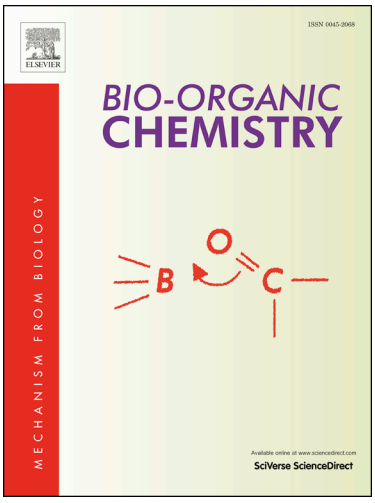

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The Novel Economical Synthesis and Antimicrobial Activity of a Trithiocarbonate Derivative

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Abstract

The compound diethyl 2,2'-(thiocarbonyl-bis(sulfanediyl))-diacetate **4** belongs to the trithiocarbonate class containing a trithiocarbonate function group flanked by ethyl acetate. In this procedure, a novel economic synthesis route to obtain compound **4** is described. This compound proved to possess broad-spectrum antimicrobial activity both *in vitro* and *in vivo,* and could be used as a lead compound. It is worth mentioning that this compound has been patented [No. US 9,988,348 B1; date of patent: June 5, 2018]

Keywords: Antimicrobial activity, diethyl 2,2' (thiocarbonyl bis (sulfanediyl)) diacetate; synthesis, trithiocarbonate.

1. Introduction

The trithiocarbonates have been investigated for years, with various synthetic routes having been employed for their synthesis [1]. They are simply and efficiently incorporated into many organic compounds and pharmaceutical therapeutic agents. In

organic synthesis, for instance, they have been used in surface and colloidal nanotechnology [2], as reagents for platinum group metals flotation [3], as an electrolyte additive in lithium batteries [1], for monodisperse surface modification [4] and for polymer formation [5]. As pharmaceutical therapeutic agents, they represent a promising class. As leads for novel biologically active compounds, they act as biological toxicants [6], anti-radiation agents in mice [7], antitumor agents [8,9], inhibitors for carbonic anhydrase, and anti-glaucoma effects *in vivo* [10]. Their activity as an inhibitor for carbonic anhydrase (CA) is seen through the formation of a mono-coordination bond of its sulfur atoms with the Zn(II) ion from the CA enzyme active site, as proved by X-ray crystal structure [11-13].

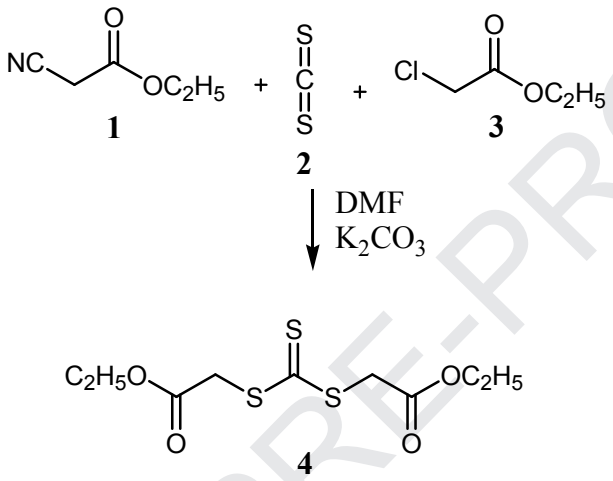
The development of antimicrobial agents never stops, but there is no doubt that the major microbial pathogens are increasingly developing resistance to the current antimicrobial drugs. Broad-spectrum antimicrobial agents must kill or inhibit specific targets in pathogenic microorganisms. These targets exist in most human microbial pathogens as part of an essential pathway. Absent from animal cells, not inhibited by broadly applied antimicrobial drugs, easy to evaluate *in vitro* and *in vivo*, highly specific for the pathogenic microorganisms, non-toxic for animals or humans, not result in the quick gaining of resistance [14,15].

Broad-spectrum antimicrobials have several advantages in initial empirical therapy, particularly against serious microbial infections. In a severe microbial disease, a therapy must be administered before the identification of the pathogenic microbe, because the time of diagnosis is a powerful predictor of mortality [16,17]. The patents of novel antifungal drugs include azole compounds (new imidazole, fuconazole analogs, azole with 2,6-di-tert-butyl-4-methylphenol), enzyme inhibitors (chitin inhibitors, β-1,3-D-glucan synthases inhibitors), peptides (fusion peptides), phenylalkane nitriles (esters of 2-phenylalkane nitriles), boron compounds, pyridine derivatives, cyclic guanidines and quinolinium compounds, and arylalkyl (azole derivatives in the structure of oxime ester) [18-20]. In the present research, we report on a simple and economical synthesis of diethyl 2,2' (thiocarbonyl bis (sulfanediyl)) diacetate **4** and investigate its biological activity as a novel broad-spectrum antimicrobial agent *in vitro* and *in vivo*.

2. Results and discussion

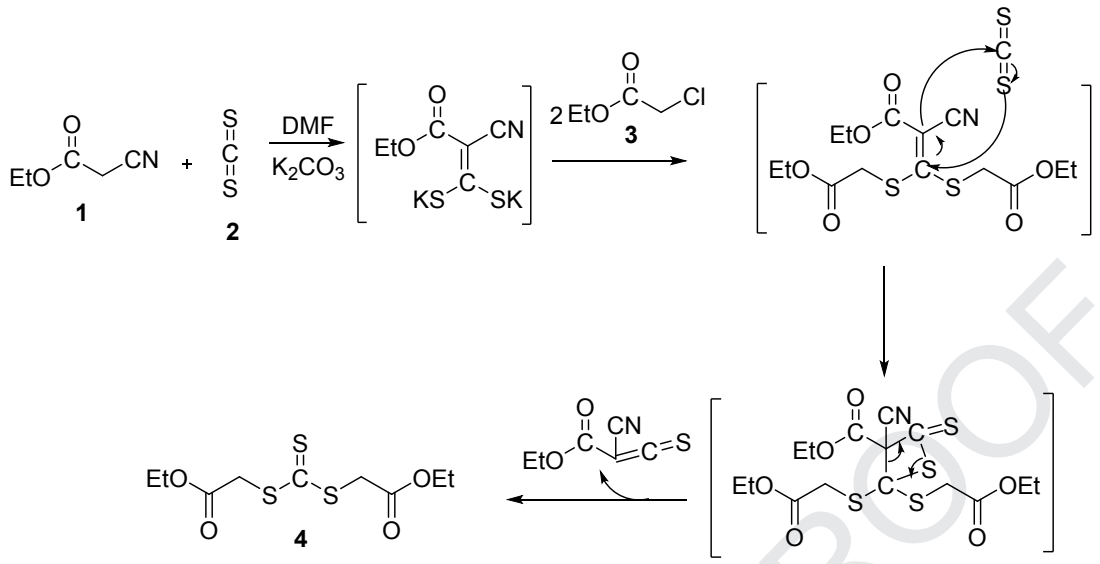
2.1. Chemistry

Trithiocarbonate **4** has been synthesized through a novel, simple and economical route from commercially available materials. Although compound **4** is available from the Sigma Company, it is very expensive; 5 mg costs $126.50 EUR. In comparison, the production of 5 mg costs $0.125 EUR, according to our synthetic route. Furthermore, Sigma noted on its website that compound **4** is rare and unique. However, that is not the case. The reaction of ethyl cyanoacetate **1**, carbon disulfide (CS_2) **2** and ethyl 2-chloroacetate **3** was used to obtain the trithiocarbonate **4,** as illustrated in Scheme 1.



