Ltd, 735 (1978).

Wikel, J. H., Paget, C. J., Delong, D. C., Nelson, J. D., Wu, C. Y. E., Paschal, J. W., Dinner, A., Templeton, R. J., Chaney, M. O., Jones, N. D. and Chamberlin, J. W., Synthesis of Syn and Anti isomers of 6-{[(Hydroxyimino)phenyl]methyl}-1-[(1-methylethyl) sulfonyl]-1H-benzimidazole-2-amine Inhibitors of Rhinovirus Multiplication. *J. Med. Chem.*, 23, 268-272 (1980).

Wolly, D. W., Some biological effects produced by benzimidazole and their reversal by purines. *J. Biol. Chem.*, 152, 225 (1944).

Zaki, M. E. A. and Fathalla, O. A., Studies on ketene S,S, Acetal Part 1. Synthesis of new pyrazolo[1,5-a]pyrimidines and pyrazolo[1,5-a] triazines utilizing ketene S,S Acetals. *Egypt. J. Pharm. Sci.*, 38(4-6), 363-376 (1997).

Zayed, A., Metri, J. and El-Hawary, S., Reactions on 6,7-benzindazole-3-carboxylic acid hydrazide, Synthesis of some heterocycles of potential biological activity. *Egypt. J. Chem.*, 24(5), 389-396 (1981).