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Design, Synthesis and Antimicrobial Evaluation of Novel Carbendazim Dithioate Analogs.

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ABSTRACT

A novel series of carbendazim dithioate analogs was synthesized by the reaction of 2-aminobenzimdazole with CS_2 and different alkyl halide in one pot reaction. All the chemical structure of carbendazim analogs were elucidated with all the spectroscopical tools IR, ^{1}H, ^{13}C NMR and mass-spectroscopy. All the synthesized carbendazim analogs were screened for their *in vitro* antimicrobial activity against different Gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*), Gram-negative (*Escherichia coli and Klebsiella pneumonia*) bacteria, fungi (*Fusarium solani* and *Fusarium oxysporu*) and *in vivo* against soil dehydrogenase activity. Among the carbendazim dithioates, some analogs were the most effective analogs against all the Gram-negative and positive-bacteria. While the most of them showed slight activities against *Fusarium solani* and *Fusarium oxysporum.* One of these analogs seemed to be more suppressive for dehydrogenase activity of soil microorganisms compared to all carbendazim sulfur analogs.

Keywords: Carbendazim; dithioate; antimicrobial; soil dehydrogenase activity.

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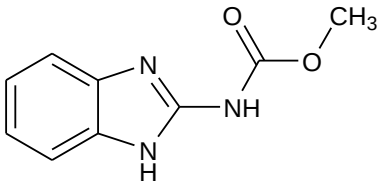
INTRODUCTION

Chemical pesticides are commonly used by farmers and corporate in agriculture to protect the crops, but these activities are depleting the soil fertility and Soil health. The use of chemical pesticides in agriculture drastically increased in recent years. The word pesticides include a heterogeneous group of chemicals developed to control a variety of pests. Soil enzymes are known to play a substantial role in maintaining soil health and its environment. The enzymatic activities in soil are mainly microbial origin, and derive from intracellular, cell associated or free enzymes. Soil enzymes are a group of enzymes whose inhabitants are soil and are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health. Soil enzymes act as mediators and catalysts of biochemical processes, therefore play important roles in soil functions, such as carbon and nutrient cycling, decomposition and soil organic matter formation [1, 2].The activity of soil enzyme can also indicate microbial activity and substrate availability and respond immediately to change in soil environment [3]. Soil enzymes play a fundamental role in establishing biogeochemical cycles.

Enzyme activity in soil results from the activity of accumulated enzymes and from enzymatic activity of proliferating microorganisms [4]. Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles.

The soil dehydrogenase activity in soil provides correlative information on the biological activity and microbial populations in soil in other words dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soil [5]. Soil dehydrogenase is not residing extracellular in the soil but exist in soils as integral parts of intact cells.

Carbendazim (MBC, FB642) [methyl 1H-benzo[d]imidazol-2-ylcarbamate] **1** (Figure 1) is one of the most important fungicides in the world. It can occupy a head of a highly biological active family like benzimidazole [6, 8]. As well as MBC is the hydrolytic product and active component of some other widely used benzimidazole fungicides such as benomyl and thiophanate methyl. It is used globally to protect agricultural crops as cereals, oils, fruits, vegetables, and ornamentals from decay caused by various fungal pathogens [9].



1

Methyl 1*H*-benzo[*d*]imidazol-2-ylcarbamate

Figure 1: Chemical structure of carbendazim

In addition, it is reported that carbendazim has a significant inhibitory effect on soil biological processes, including soil ammonium-N and nitrate-N concentrations and soil dehydrogenase activity [10].

More recently, a temporary alteration in the structure of the soil bacterial community caused by successive applications of carbendazim was observed [11].

The application of carbendazim in agricultural soil particularly groundnut crop cultivating soil leads to decrease the total numbers of soil microorganisms at initial period and as well as negatively affect the soil enzymes activities especially urease, dehydrogenase and phosphatase [12].