Scheme 1. Synthesis of trithiocarbonate **4**

This reaction was carried out in the presence of anhydrous K_2CO_3 as a base in DMF at room temperature. An unsuccessful attempt was implemented to obtain trithiocarbonate by reacting CS_2 **2** and ethyl 2-chloroacetate **3** in the absence of ethyl cyanoacetate **1**. A plausible mechanism was then proposed to rationalize the formation of trithiocarbonate **4,** as depicted in Scheme 2.



Scheme 2. Mechanism proposed for the formation of trithiocarbonate **4**

The structure of the synthesized compound **4** was revealed by using IR, ^{1}H-NMR, ^{13}C-NMR, HRMS and X-ray single crystallography. The IR spectrum of this compound displayed the characteristic absorption of the carbonyl and thiocarbonyl groups at υ 1732 and 1155 cm^{-1}, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra showed the symmetrical pattern of compound **4.** The ^{1}H-NMR spectrum exhibited a triplet signal at δ 1.23 ppm, with coupling constants J = 7 Hz integrated for the protons assigned for the methyl group (-OCH$_2$***CH$_3$***), and a quartet signal at δ 4.16 ppm with coupling constants J = 7 Hz integrated for the protons assigned for the methylene group of the ester functional group (-O***CH$_2$***CH$_3$). The signal of protons assigned for the other methylene group (-S-***CH$_2$***CO-) appeared as a singlet at δ 4.14 ppm. In the ^{13}C-NMR spectrum, the peaks which appeared at δ 14.0, 38.9 and 62.1 ppm were attributed to (-OCH$_2$***CH$_3$***), (-S-***CH$_2$***CO-) and (-O***CH$_2$***CH$_3$), respectively. The carbonyl group peak appeared at δ 167.0 ppm, while the peak of thiocarbonyl downshifted and appeared at δ 220.2 ppm.

The x-ray single diffraction analysis clearly elucidated the molecular structure of compound **4**. The crystallographic data and refinement information for this compound are summarized in **Table 1**. The selected bond lengths and bond angles are listed in **Table 2**. The asymmetric unit contains one independent molecule, as shown in **Figure 1**. All the bond lengths and angles are within normal ranges [21]. Regarding the crystal packing, shown in **Figure 2**, the molecules are linked *via* three non-classical intermolecular hydrogen bonds (**Table 3)**.

Table 1. Experimental data

Crystal data	
Chemical formula	$C_9H_{14}O_4S_3$
Mr	282.38
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	293
a, *b*, *c* (Å)	15.1354 (7), 4.8723 (2), 18.6434 (8)
β (°)	91.866 (3)
V (Å3)	1374.11 (10)
Z	4
Radiation type	Cu *K*α
μ (mm^{-1})	4.93
Crystal size (mm)	0.37 × 0.19 × 0.12
Data collection	
Diffractometer	Bruker APEX-II D8 venture diffractometer
Absorption correction	Multi-scan SADABS Bruker 2014
Tmin, Tmax	0.266, 0.584
No. of measured, independent and observed [I > 2σ(I)] reflections	12063, 2196, 1348
R_{int}	0.060
Refinement	
R[F^2 > 2σ(F^2)], wR(F^2), S	0.077, 0.243, 1.03
No. of reflections	2196
No. of parameters	147
No. of restraints	2
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e $Å^{-3}$)	0.47, −0.36

Table 2. Selected geometric parameters (Å, °)

S1—C4	1.788 (5)	O1—C3	1.308 (9)
S1—C5	1.729 (5)	O2—C3	1.198 (8)
S2—C5	1.617 (4)	O3—C7	1.213 (8)
S3—C5	1.740 (5)	O4—C7	1.313 (9)
S3—C6	1.784 (5)	O4—C8	1.486 (11)
O1—C2	1.450 (12)		
C4—S1—C5	103.6 (2)	S1—C5—S2	126.5 (3)
C5—S3—C6	103.1 (2)	S1—C5—S3	108.1 (2)
C2—O1—C3	116.6 (7)	S2—C5—S3	125.5 (3)
C7—O4—C8	118.5 (6)	S3—C6—C7	114.4 (4)
O1—C2—C1	117.2 (10)	O3—C7—O4	124.8 (6)
O1—C3—O2	124.4 (6)	O3—C7—C6	124.6 (6)
O1—C3—C4	108.9 (5)	O4—C7—C6	110.6 (5)
O2—C3—C4	126.6 (6)	O4—C8—C9	111.7 (9)
S1—C4—C3	113.2 (4)		

Table 3. Hydrogen-bond geometry (Å, °)

D—H···A	D—H	H···A	D···A	D—H···A
C4—H4A···O2i	0.9900	2.4600	3.319 (7)	145.00
C6—H6A···O3ii	0.9900	2.5800	3.353 (7)	135.00
C6—H6A···O3iii	0.9900	2.6000	3.260 (8)	124.00
Symmetry codes: (i) x, y−1, z; (ii) x, y+1, z; (iii) −x+1, y+1/2, −z+3/2.				

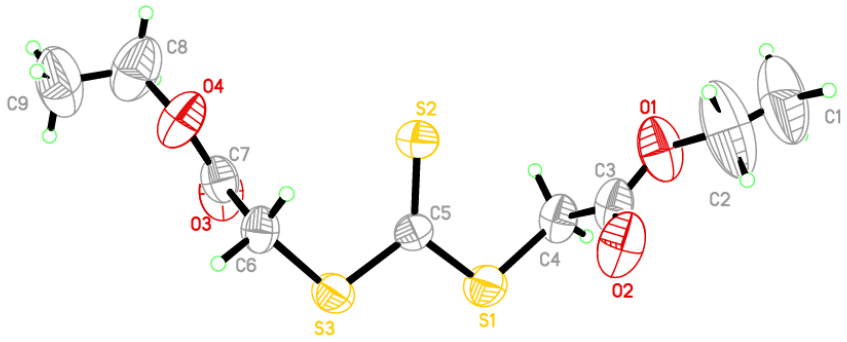


Figure 2. Molecular packing of compound **4** viewed as hydrogen bonds drawn as dashed lines along the *b* axis.

2.2. Biological activity tests

Antimicrobial agents are used in the treatment and prevention of microbial infections. These agents (drugs) have been produced by both biological and chemical methods. Many antimicrobial agents, such as sulfonamids, oxazolidinones and quinolones, are produced via chemical synthesis [22]. The production of a broad-spectrum antimicrobial agent is extremely significant when countering the threat of pathogenic microorganisms, particularly antibiotic resistant microorganisms. The production of broad-spectrum antibacterial and antifungal agents through chemical synthesis was the principle objective of this work.

The biological activity of compound **4** as an antimicrobial and anticancer agent was first evaluated. The results indicated that the compound showed no biological activity as an anticancer against the MCF-7 human breast cancer cell line. The data in **Table 4** did confirm that compound **4** showed biological activity as an antibacterial and antifungal agent against all pathogenic microorganisms that were used in this research. Both the water- and DMSO-soluble forms indicated efficacy against the microorganisms.