The biological activity of benzimidazole ring isn't limited to its occurrence as anti-fungi but benzimidazole moiety and its derivatives play a significant role in pharmaceutical and agricultural fields due to their broad spectrum of biological activities. Such a moiety has a various biological activities such as an

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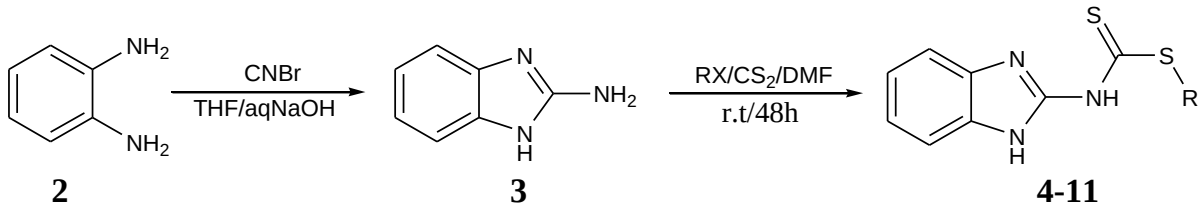
inflammatory [13], anticonvulsant [14], antibacterial [15, 16], antiviral [17], insecticides [18], acaricides [18], nematocides [19], herbicides [20] and other plant-protective agents in the field of pest control as well as agricultural compositions [21].

In our previous work on enhancing the biological activity of different heterocyclic compounds by inducing dithiocarbamate or dithioate group, it is found that there is a perceptible improvement of its biological activities [22, 25]. On the other hand dithioate derivatives have also associated with tremendous fields of biological activity such as antifungal [26] antibacterial [27, 28], anticancer [22] and carbonic anhydrase inhibitors [29, 30].

In spite of the wide spectrum of biological activity for benzimidazole as well as their dithioate or dithiocarbamate analogs, there are only few relevant searches that merge dithioate with benzimidazole ring and evaluation of their biological activity [31, 32].

Based on the previously mentioned benzimidazole activities, the function of dithioate compounds as good antimicrobial agents and in order to enhance the antimicrobial activity of carbendazim **1**, a new series of benzimidazole dithioates (carbendazim sulfur analogs) [**4-11**] have been synthesized by introducing two sulfur atoms in the side chain at the amino group (NH_2) of the 2-amino benzimidazole moiety **3** and investigated against their antimicrobial activity.



2 **3** **4-11**

Scheme 1: Synthesis of carbendazim dithioate analogs 4–11.

EXPERIMENTAL

General

The progress of all reactions and synthesized product were monitored via analytical silica gel TLC plates 60 F_{254} and spots were located by UV light which purchased from Merck and spots were located by UV light. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 400 MHZ spectrometers (Nagasaki University-Japan, University of Southern Denmark). Chemical shifts are reported in parts per million (ppm) relative to the respective deuterated solvent peak DMSO-d_6 (δ 2.50 ppm) for ^{1}H NMR and (δ 39.44 ppm) for ^{13}C NMR. MALDI mass spectra of the synthesized compounds were recorded on a Fourier transform Ion Cyclotron Resonance Mass Spectrometer (IonSpec, Irvine, CA). For accurate ion mass determinations, the (MH^+) or (MNa^+) ion was peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix, Nagasaki University-Japan. The mass Spectrometer is controlled by the OMEGA Data System. Electrospray ionization high resolution mass spectra (ESIHRMS) were performed on PE SCIEX API Q-Star Pulsar Mass Spectrometer. For accurate ion mass determinations, the (MH^+) or (MNa^+) ion was peak matched by calibration with NaI, University of Southern Denmark. IR spectra were recorded (KBr) on a Pye-Unicam Sp-883 Perkins-Elmer spectrometer at CairoUniversity. Melting points were determined on a Büchi melting point apparatus and were uncorrected. Solvents were distilled prior to use, while reagents were used as purchased. The starting material, 2-Aminobenzimdazole **3** was prepared according to the reported procedure [33]. All chemicals and solvents were purchased from E. Merck (Darmstadt, Germany) and Sigma–Aldrich.