All tested fungi (*C. albicans, Cr. neoformans, A. niger, P. chrysogenum, Fusairum sp.)* except *C. parapsilosus* were significantly (P <0.05) susceptible to the compound. Regarding the *A. fumigatus* mold, the inhibition shown by the compound was significantly (P <0.05) stronger than that of the standard antifungal drugs Cycloheximide, Canesten and Caspofungin. The *A. fumigatus* used in this work is one of the strains isolated from humans who suffered from aspergillosis disease in the city of Riyadh, KSA, as seen in our previous study [23]. The *A. fumigatus* mold, which lives both outdoors and indoors, is considered a real risk for individuals with immunodeficiency or lung illnesses [24]. Compound **4** also showed high biological activity as an antifungal agent against *Cr. neoformans*, with the inhibition zone and minimal inhibitory concentration reaching 30 mm and 0.03125 mg/ml, respectively. The *Cr. neoformans* strain used in this study confirmed that Caspofungin has limited activity against this fungi [25]. This pathogenic encapsulated yeast causes several diseases called cryptococcosis [26]. The results in **Table 4** refer to the efficiency of compound **4** to inhibit both pathogenic yeasts and molds, particularly its effectiveness in inhibiting Cycloheximide, Canesten and Caspofungin resistant *C. parasilosus*. Both the gram-positive and -negative bacteria which were used in this research were

inhibited by compound **4** (**Table 4**). Additionally, the biological activity against *S. mutans* (plays a major role in tooth decay) and *N. meningitides* (can cause meningitis) was also very good.

Regarding *in vitro* uses, the present work concluded that compound **4** exhibits biological activity as a novel broad-spectrum antimicrobial agent.

To confirm the results obtained from the *in vitro* study, an animal experiment was also implemented. In this stage, the role of the water-soluble compound **4** was evaluated to treat mice infected with *C. albicans* (candidemia). The animal experiments add abundant evidence to support the use of Compound **4**. In the following results, Co1, Co2, FluCal, ComCal and Comp are given to several groups of mice, treated with sterile normal saline, infected with *C. albicans*, treated with Diflucan (fluconazole) (Pfizer Inc. New York, N.Y, U.S.A), treated with compound **4** or injected only with the chemical, respectively. In **Table 5**, a percentage change refers to the change in weight of the treated groups compared to the control group (Co1). At the end of the 15th day, the data showed that the percentage change of groups treated with fluconazole and compound **4** were convergent changes, whereas the percentage change of the group infected with *C. albicans* (Co2) was the highest. Food consumption (g/day/mice) and water consumption (ml/day/mice) were arranged as noted in **Tables 6** and **7**. Both fluconazaole and compound **4** had nearly identical effects on food and water consumption. The investigated compound **4** had no negative side effects on food and water consumption compared to the control group (**Tables 6, 7**). **Table 8** shows the daily mortality rate (%) in albino male mice infected with *C. albicans* and treated with fluconazole or compound **4**. The *C. albicans* caused dramatic increases in daily mortality compared to the control group. The results demonstrated that compound **4**, at a loading dose of 800 mg, followed by 400 mg/d for 2 weeks, reduced the mortality (%) caused by *C. albicans*. The results confirmed that compound **4** has very similar effects to fluconazole (**Table 8**). The mortality in the group treated only with compound **4** was also very similar to the control group.

The effectiveness of the previous treatments on the serum marker enzymes of the mice is shown in **Table 9**. The activity of the serum marker enzymes (SGOT, SGPT, alkaline phosphatase, GGT and bilirubin) significantly ($p<0.001$) increased in the group infected with *C. albicans* (negative control) when compared with the control group. The activity of serum marker enzymes also significantly ($p<0.001$) increased in the

group infected with *C. albicans* and treated with fluconazole, more so than the group only infected with *C. albicans*. The results obtained from the group treated with compound **4** showed significantly ($p<0.001$) enhancement in activity, with the exception of SGOT activity. In the group injected only with compound **4**, there was significantly ($p<0.01$) less SGOT activity than in the control group. **Table 10** includes the concentration of serum lipoproteins (mg/dl) in the tested mice. The data confirmed that the presence of *C. albicans* led to a significant ($p<0.001$) rise in cholesterol, triglycerides, VLDL and LDL level, whereas it caused a significant ($p<0.001$) decrease in the HDL level. These effects of *C. albicans* significantly ($p<0.001$) decreased in the groups treated with compound **4** and fluconazole. The compound **4** group showed a significant ($p<0.001$) decrease in cholesterol, triglycerides, VLDL and LDL levels, along with a non-significant increase in HDL, compared to the control group. The data in **Table 11** demonstrates that *C. albicans* caused a significant ($p<0.001$) increase in glucose and LDH, whereas it caused a significant ($p<0.001$) decrease in the total protein level in the serum blood of treated mice. The effects of *C. albicans* (**Table 11**) significantly decreased in the groups treated with compound **4** and fluconazole ($p<0.01$, $p<0.001$ respectively). The glucose and total protein level in serum blood of mice injected only with the compound **4** significantly decreased ($p<0.01$) and increased ($p<0.05$) respectively, while no significant changes were seen in LDH level.

The MDA, total protein and NP-SH in the liver tissues of the mice were estimated in the previous treatments (**Table 12**). Significant ($P<0.001$) differences were found between the control group and the negative control group infected with *C. albicans*. Compound **4** and fluconazole demonstrated similar results in reducing the biological effects of *C. albicans* (**Table 12**). **Table 13** illustrates that all hematologic parameters in the mice decreased in the group infected with *C. albicans*, when compared with those injected with sterile normal saline. The lymphocytes excludes from the previous results in **Table 13** showed significant ($p<0.001$) increase. The compound **4** and fluconazole groups noted a decrease in the hematologic changes resulting from *C. albicans,* with the exception of monocytes (%).

The data obtained from the in *vivo* test confirmed that compound **4** significantly reduces the activity of aspartate aminotransferase (AST), cholesterol, triglycerides, VLDL, LDL, and glucose, MDA, WBC and lymphocytes in the serum of mice. Additionally,

it significantly increases other hematologic parameters, the total protein of serum and the total protein of liver tissue.

Antimicrobial agents can be grouped based on their chemical structures into aminoglycosides, anasamacrolides, beta-lactams, chloramphenicol and analagues, lincosamides, macrolides, nucleosides, puromycin, peptides, phenazines, polyenes, polyethers and tetracyclines [27]. Recent scientific research has focused on the function and inhibition of fungal β-carbonic anhydrases and bacterial carbonic anhydrases (α-, β-, and/or γ- carbonic anhydrases familie) as novel antifungal and antibacterial targets [28,29]. Based on its chemical structure, compound **4** belongs to the trithiocarbonates class. This class is considered a strong inhibitor of carbonic anhydrases [10,30], which may explain the effects of compound **4** on the tested microorganisms.

The present results obtained from *in vitro* and *in vivo* experiments suggest that compound **4** may be a novel antibacterial and antifungal drug.

Table 4. Biological activity of soluble water compound **4** as an antimicrobial agent against some medical microorganisms.

		Compound **4** (4 mg/disk)		Inhibition zone diameter (mm) of standard antimicrobial agents					
				Standard antibacterial agent			Standard antifungal agent		
	Microorganisms	Inhibition zone diameter (mm)	Minimal inhibitory concentration (mg/ml)	Tobramycin (10 µg/disk)	Chloramphenicol (30 µg/disk)	Fusidic acid (10 µg/disk)	Cycloheximide (30 µg/disk)	Canesten (10 µg/disk)	Caspofungin (10 µg/disk)
Gram positive bacteria	*S. aureus*	10.6±0.5 F*	2	14	30	23	N.T	N.T	N.T
	S. mutans	20±1 D	0.0625	35	20	10	N.T	N.T	N.T
Gram negative bacteria	*E. coli*	11.6±0.5 E	1	20	40	0	N.T	N.T	N.T
	N. meningitides	22.6±0.5 C	0.0625	21	34	20	N.T	N.T	N.T
	Sa. typhimurium	8.6±0.5 F	2	14	30	0	N.T	N.T	N.T
Yeasts	*C. albicans*	20±1 D	0.0625	N.T	N.T	N.T	0	26	20
	C. neoformasn	30±1 B	0.03125	N.T	N.T	N.T	32	25	0
	C. parasilosus	13±1 E	0.05	N.T	N.T	N.T	0	0	0
Molds	*P. chrysoginum*	30.3±.5 B	0.03125	N.T	N.T	N.T	23	30	0
	A. fumigatus	34.6±0.5 A	0.03125	N.T	N.T	N.T	22	23	20
	Fusarium sp.	30±1 B	0.03125	N.T	N.T	N.T	30	15	0

*The mean of inhibition zone diameter (mm) ± Standard division. In the same column, the means with different letters are significant at $P< 0.05$.

Table 5. Daily body weight (gm) of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4** for 15 days.

Days↓	Co1*	Co2	FluCal	Comp.	CompCal.
0	19.20±0.35**	19.9±0.43	19.70±0.47	20.20±0.38	21.00±0.36
1	20.10±0.31	19.9±0.37	19.80±0.35	20.30±0.36	21.50±0.26
	4.68↑**	0.00	0.50	0.49	2.38
2	21.1±0.31	20.8±0.38	21.80±0.24	22.10±0.23	22.00±0.25
	9.89	4.52	10.65	9.40	4.76
3	21.7±0.21	21.7±0.3	22.30±0.21	23.60±0.33	22.3±0.21
	13.02	9.04	13.19	16.83	6.19
4	22.2±0.20	22.22±0.27	22.40±0.22	24.20±0.20	22.90±0.27
	15.62	11.66	13.70	19.80	9.04
5	22.3±0.21	24.00±0.26	23.90±0.31	24.90±0.23	23.55±0.37
	16.14	20.60	21.31	23.26	12.16
6	23.2±0.24	25.37±0.26	24.00±0.29	25.70±0.21	24.22±0.32
	20.83	27.51	21.82	27.22	15.34
7	23.9±0.23	25.12±0.29	24.22±0.22	26.20±0.24	24.66±0.23
	24.47	26.25	22.95	29.70	17.46
8	24.2±0.24	26.12±0.29	24.44±0.24	26.70±0.26	24.87±0.29
	26.04	31.28	24.08	32.17	18.45
9	24.9±0.23	27.00±0.48	24.50±0.18	26.80±0.29	25.25±0.25
	29.68	35.67	24.36	32.67	20.23
10	25.00±0.22	27.42±0.48	24.87±0.29	27.00±0.27	25.37±0.26
	30.20	37.83	26.29	33.66	20.83
11	25.70±0.22	27.85±0.40	25.25±0.25	27.40±0.35	26.14±0.26
	33.85	39.98	28.17	35.64	24.48

12	26.3±0.27	27.83±0.40	25.37±0.26	27.66±0.28	26.28±0.28
	36.97	39.86	-28.80	36.96	25.17
13	26.7±0.38	29.50±0.42	26.00±0.30	27.77±0.27	27.28±0.28
	39.06	48.24	31.97	35.51	29.93
14	27.55±0.29	29.16±0.47	26.28±0.28	28.00±0.37	28.33±0.49
	43.51	46.56	33.43	38.61	34.92
15	28.00±0.40	30.20±0.73	26.71±0.28	28.62±0.41	28.66±0.33
	45.83	51.78	35.60	41.70	36.50

* Co1= control group treated with sterile normal saline, Co2= negative control infected with *C. albicans*, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. **Mean±SE, ***% change in daily body weight (gm) compared with control group .

Table 6. Food consumption (g/day/mouse) of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

	Days														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Co1*	4.5**	4.6	4.6	4.7	4.8	5.0	5.2	5.5	5.7	5.7	5.9	5.9	6.0	6.1	6.2
Co2	4.1	4.1	4.3	4.2	4.5	4.6	4.8	4.8	4.5	4.8	5.5	5.6	5.5	5.8	6.0
FluCal	4.3	4.3	4.5	4.6	4.6	4.7	5.0	5.1	4.7	5.00	5.1	5.62	5.2	5.2	5.5
Comp.	4.2	4.5	4.4	4.5	4.6	4.6	4.8	4.9	4.9	5.1	5.2	6.0	6.0	6.1	6.1

CompCal.	4.5	4.6	4.7	4.9	4.00	4.2	4.2	4.3	4.5	4.5	4.7	5.0	5.14	5.3	5.5

* Co1= control group treated with sterile normal saline, Co2= negative control infected with *C. albicans*, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. ** Food consumption (g/day/mice).

Table 7. Water consumption (ml/day/mouse) of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

	Days														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Co1*	5.1**	5.1	5.2	5.3	5.3	5.4	5.4	5.6	5.8	5.7	5.8	5.8	5.9	6.1	6.2
Co2	4.4	4.5	4.5	4.4	4.6	4.8	5.0	5.1	5.0	5.1	5.1	5.0	5.3	5.5	6.0
FluCal	4.8	4.8	4.9	5.0	5.2	5.2	5.1	5.2	5.2	5.3	5.3	5.5	5.4	5.4	5.7
Comp.	5.2	5.2	5.4	5.5	5.5	5.7	5.8	5.8	5.9	5.9	6.0	6.11	6.2	6.2	6.3
CompCal.	4.5	4.5	4.7	4.9	4.2	4.6	5.7	4.6	4.6	4.8	5.1	5.4	5.5	5.5	5.8

* Co1= control group treated with sterile normal saline, Co2= negative control infected with *C. albicans*, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. ** Water consumption (g/day/mice).

Table 8. Daily mortality (%) in albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

	Days														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Co1*	0**	0	0	0	0	0	0	0	0	0	0	0	0	10	10
Co2	0	0	0	10	20	20	20	20	30	30	30	40	40	40	50
FluCal	0	0	0	0	0	0	10	10	20	20	20	20	30	30	30
Comp.	0	0	0	0	0	0	0	0	0	0	0	0	10	10	10
CompCal.	0	0	0	0	10	10	10	20	20	20	30	30	30	30	30

* Co1= control group treated with sterile normal saline, Co2= negative control infected with *C. albicans*, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. **Mortality (%).