General Procedure forThe synthesis of Benzimidazole Dithioate Analogs [4-11]

A mixture of 2-amino benzimidazole **(3)** (1 mmol), carbon disulfide (2 mmol), sodium carbonate (2mmol) and the appropriate alkyl halide RX **(a-h)** (1 mmol) (table 1) in DMF (4 mL) was stirred for 48 h at room temperature. The reaction mixture was poured onto ice water. The precipitate was collected by

filtration, draying and recrystallized to afford benzimidazole dithioate analogs **[4-11]**.

Methyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (4)

Light green solid; mp 215–218°C (ethanol); IR (KBr) υ_{max} 3443, 3063, 2986, 1626, 1469, 1339, 1187 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 13.06 (br s, 2H), 7.52–7.54 (m, 2H), 7.27–7.29 (m, 2H), 2.48 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 205.1 (C), 150.5 (C), 128.4 (2C), 123.5 (2CH), 112.2 (2CH), 18.0 (CH_3) ppm; HRMS (ESI, m/z) 246.0130 calcd for $C_9H_9N_3S_2$ (M+Na) found 246.0129.

Ethyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (5)

Pale yellow solid; mp 213–215°C (ethanol); IR (KBr) υ_{max} 3417, 3073, 2979, 1623, 1464, 1339, 1183 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 13.09 (br s, 2H), 7.52-7.54 (m, 2H), 7.27–7.29 (m, 2H), 3.07 (q, 2H), 1.27 (t, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 204.5 (C), 150.8 (C), 128.6 (2C), 123.6 (2CH), 112.3 (2CH), 28.3(CH_2), 14.4 (CH_3) ppm; HRMS (MALDI, m/z) 238.0473 calcd for $C_{10}H_{11}N_3S_2$ (M+H) found 238.0472.

Propyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (6)

Yellow solid; mp 205-207°C (ethanol); IR(KBr) υ_{max} 3450, 3052 , 2940, 2897, 1635, 1445, 1343, 1188 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.71 (br s, 2H), 7.52–7.54 (m, 2H), 7.27–7.29 (m, 2H), 3.07 (t, 2H), 1.62–1.67 (m, 2H), 0.97 (t, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 205.1 (C), 150.6 (C), 128.4 (2C), 123.5 (2CH), 112.2 (2CH), 36.1 (SCH_2), 22.2 (CH_2), 13.5 (CH_3) ppm; HRMS (ESI**,** m/z) 252.0629 calc for $C_{11}H_{13}N_3S_2$ (M+H) found 252.0615.

Isopentyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (7)

Light yellow crystalline solid; mp 209-211°C; IR(KBr) υ_{max} 3434, 3068, 2963, 2923, 1624, 1470, 1334, 1207, 1150 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 13.05 (br s, 2H), 7.52–7.54 (m, 2H), 7.26–7.28 (m, 2H), 3.10 (t, 2H), 1.63-1.70 (m , 1H), 1.48–1.52 (m, 2H), 0.92 (s, 3H), 0.90 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 204.5 (C), 150.6 (C), 128.4 (2C), 123.5 (2CH), 112.1 (2CH), 37.8 (SCH_2), 32.3 (CH_2), 27.1 (CH), 22.3 ($2CH_3$) ppm; HRMS(ESI**,** m/z) 280.0942 calc for $C_{13}H_{17}N_3S_2$ (M+H) found 280.0930.

Octyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (8)

Yellowish-white soild; mp 261-263°C (toluene); IR(KBr) υ_{max} 3411, 3095, 2960, 2832, 1622, 1467, 1353, 1208, 1181 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ:13.06 (br s, 2H), 7.52–7.54 (m, 2H), 7.26–7.29 (m, 2H), 3.08 (t, 2H), 1.57–1.65 (m, 2H), 1.33–1.38 (m, 2H), 1.23-1.29 (m, 8H), 0.84 (t, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 204.5 (C), 150.6 (C), 128.4 (2C), 123.5 (2CH), 112.1 (2CH), 34.1 (SCH_2), 31.2 (CH_2), 28.8 (CH_2), 28.7 ($2CH_2$), 28.4 (CH_2), 22.1 (CH_2), 13.9 (CH_3) ppm; HRMS (ESI**,** m/z) 322.1412 calc for $C_{16}H_{23}N_3S_2$ (M+H) found 322.1403.

Allyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (9)

Yellow soild; mp 191-193°C (methanol); IR(KBr) υ_{max} 3245, 3111, 3092, 2999, 2937, 1686, 1625, 1470, 1334, 1178 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.83 (br s, 2H), 7.53–7. 55 (m, 2H), 7.28–7.30 (m, 2H), 5.90–5.97 (m, 1H), 5.27 (d, J = 12 Hz, 1H),5.08 (d, J = 8 Hz, 1H), 3.80 (d, J = 8 Hz, 2H)ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 205.0 (C), 150.5 (C), 129.3 (CH), 128.4 (2C), 123.6 (2CH), 117.2 (CH_2), 112.2 (2CH), 37.4 (SCH_2) ppm; HRMS (ESI, m/z) 250.0473 calc for $C_{11}H_{11}N_3S_2$(M+H) found 250.0470.

Hex-5-en-1-yl 1H-benzo[d]imidazol-2-ylcarbamodithioate (10)

Yellow solid; mp 181-183°C (ethanol); IR(KBr) υ_{max} 3399, 3065, 2975, 2930, 1686, 1625, 1473, 1342, 1183 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 13.01 (br s, 2H), 7.52–7.54 (m, 2H), 7.27–7.29 (m, 2H), 5.77–5.85 (m, 1H), 4.93-5.04 (m, 2H), 3.10 (t, 2H), 2.03–2.08 (m, 2H) , 1.59–1.67 (m, 2H) 1.43–1.50 (m, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6)δ: 204.6 (C), 150.7 (C), 138.7 (CH), 128.5 (2C), 123.6 (2CH), 115.0 (CH_2), 112.3 (2CH), 34.0 (SCH_2), 32.8 (CH_2), 28.4 (CH_2), 27.6 (CH_2); HRMS (MALDI, m/z) 292.0942 calc for $C_{14}H_{17}N_3S_2$ (M+H) found 292.0938.

ISSN: 0975-8585

Benzyl 1H-benzo[d]imidazol-2-ylcarbamodithioate (11)

Light green crystalline solid; mp 200-201°C (ethanol); IR(KBr) υ_{max} 3342, 3145, 2923, 1626, 1460, 1331, 1190 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 13.15 (br s, 2H), 7.55–7.58 (m, 2H), 7.39-7.42 (m, 2H), 7.27-7.32 (m, 4H), 7.21-7.24 (m, 1H), 4.41 (s, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 202.9 (C), 150.5 (C), 138.5 (C), 129.0 (2CH), 128.4 (CH), 128.3 (2C), 126.7 (2CH), 123.6 (2CH), 112.3 (2CH), 38.6 (SCH_2) ppm; HRMS (ESI, m/z)300.0629 calc for $C_{15}H_{13}N_3S_2$(M+H) found 300.0627.

Biological Activity

Antimicrobial activity was carried out using four different bacterial strains *klebsiella pneumonia*, *Escherichia coli* (Gram-negative), *Staphylococcus aureus* and *Micrococcus luteus* (Gram-positive). On the other hand, two pure fungi strains *Fusarium oxysporum* and *Fusarium solani* as well as one enzymatic active toward dehydrogenase activity (DHA) which used to determine the biological activity. The cultures were purchased from Agric. Microbiology Department, National Research Centre Dokki, Cairo, Egypt.