Table 9. Activity of serum marker enzymes of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

	SGOT(AST) U/L		SGPT(ALT) U/L		Alkaline phosphatase E(U/L)		GGT (U/L)		Bilirubin (mg/dl)	
	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change
Co1*	107.45 ±2.71		27.07 ±0.53		296.88 ±4.35		4.94 ±0.14		0.54 ±0.01	
Co2	204.8 ±5.86 ***a	90.59 ↑	125.20 ±7.45 ***a	362.37 ↑	414.80 ±6.75 ***a	39.71 ↑	9.70 ±0.29 ***a	9617 ↑	1.67 ± 0.04 ***a	209.46↑
FluCal	143.28 ±2.88 *** b	30.03 ↓	50.48 ±3.17 ***b	59.67 ↓	321.28 ±6.91 ***b	22.54 ↓	5.60 ±0.17 ***b	42.26 ↓	0.75 ±0.03 ***b	55.13 ↓
Comp.	89.07 ±3.82 ** a	17.10 ↓	28.67 ±1.15 [a]	5.89 ↑	286.62 ±4.67 [a]	3.45 ↓	4.40 ±0.13 a	11.10 ↓	0.57 ±0.017 a	6.04 ↑
CompCal.	169.33 ±2.69 ***b	17.31 ↓	83.50 ±2.35 ***b	33.30 ↓	354.66 ±5.57 ***b	14.49 ↓	7.18 ±0.20 ***b	25.94 ↓	1.15 ± 0.03 ***b	31.16 ↓

* Co1= control group treated with sterile normal saline, Co2= negative control infected with C. albicans, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. All values represent mean ± SEM. ***p<0.001; Student's- t-test. [a] As compared with control group. [b] As compared with *C. albicans* group.

Table 10. Serum lipoproteins of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

Treatments	Cholesterol (mg/dl)		Triglycerides (mg/dl)		HDL(mg/dl)		VLDL(mg/dl)		LDL(mg/dl)	
	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change
Co1*	98.17 ± 2.1		85.3 ± 2.7		39.0 ± 0.7		17.0 ± 0.5		42.0 ± 2.2	
Co2	212.8 ± 6.0 ***a	116.7 ↑	186.8 ± 6.0 ***a	118.8 ↑	21.1 ± 0.9 ***a	45.8 ↓	37.3 ± 1.21 ***a	118.8 ↑	154.2 ± 5.7 ***a	267.1↑
FluCal	134.8 ± 3.6 ***b	36.6↓	105.1 ± 2.2 ***b	43.7↓	29.8 ± 0.9 ***b	41.1 ↑	21.0 ± 0.4 ***b	43.7↓	83.9 ± 3.3 ***b	45.5↓
Comp.	78.68 ± 3.2 ***a	19.85↓	68.5 ± 1.6 ***a	19.6↓	41.6 ± 2.1[a]	6.42 ↑	13.7 ± 0.3 ***a	19.6↓	23.3 ± 3.0 ***a	44.35↓

CompCal.	159.8 ± 3.4 ***b	24.8↓	134.8 ± 3.8 ***b	27.8↓	26.9 ± 1.1 **b	27.2 ↑	26.9 ± 0.7 ***b	27.8↓	105.9 ± 4.6 ***b	31.3↓

* Co1= control group treated with sterile normal saline, Co2= negative control infected with C. albicans, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. All values represent mean ± SEM. **p<0.01; ***p<0.001; Student's- t-test. [a] As compared with control group. [b] As compared with *C. albicans* group.

Table 11. Serum glucose, total protein and LDH of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

Treatments	**Glucose level (mg/dl)**		**Total Protein (mg/dl)**		**LDH**	
	Mean±SE	**% Change**	**Mean±SE**	**% Change**	**Mean±SE**	**% Change**
Co1*	113.95 ±2.96		7.11 ±0.15		158.84 ±3.00	
Co2	204.2 ±7.58***a	79.19↑	3.55 ±0.08***a	50.05↓	245.18 ±5.12***a	54.35↑
FluCal	143.85 ±2.47***b	29.55↓	5.64 ±0.11***b	58.79↑	184.92 ±5.08***b	24.57↓
Comp.	96.38 ±3.62** a	15.41↓	7.66 ±0.18* a	7.69↑	162.50 ±1.84 a	2.29↑
CompCal.	166.83 ±4.32**b	18.29↓	4.91 ±0.09***b	38.14↑	220.98 ±3.96**b	9.86↓

* Co1= control group treated with sterile normal saline, Co2= negative control infected with C. albicans, Flucal= group infected with C. albicans and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with C. albicans and treated with

compound **4**. All values represent mean ± SEM. **p<0.01; ***p<0.001; Student's- t-test. [a] As compared with control group. [b] As compared with *C. albicans* group.

Table 12. MDA, Total Protein and NP-SH in liver tissue of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

Treatments	MDA (nmol/g)	Total Protein (g/l)	NP-SH (nmol/g)
Co1*	1.24±0.02	115.23±1.80	5.70±0.14
Co2	6.36±0.25***[a]	65.14±2.06***[a]	1.61±0.14***[a]
FluCal	2.33±0.07***[b]	92.72±1.54***[b]	4.21±0.18***[b]
Comp.	0.94±0.03***[a]	129.04±2.80**[a]	6.88±0.22***[a]
CompCal.	4.57±0.25***[b]	77.84±2.37**[b]	3.56±0.16***[b]

* Co1= control group treated with sterile normal saline, Co2= negative control infected with C. albicans, Flucal= group infected with C. albicans and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. All values represent mean ± SEM. **p<0.01; ***p<0.001; Student's- t-test. [a] As compared with control group. [b] As compared with *C. albicans* group.

Table 13. Hematological studies of albino male mice infected with *C. albicans* and treated with fluconazole and compound **4**.

Treatments	Co1*	Co2		FluCal		Comp.		CompCal.	
	Mean±SE	Mean±SE	% Change	Mean±SE	% Change	Mean±SE	% Change	Mean± SE	% Change
WBC (Cell/mm^3)	8582.2 ±17.7	6513.6 ±24.0 ***a	24.1↓	8233.2 ±83.7 *** b	26.4↑	8795 ±45.9 ** a	2.48↑	7540.8 ±87.3 *** b	15.9 ↑
RBC (10^6/$_{mm}^3$)	8.14 ±0.17	4.46 ±0.19 *** a	45.23 ↓	7.50 ±0.23 *** b	68.16 ↑	9.26 ±0.1 ** a	13.72 ↑	6.16 ±0.1 *** b	38.2 ↑
HGB (g/dL)	11.9 ±0.18	7.62 ±0.16* ** a	17.9↓	11.1 ±0.18 *** b	46.7↑	12.4 ±0.18 a	3.4↑	9.8 ±0.14 *** b	28.6 ↑
HCT	42.48 ±1.47	23.3 ±1.54 *** a	44.9↓	35.05 ±1.5 *** b	49.9↑	45.9 ±1.51 a	8.2↑	31.3 ±0.42 *** b	33.8 ↑
PLT (10^3/$_{mm}^3$)	618.0 ±15.5	441.2 ±24.7 *** a	28.6↓	563.8 ±17.4 ** b	27.8↑	670.7 ±15.3 * a	8.5↑	522.3 ±10.1 * b	18.3 ↑
MCV (fL)	75.84 ±3.72	43.5 ±2.5 *** a	42.5↓	72.04 ±2.6*** b	65.4↑	87.31 ±1.74 * a	15.1↑	53.7 ±2.0 * b	23.4 ↑
MCH (pg)	29.9 ±0.97	21.2 ±0.5 *** a	28.9↓	28.2 ±0.7*** b	32.6↑	39.0 ±1.0 *** a	30.4↑	26.3 ±0.5 *** b	23.6 ↑

MCHC (g/dL)	27.87 ±0.94	19.3 ±0.7 *** a	30.6↓	24.3 ±0.5*** b	25.9↑	32.6 ±0.9 ** a	17.0↑	23.4 ±1.0 * b	21.1 ↑
Neutrophils (%)	28.88 ±0.71	21.0 ±0.4 *** a	27.3↓	25.9 ±0.5 *** b	23.3↑	33.3 ±1.1 ** a	15.3↑	24.1 ±0.2 *** b	15.0 ↑
Lymphocytes(%)	5.49 ±0.10	8.8 ±0.2 *** a	60.7↑	6.2 ±0.1 *** b	29.2↑	4.8 ±0.09 ** a	11.3↓	7.10 ±0.07 *** b	19.4 ↓
Eosinophils (%)	1.74 ±0.04	0.96 ±0.05 *** a	44.8↓	1.61 ±0.10 *** b	68.3↑	2.38 ±0.09 *** a	36.7↑	1.30 ±0.02 *** b	36.1 ↑
Monocytes (%)	1.04 ±0.02	0.96 ±0.02* a	7.98 ↓	1.06 ±0.02* b	11.16 ↑	1.15 ±0.01** a	10.7 ↑	0.88 ±0.07 b	7.9 ↓

* Co1= control group treated with sterile normal saline, Co2= negative control infected with C. albicans, Flucal= group infected with *C. albicans* and treated with fluconazole, Comp= group treated with compound **4** and CompCal= group infected with *C. albicans* and treated with compound **4**. All values represent mean ± SEM. **p<0.01; ***p<0.001; Student's- t-test. [a] As compared with control group. [b] As compared with *C. albicans* group.

3. Material and methods

3.1. Chemistry

All chemicals were obtained from commercial sources, including Sigma-Aldrich (St. Louis,MO, USA), and were used as received without further purification, unless otherwise stated. Melting points were measured on a Gallenkamp melting point apparatus (Thermo Fisher Scientific, Paisley, UK) in open glass capillaries and are uncorrected. Infrared spectra (IR) were recorded using the KBr disc technique on a Perkin Elmer FT-IR spectrophotometer 1000 (PerkinElmer, Waltham, MA, USA). ^{1}H-

and^{13}C-NMR spectra were obtained with either a JEOL ECP 600 NMR spectrometer (Tokyo, Japan) operating at 600 MHz z in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6). Chemical shifts are expressed in δ units and J-coupling constants are given in Hz. Mass spectra were acquired with the aid of a Shimadzu GCMS-QP 1000 EXmass spectrometer (Tokyo, Japan) at 70 eV. Elemental analysis was carried out on a Perkin Elmer 2400Elemental Analyzer; CHN mode.

3.1.1. Synthesis of diethyl 2,2'-(thiocarbonylbis(sulfanediyl))diacetate 4

A mixture of ethyl cyanoacetate **1** (0.1 mol) and anhydrous K_2CO_3 (30 g) in DMF (35 mL) was stirred vigorously at 25 ^{0}C for 10 min, and then CS_2 **2** (0.2 mol) was added with stirring for 50 min. The mixture reaction was cooled in an ice bath. Then, ethyl 2-chloroacetate **3** (0.2 mol) was added with continued stirring for 15 min. The cooling bath was subsequently removed, and the mixture was stirred for 3 h. The solid product was precipitated by the addition of H_2O collected by filtration, washed with H_2O, and dried. The compounds were recrystallized from EtOH to afford two crystalline materials: ethyl 3-amino-4-cyano-5-((2-ethoxy-2-oxoethyl)thio)thiophene-2-carboxylate, Yield: 60%; m. p. 93° and diethyl 2,2'-(thiocarbonylbis(sulfanediyl))diacetate **4.** These compounds had the following characteristics: Yield: 20%; m. p. 40 ºC; IR (KBr, cm^{-1}) ύ = 2978, 2930, 1732, 1475, 1367, 1300, 1202, 1155, 1095, 1069; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 1.23 (t, J = 7 Hz, 3H, -CH_3), 4.14 (s, 2H, S-CH_2-CO), 4.16 (q, 2H, J = 7 Hz, -O-CH_2-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ = 14.0, 38.9, 62.1, 167.0, 220.2; MS m/z: 283.0 $[M+H]^+$, 267.1, 239.1, 207.0; Elemental Analysis: C, 38.28; H, 5.00; S, 34.06, Found: C, 38.31; H, 5.12; S, 33.97

3.1.2. Single-Crystal X-ray Diffraction of Compound 4

Compound **4** was obtained as single crystals by slow evaporation from an ethanol solution of the pure compound at room temperature. Data were collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Cu *K*α radiation, λ = 1.54178 Å at 293 (2) K. Cell refinement and data reduction were carried out by a Bruker SAINT. SHELXT [31,32] was used to reveal the structure. The final refinement was carried out using the full-matrix least-squares technique with anisotropic thermal data for non-hydrogen atoms on ??. CCDC 1522608 contains the supplementary crystallographic data for this compound and can be obtained free of charge

from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

3.2. Biological activity tests

3.2.1. *In vitro*, antimicrobial activity

To evaluate the antimicrobial activity of compound **4** as a novel anti-bacterial and anti-fungal agent, the inhibition zone diameter (mm) and the minimal inhibitory concentration were determined according to literature reports [33,34]. The tests were carried out on several clinical microorganisms, including gram positive bacteria (*Staphylococcus aureus* ATCC and *Streptococcus mutans* ATCC 35668), gram negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Neisseria meningitides* ATCC 1302), yeasts (*Candid albicans* ATCC 60193, *Cryptococcus neoformans* Wild strain and *Candida parapsilosus* ATCC 22019), and molds (*Aspergillus fumigatus* AUMC 8794, *Penicillum chrysogenum* AUMC 9476 and *Fusarium* sp.).

The clinical microorganisms were cultivated at least three times with suitable culture mediums and incubating conditions before the susceptibility tests. The concentrations of microbial inoculation suspensions in sterile sodium chloride solution (0.89 %) were adjusted to 0.65 at 620 nm by spectrophotometer. In the first stage, 150 mg of compound **4** was dissolved in 2000 µL of DMSO, then 40 µL of solution was added on a sterile filter disk (6 mm) to obtain 3 mg per disk. To prepare a water soluble form of compound **4**, 150 mg of compound **5** was treated with sodium ethoxide and dissolved with 2000 µL of sterile distilled water; then, 40 µL of solution was added to the filter disk (3 mg/disk). DMSO- and water-soluble forms of compound **4** were investigated using disk diffusion assay to determine the inhibition zone (mm) resulting from 3 mg of compound per disk.

The disks were then dried at 25^0 C under sterile conditions using a safety biological cabinet. Mueller-Hinton agar (MHA) (Scharlau Microbiogy, Spain) and potato dextrose agar (PDA) (Scharlau, Spain) were used to perform antibacterial and antifungal tests, respectively. The media were prepared according to the manufacturer's guidelines. To prepare one liter of MHA and one liter of PDA, 21 grams of MHA and 39 grams of PDA were dissolved in one liter of sterile distilled water, then sterilized at 121 °C for

15 minutes by autoclave (HL-321, Taiwan). The sterilized media were poured into sterile plastic petri dishes at 50 ºC. The media were allowed to solidify at room temperature. Next, 0.1 ml of microbial inoculation suspension was spread on the dried surface of the medium and kept at 25 ^{0}C for 5-10 minutes. Compound **4** and the standard antimicrobial drug discs (Table 1) were dispensed on the surface of the inoculated media. The bacteria were incubated at 37±1 ºC for 19-22 hours and the fungi at 25±1 ºC for 48-72 hours. After incubation, the diameters of the inhibition zones (millimetre) resulting from the biological activity of the compound were recorded.