The antibacterial activities of tested compounds were investigated by disc diffusion LB agar plates, and zone of inhibition after incubation has been measured. The antifungal activities of tested compounds were measured by incubation into100ml Czapek-Dox liquid media supplemented with sterile disc, dipped in each composite. The aliquot (0.5 ml) of spore suspension (prepared by overtaxing a 7 mm disc, from the margin 7 days old colony in 10 ml sterile distilled water)were used as inoculum and incubated for 7 days in an orbital rotary shaker at 120 rpm and 27 °C. Mycelia were harvested, washed twice with distilled water and dried at 60°C for 3 days sing aluminum foil cups for determination of mycelia dry weight. Dehydrogenase activity (DHA) was determined according to Pepper et al (1995) [34]. Nalidixic acid and Carbendazim were used as standard drugs (positive control) for antimicrobial activity, while Dimethyl sulfoxide (*DMSO*) was used as negative control. All compounds were tested at 150µg/ml in *DMSO*.

Statistical Analysis

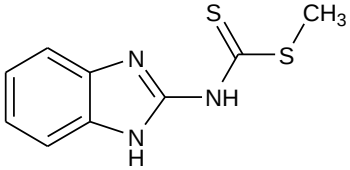
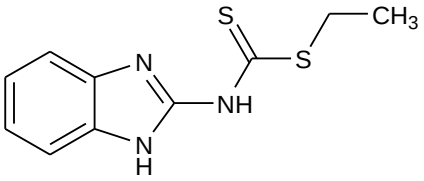
All statistical analyses were performed using SPSS software package (SPSS In. U.S.A.) and Excel (Microsoft, U.S.A.). The data were subjected to analysis of variance, and significant differences between means were determined by Duncan's multiple range tests ($p < 0.05$).

RESULTS AND DISCUSSION

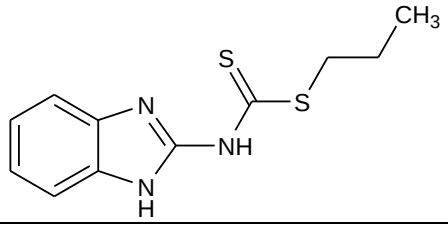
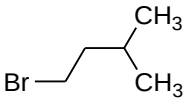
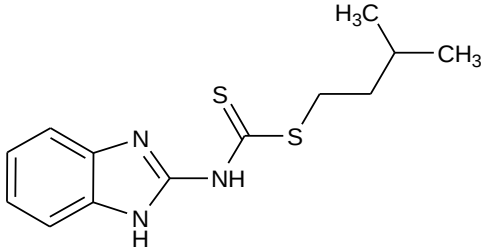
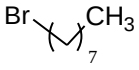
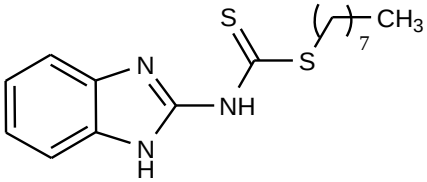
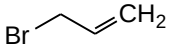
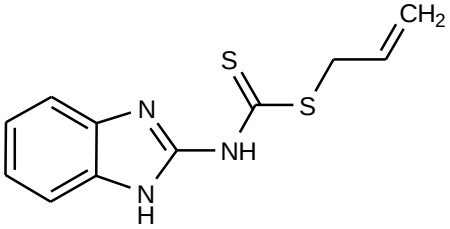
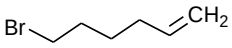
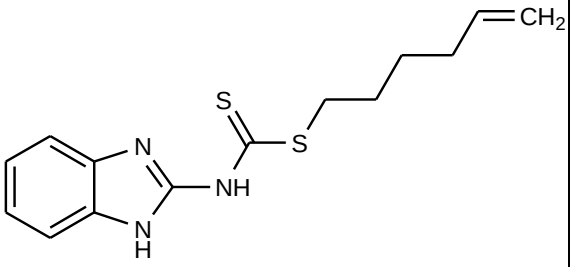
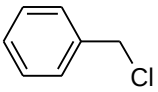
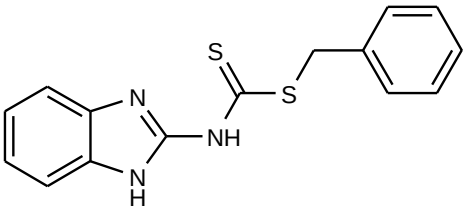
Chemistry