The minimal inhibitory concentration (MIC) of compound **4** (water-soluble form) was determined by microdilution assay, using a Mueller-Hinton broth for bacteria and a potato dextrose broth for fungi. Compound **4** was dissolved in a sterile culture broth (2 mg/mL). Two-fold serial dilutions were carried out to obtain concentrations ranging from 2 to 0.008 mg/mL. Each dilution of 95 µL was added to 96-well plates, then each well was inoculated with 5 µL of inoculation microbial suspension (5×10^8 colony form unit (CFU)/ml). The plates were incubated at conditions suitable for the tested microorganism. After incubation, microbial growth was detected in each well by a *p*-iodonitrotetrazolium violet reagent (Sigma, USA). Each well received 20 µL of the reagent (0.5 mg/ml), and was then incubated at 37±1 ºC for 30 min. A violet color indicated microbial growth in the well. The minimal inhibitory concentration (mg/ml) of compound **4** was estimated as the lowest concentration that inhibited microbial growth.

3.2.2. Anticancer test

The biological activity of compound **4** (water soluble form) as an anticancer agent for human breast cancer was evaluated according to a previous study [35]. Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma, USA) was prepared according to the manufacturer's guidelines and supplemented with fetal bovine serum (10%). To avoid microbial contamination, streptomycin and penicillin were added to RPMI-1640. Several concentrations of compound **5**, ranging from 4 to 0.008 mg/mL**,** were tested.

3.2.3. *In vivo*, antifungal activity

To confirm that the water-soluble form of compound **4** has biological activity as a novel antimicrobial agent, mice were treated with candidemia and the effectiveness of

compound **4** was investigated. The experiment was performed in the Experimental Animal Care Center (EACC), College of Pharmacy, King Saud University, Riyadh. Fifty albino male mice weighing nearly 30±2 gram were used. The mice were arranged in five groups, ten mice in each cage, and given water and feed ad libitum. The negative control group was infected intravenously with 0.2 ml *of C. albicans* suspension (10^6 CFU/mouse), while the control groups received 0.2 ml of sterile normal saline (0.89% Nacl). The group treated with the standard drug (fluconazole) was infected intravenously with 0.2 ml *of C. albicans* suspension (10^6 CFU/mouse) and treated (orally) by 12 mg/kg of fluconazole (loading dose) on the first day, followed by 6 mg/ml for 2 weeks. The group treated with the investigated compound **4** was infected intravenously with *C. albicans* at 10^6 CFU/mouse (0.2 ml) and treated (orally) by 12 mg/kg of compound **4** (loading dose) on the first day, followed by 6 mg/ml for 2 weeks. To study the biological effects of compound **4**, a fifth group was designated. In this group, the mice were treated intravenously (I.V) with 12 mg/kg of compound **4** on the first day, followed by 6 mg/ml for 2 weeks. All doses of the standard drug and compound **4** were given twice daily.

The effectiveness of the treatments on some biological aspects was evaluated. The biological parameters included daily body weight (gm), mortality (%), food consumption (g/day/mouse), water consumption (ml/day/mouse), serum glutamic oxaloacetic transaminase [SGOT(AST) (U/L)], serum glutamic-pyruvic transaminase [SGPT(ALT)(U/L)], alkaline phosphatase (U/L), gamma-glutamyl transferase (GGT) (U/L), bilirubin (mg/dl)], cholesterol (mg/dl), triglycerides (mg/dl), high-density lipoprotein (HDL) (mg/dl), very-low-density lipoprotein (VLDL) (mg/dl), low-density lipoprotein (LDL) (mg/dl), lactate dehydrogenase (LDH) (U/L), glucose (mg/dl), total protein (mg/dl), malondialdehyde (MDA) (nmol/g), nonprotein sulfyhydryl (NP-SH) (nmol/g). Additional hematologic constituents, including white blood cells (WBC) (cell/mm^3), red blood cells (RBC) (10^6/mm^3), hemoglobin (HGB) (g/dL), HCT, platelets (PLT) (10^3/mm^3), mean corpuscular volume (MCV) (fL), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dL), neutrophils (%), lymphocytes (%), eosinophils (%) and monocytes (%)] were also evaluated [36-38]. SGOT (AST), SGPT (ALT), ALP, GGT, bilirubin LDH and total protein were estimated using a Reflotron Plus Analyzer and Roche kits (Roche Diagnostics GmbH, Mannheim Germany) and United diagnostic kits assay.

Hematological tests were performed by Mindray BC-2800VET Auto Hematology Analyzer.

3.2.4. Experimental design and statistical analysis

The present experiment was designed as a completed random design (CRD). The data are arranged as mean± standard deviation (SD) or standard error of mean (SEM). The Statistical Package for the Social Sciences (SPSS 13.0) was used to analyze the mean of the biological parameters by using the Tukey post-hoc and Student's- t-test.

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References

[1] H. S. Kim, D. Q. Nguyen, M. Cheong, H. Kim, H. Lee, N. H. Ko, J. S. Lee. One-pot synthesis of ethylene trithiocarbonate from ethylene carbonate. Applied Catalysis A: General 337(2) (2008) 168-172.

[2] S. Efrima, N. Pradhan Xanthates and related compounds as versatile agents in colloid science. Comptes Rendus Chimie 6(8) (2003) 1035-1045.

[3] C. Vos, J. Davidtz, J. D. Miller. Trithiocarbonates for PGM flotation. J. South African Institute of Mining and Metallurgy, 107(1) (2007) 23.

[4] K. Ohno, Y. Ma, Y. Huang, C. Mori, Y. Yahata, Y. Tsujii, T. Maschmeyer, J. Moraes, S. Perrier. Surface-initiated reversible addition–fragmentation chain transfer (RAFT) polymerization from fine particles functionalized with trithiocarbonates. Macromolecules 44(22) (2011) 8944-8953.

[5] X. Wang, Y. Shi, R. W. Graff, X. Cao, H. Gao. Synthesis of Hyperbranched Polymers with High Molecular Weight in the Homopolymerization of Polymerizable Trithiocarbonate Transfer Agent without Thermal Initiator. Macromolecules 49(17) (2016) 6471-6479.

[6] Jr. H. Godt, R. Wann The Synthesis of Organic Trithiocarbonates1. The Journal of Organic Chemistry 26(10) (1961) 4047-4051.

[7] W. O. Foye, J. Mickles, R. N. Duvall, J. R. Marshall. Antiradiation Compounds. IV. Trithiocarbonates of β-Mercaptoethylguanidines1. Journal of medicinal chemistry 6(5) (1963) 509-512.

[8] Z. M. Rzayev, M. Tűrk, E. A. Söylemez. Bioengineering functional copolymers. XXI. Synthesis of a novel end carboxyl-trithiocarbonate functionalized poly (maleic anhydride) and its interaction with cancer cells. Bioorganic & medicinal chemistry 20(16) (2012) 5053-5061.

[9] F. Dehmel, T. Ciossek, T. Maier, S. Weinbrenner, B. Schmidt, M. Zoche, T. Beckers. Trithiocarbonates—exploration of a new head group for HDAC inhibitors. Bioorganic & medicinal chemistry letters 17(17) (2007) 4746-4752.