2-Aminobenzimdazole **(3)**, carbonyl sulfide, sodium carbonate and the appropriate alkyl halide (aliphatic, alicyclic and aromatic) (**a-h**) (table 1) were dissolved in DMF and stirred for 48 h at room temperature to afford benzimidazole dithioate (carbendazim sulfur analogs) **[4-11]** in good to excellent yields.

Table 1: Appropriate alkyl halide entries (A–H) and the corresponding carbendazim dithioateanalogs 4–11

Entry	RX	Product	Structure	Yield (%)
A	CH_3I	**4**		85
B	CH_3CH_2Br	**5**		62

ISSN: 0975-8585

C	$CH_3CH_2CH_2Cl$	**6**		82
D		**7**		88
E		**8**		81
F		**9**		75
G		**10**		66
H		**11**		62

Several trials had been done using different solvents as chloroform, methanol and dioxane. But unfortunately these reactions were failed to proceed properly. The chemical structure of all the synthesized compounds were elucidated on the basis of their spectral data IR, ^{1}H, ^{13}C NMR and Fourier transform Ion Cyclotron Resonance (MALDI), PE SCIEX API Q-Star Pulsar (ESI). The IR spectra of carbendazim sulfur analogs **[4-11]** supported the expected structures and showed absorption bands in the region 3500-3400 cm^{-1} resulting from the 2NH function, 1030-1200 cm^{-1} region resulting from C=S function, and 1647-1655 cm^{-1} region resulting for C=N function. The ^{1}H NMR spectra of carbendazim sulfur analogs **[4-11]** displayed signals about 2.8 ppm which were associated with methylene protons (S-CH_2-R) as singlet, doublet, triplet or multiplet according to the corresponding alkyl halide. The ^{13}C NMR spectra of analogs **[4-11]** display signals about δ 112.3, 123.3 and 128.6 ppm which were associated with the phenyl ring of benzimidazole. The ^{13}C NMR spectra of analogs **[4-11]** display signals about δ150.2 and 204.5 ppm which was associated with (-N=CN)and

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(C=S), respectively. ESI and MALDI mass spectra of carbendazim sulfur analogs **[4-11]** display molecular ions $[M+H]^+$ and $[M+Na]^+$ which confirmed their molecular weights. The mass data confirmed the structure of carbendazim sulfur analogs **[4-11]**

All the analytical data were in complete accordance with the proposed structures. Reagents and analytical data were presented in Experimental Section.

Antimicrobial Activity

Antibacterial Activity

Novel eight carbendazim dithioate analogs were tested against standard strains of Gram-positive and Gram-negative bacteria in a comparative way with standard antibiotic nalidixic acid. As shown in **Table 2**, the analog **5** showed high activity against *K. pneumonia* (Gram-negative bacteria), while compounds **4**, **9** and **11** showed high potency against *E. coli* (Gram-negative bacteria) compared to nalidixic acid. The rest of carbendazim sulfur analogs showed poor to moderate activity against the two types of Gram-negative bacteria than nalidixic acid (standard antibiotic). *S. aureus* (Gram-positive bacteria) showed high resistant to all novel carbendazim sulfur analogs and significant decrease antimicrobial activity. The two sulfur analogs **9** and **11** showed high potent antimicrobial activity against *M. luteus* (Gram-positive bacteria), which is comparable to nalidixic acid, while sulfur analogs **8** and **4** showed the same activity compared with nalidixic acid. Over all, the sulfur analogs **4**, **5**, **9** and **11** were the most effective compounds against the four selected bacteria.

Table 2: The Antimicrobial activity of carbendazim dithioate analogs 4-11.

Compound	Gram-negative		Gram-positive		*Fusariumsolani*		*Fusariumoxysporum*		Dehydrogenase activity µlH2g-1 soil-124h	Change in dehydrogenase activity(%) compared to Untreated
	K. pneumonia	*E. coli*	*S. aureus*	*M. luteus*						
	Inhibition Zone (mm)				Mycelium dry weight(mg 100ml media^{-1})	Stimulation or inhibition (%)	Mycelium dry weight(mg 100ml media^{-1})	Stimulation or inhibition (%)		
Untreated	-	-	-	-	680^{e}*	-	807^{bc}	-	22.59^{a}*	-
Nalidixic	20.0^{b}	25.0^{e}	22.0^{a}	22.0^{c}						
Carbendazi	-	-	-	-	187^{g}	-72.50	305^{g}	-62.21	8.75^{e}	-61.27
DMSO	-	-	-	-	-	-	-	-	-	-
4	12.5^{d}	33.5^{a}	13.0^{c}	21.0^{c}	505^{f}	-25.74	796^{cd}	-1.36	12.00^{c}	-46.88
5	22.5^{a}	21.0^{d}	12.0^{cd}	11.5^{e}	853^{a}	25.44	786^{d}	-2.60	7.93^{fg}	-64.90
6	12.3^{d}	1.90^{g}	16.0^{b}	19.0^{d}	860^{a}	26.47	752^{e}	-6.82	10.55^{d}	-53.30
7	11.0^{de}	10.5^{e}	10.5^{e}	18.2^{d}	774^{d}	13.82	853^{a}	5.70	4.42^{h}	-80.47
8	13.5^{c}	1.90^{g}	11.0^{de}	21.5^{c}	687^{e}	1.03	846^{a}	4.83	8.47^{ef}	-62.51
9	10.5^{e}	32.5^{b}	10.8^{e}	25.8^{a}	827^{b}	21.62	739^{f}	-8.43	11.99^{c}	-46.92
10	9.5^{f}	8.0^{f}	8.0^{f}	6.5^{f}	866^{a}	27.35	820^{b}	1.61	12.81^{b}	-43.29
11	9.0^{f}	27.3^{c}	11.5^{d}	23.8^{b}	779^{d}	14.56	752^{e}	-6.82	7.04^{g}	-68.84

*Means followed by the same letter within a columns are not significantly different at P= 0.05 (Duncan's)
Simulation (+),Inhibition (-)

Antifungal Activity

Unexpectedly, the antifungal activity has been found less than the antibacterial activity for all the synthesized sulfur analogs. Interestingly, some of the synthesized carbendazim sulfur analogs exhibited stimulation fungal growth compared to standard carbendazim. Only analog **4** (25.74%) showed inhibition activity in the case of *Fusarium solani* (table 2). The rest of synthesized carbendazim sulfur analogs were showed stimulation hyphal growth against *Fusarium solani*. Meanwhile, the analogs **6**, **9** and **11** were showed slight growth inhibitory activity (6.82, 8.43, 6.82 and 9.05 %) against *Fusarium oxysporum*, while analogs **4** and

5 showed poor inhibition (1.36 and 2.60%). The rest of the synthesized carbendazim sulfur analogs were showed stimulation hyphal growth against *Fusarium oxysporum*.