[10] F. Carta, A. Akdemir, A. Scozzafava, E. Masini, C. T. Supuran. Xanthates and trithiocarbonates strongly inhibit carbonic anhydrases and show antiglaucoma effects in vivo. Journal of medicinal chemistry 56(11) (2013) 4691-4700.

[11] A. Innocenti, A. Scozzafava, C. T. Supuran. Carbonic anhydrase inhibitors. Inhibition of cytosolic isoforms I, II, III, VII and XIII with less investigated inorganic anions. Bioorganic & medicinal chemistry letters 19(7) (2009) 1855-1857.

[12] A. Innocenti, A. Scozzafava, C. T. Supuran. Carbonic anhydrase inhibitors. Inhibition of transmembrane isoforms IX, XII, and XIV with less investigated anions including trithiocarbonate and dithiocarbamate. Bioorganic & medicinal chemistry letters 20(5) (2010) 1548-1550.

[13] C. Temperini, A. Scozzafava, C. T. Supuran. Carbonic anhydrase inhibitors. X-ray crystal studies of the carbonic anhydrase II–trithiocarbonate adduct—An inhibitor mimicking the sulfonamide and urea binding to the enzyme. Bioorganic & medicinal chemistry letters 20(2) (2010) 474-478.

[14] T. Meinnel, Developing a rational strategy for new antibacterial agents. Pathologie-biologie 47(8) (1999) 780.

[15] C. Giglione, M. Pierre, T. Meinnel. Peptide deformylase as a target for new generation, broad spectrum antimicrobial agents. Molecular microbiology 36(6) (2000) 1197-1205.

[16] L. A. Mandell, , R. G. Wunderink, A. Anzueto, J. G. Bartlett, G. D. Campbell, N. C. Dean, S. F. Dowell, T. M. File, Jr. D. M. Musher, M. S. Niederman, A. Torres, C. G. Whitney. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clinical infectious diseases 44(Supplement 2) (2007) S27-S72.

[17] M. H. Kollef, Broad-spectrum antimicrobials and the treatment of serious bacterial infections: getting it right up front. Clinical infectious diseases 47(Supplement 1) (2008) S3-S13.

[18] G. L. I. Roger, E. Lim, M. B. Fadem, Anti-fungal peptides, Google Patents (2003).

[19] M. V. Castelli, E. Butassi, M. C. Monteiro, L. A. Svetaz, F. Vicente, S. A Zacchino. Novel antifungal agents: a patent review (2011–present). Expert opinion on therapeutic patents 24(3) (2014) 323-338.

[20] S. Dalkara, S. S. Tarhan, A. Karakurt. Antifungal compounds of (arylalkyl) azole derivatives in the structure of oxime ester, Google Patents (2014).

[21] F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, R. Taylor. Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds. Journal of the Chemical Society, Perkin Transactions 2(12) (1987) S1-S19.

[22] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich. Antibacterial natural products in medicinal chemistry—exodus or revival? Angewandte Chemie International Edition 45(31) (2006) 5072-5129.

[23] K. Niazi, J. M. A. Khaled, S. A Kandeal, A. S. Khalel. Assessment Techniques to Detect Aspergillus fumigatus in Different Samples of Immunosuppressed Male Western Albino Rats. Jundishapur journal of microbiology 7(11) (2014) 1-5.

[24] C. M. O'Gorman, H. T. Fuller, P. S. Dyer. Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature 457(7228) (2009) 471-474.

[25] E. J. Ernst, M. E. Klepser, M. Pfaller. Postantifungal Effects of Echinocandin, Azole, and Polyene Antifungal Agents against Candida

albicans andCryptococcus neoformans. Antimicrobial agents and chemotherapy 44(4) (2000) 1108-1111.

[26] K. Tripathi, V. Mor, N. K. Bairwa, M. D. Poeta, B. K. Mohanty Hydroxyurea treatment inhibits proliferation of Cryptococcus neoformans in mice. Frontiers in Microbiology 3(187) (2012)1-7.

[27] N.Okafor, Modern industrial microbiology and biotechnology, CRC Press. (2016).

[28] C. T. Supuran, Bacterial carbonic anhydrases as drug targets: toward novel antibiotics? Frontiers in pharmacology 2 (2011) 34.

[29] Lehneck, R. and S. Pöggeler Fungal Carbonic Anhydrases and Their Inhibition. (2016).

[30] D. Vullo, M. Durante, F. S. Di Leva, S. Cosconati, E. Masini, A. Scozzafava, E. Novellino, C. T. Supuran, F. Carta. Monothiocarbamates strongly inhibit carbonic anhydrases in vitro and possess intraocular pressure lowering activity in an animal model of glaucoma. Journal of medicinal chemistry (2016).

[31] G. M. Sheldrick, SHELXTL-PC (Version 5.1) Siemens Analytical Instruments Inc., Madison, WI, USA (1997)

[32] G. M. Sheldrick, A short history of SHELX. Acta Crystallographica Section A: Foundations of Crystallography 64(1) (2008) 112-122.

[33] J. M. Andrews, Determination of minimum inhibitory concentrations." Journal of antimicrobial Chemotherapy 48(suppl 1) (2001) 5-16.

[34] J. Hudzicki, Kirby-Bauer disk diffusion susceptibility test protocol." American Society for Microbiol, (2009).

[35] P. S. Kumar, R. M. Febriyanti, F. F. Sofyan, D. E. Luftimas, R. Abdulah. Anticancer potential of Syzygium aromaticum L. in MCF-7 human breast cancer cell lines. Pharmacognosy research 6(4) (2014) 350-354.

[36] O. Classics Lowry, N. Rosebrough, A. L. Farr, R. J. Randall. Protein measurement with the Folin phenol reagent. J biol Chem 193 (1951) 265-275.

[37] H. G. Utley, F. Bernheim, P. Hochstein. Effect of sulfhydryl reagents on peroxidation in microsomes. Archives of Biochemistry and Biophysics 118(1) (1967) 29-32.

[38] J. Sedlak, R. H. Lindsay Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry 25 (1968) 192-205.

Graphical abstract

The Novel Economical Synthesis and Antimicrobial Activity of a Trithiocarbonate Derivative

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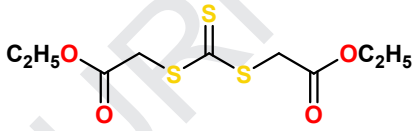

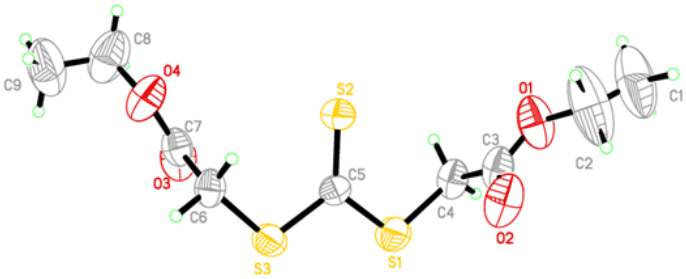

Highlights

- A novel economic synthesis route to obtain diethyl 2,2'-(thiocarbonyl-bis(sulfanediyl))-diacetate is described.

- Antimicrobial activity of this compound was evaluated as antimicrobial agent.
- This compound proved to possess broad-spectrum antimicrobial activity both *in vitro* and *in vivo*
- This compound has been patented [No. US 9,988,348 B1; date of patent: June 5, 2018]