Soil Dehydrogenase Activity (DHA)

Furthermore, the effect of the novel carbendazim sulfur analogs on soil dehydrogenase activity is presented in **Table 2**. Untreated soil exhibited the highest enzyme activity (22.59 ul H_2g^{-1} soil day^{-1}). Addition of either the carbendazim **1** or its sulfur analogs **[4-11]** significantly decreased the activity according to their chemical constitution. In soil receiving carbendazim, the activity decreased by 61.27%. More lethal influence of carbendazim sulfur analogs on soil microorganisms and consequently on dehydrogenase activity was displayed when the analog was incorporated into soil. Analogs **5**, **7** and **11** generally showed the highest inhibition values of dehydrogenase activity by 64.90, 80.47 and 68.84%, respectively compared to standard carbendazim. A less inhibition effect was observed with analogs **4**, **6**, **9** and **10** (average decrease of 46.88, 53.30, 46.92, 43.29, 45.68 and 54.1%, respectively). Irrespective of untreated, analog **7** showed the highest suppressive for dehydrogenase activity of soil microorganisms compared to all carbendazim **1** and its sulfur analogs **[4-11]**. On the other hand sulfur analog **8** has the same inhibition value for the reduction of dehydrogenase activity as standard carbendazim. Analysis of variance showed the differences in antibacterial, antifungal and dehydrogenase activity between carbendazim and its sulfur analogs (table 2).

Structure-Activity Relationship (SAR)

Based on the obtained *in vitro* and *in vivo* antimicrobial activity, it is noted that the change in three different antimicrobial activities has significant relation to the alkyl as well as allyl groups attached to the sulfur atom of the dithioate group. The results derived from the antibacterial activity of carbendazim sulfur analogs **4** and **5** which sharing in side chain i.e. linked to the dithiocarbamate group possesses high antibacterial activity. Analog **4** possess a methyl group showed high potency against *E coli*, which might be refer to the facilitation of the interaction of the methyl group i.e. *in vitro* condition of the experiment and might undergo methylation for the DNA with different type of bacteria. The recognition that the activity decrease in case of elongation of the side chain linked to the sulfur atom through increasing of the number of CH_2 in the analog **5** might lead to decreasing the activity due to stereochemistry factor, whereas the interaction of the terminal methyl group is quietly restricted as the chain has been elongated. While the high potency of analogs **9** and **11** towards two types of bacteria (*E. coli* and *M. luteus*) might refer to the presence of phenyl group or terminal unsaturation site attached to a methylene group.

The antifungal activity of all the carbendazim sulfur analogs was investigated, the analogs **4**, **5**, **6** and **9** showed slight activity compared to carbendazim, which might be refer to the presence of dithiote group.

Analog **7** showed the highest inhibition of dehydrogenase enzyme *in vivo* investigation, this might be refer to the presence of branched hydrocarbon chain linked to dithiocarbamate group which might be interact with the DNA of the different soil microorganisms. While analog **10** exhibited less suppressive for dehydrogenase activity of soil microorganism compared to the other analogs. Finally, the antimicrobial activity of analog **8** showed the same effect on the inhibition of carbendazim. In general most of the synthesized carbendazim sulfur analogs **(4-11)** derivatives showed high to moderate biological activity, which might be attribute to many factors, for instance the presence of dithioate group, stereochemistry of the synthesized analogs and the active sides in these analogs as well as the presence of spacers. Therefore theses class of compounds consider as an interesting chemical core for many different agrochemical application of soil as well as for discrimination of the harmful fungi or bacteria inside the soil.

CONCLUSIONS

A series of novel carbendazim dithioate analogs were designed and synthesized as potential antimicrobial agents. All the synthesized carbendazim dithioate analogs were screened for *in vitro* and *in vivo* antimicrobial activity (antibacterial, antifungal and soil dehydrogenase activity). Some of them possess high potent activity against the Gram-negative and Gram-positive bacteria. The antifungal activity has been found less than the antibacterial activity of the all synthesized carbendazim sulfur analogs. The most sulfur analogs showed slight activity against *Fusarium solani* and *Fusarium oxysporum.* But most analogs showed inhibition hyphal growth of *Fusarium oxysporum* than *Fusarium solani*. Investigation of the dehydrogenase activity of soil

for sulfur analog **7** showed the highest suppressive activity against the soil microorganisms compared to all carbendazim and the other sulfur analogs. Whereas, some of the sulfur analogs possess the same effect on the reduction of dehydrogenase activities as carbendazim.

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