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Synthesis of pentadecanyl-amino thiadiazole pharmacophores and their antimicrobial assessments

Yvette A. Issac[a], Shaaban K. Mohamed[b*], Abd El-Monem M. F. Eissa[a], Ahmed H. Tantawy[a], Abdallah A. El-Sawy[a]

[a]*Chemistry Department, Faculty of Science, Benha University, 13518-Benha, Egypt.*
[b]*Chemistry and Environmental Division, Manchester Metropolitan University, Manchester, M1 5GD, England*

ABSTRACT

*Several fatty chain palmitylthiadiazole derivatives have been synthesized by reaction of palmitic acid with thiosemicarbazide in presence of phosphorous oxytrichlorideto afford the corresponding 5-pentadecanyl-amino thiadiazole which in turn has been reacted via its nucleophilic amino group with different reagentssuch as acid chlorides, acid anhydrides, aldehydes and isocyanates to give the corresponding 5-pentadecanyl-2-amino-1,3,4-thiadiazole derivatives in an excellent yield.The antimicrobial study of all products has been screened and showed that most of thiadiazolecompounds exhibited high to moderate inhibitory effect particularly the acetamidothiadiazoloe**2***which showed the highest effect against all employed microorganisms. Structure of all products wascharacterised by IR, ^{1}H-NMR, Mass spectra and elemental analyses.*

Keywords: Amino thiadiazoles; palmitoyl chloride; fatty acids; anti-microbial; sulphur containing compounds.

INTRODUCTION

1,3,4-Thiadiazole derivatives are of great interest class of heterocyclic compounds having an important wide range of pharmacological properties. They showed anticonvulsant[1-5], analgesic[6,7], anti-secretory [8], and antimicrobialactivities[9-13]. Among these compounds 2,5-disubstituted -1,3,4- thiadiazole derivatives havebeen reported to exhibit antibacterial activity[14,15],antitypanosomal profile [16], anti-tubercular activity [17] and anticancer [18,19]. In addition, 2,5-disbstituted-1,3,4- thiadiazole derivatives have used as effective, cheap, and safe drugs for the treatment of leishmaniasis[20]. Based on these facts and on following to our strateigy in synthesis of biologically active molecules [21-23]we get prompted to utilize palmitic acid as a target material in synthesis of new thiadiazole derivatives carrying a long aliphatic chain at position (5) and studying their biological activities.

EXPERIMENTAL SECTION

Melting points are uncorrected and determined by the open capillary method using Gallen Kamp melting point apparatus.Spectrophotometer (KBr disk) of the synthesized compounds was recorded on FT/IR-BRUKER, Vector 22(Germany).Microanalyses were carried out by Micro Analytical Unit at Cairo University.^{1}HNMR Spectra were recorded in deuterated chloroform (CDCL3) or dimethylsulphoxide (DMSO-d6) on a Varian Germini-200 MHz instrument.Mass Spectra were recorded on HP-MODEL MS-5989A (U.S.A) and/or Shimadzu GCMS-QP-1000EX mass spectrometer at 70e.v.All reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60 F254 (Mark) plates. The physical properties of the newly synthesized compounds **1 -10**are tabulated in tables 1.The synthesized compounds were tested for biological activities in Botany Department, Faculty of Science, Benha University.

Materials

Starting materials: Plmitic acid, thiosemicarbazide, phosphorous oxychloride, acid anhydrides, acid chlorides and aldehydeswere used as received from chemical suppliers. All employed solvents have been used as commercially provided.

Table 1: Physical properties of thiadiazole compounds1 -10.

No.	M.F.	M.wt	Solvent of crystallsn.	Yield %	M.P (^{0}C)	Analysis data calc/ found %			
						C	H	N	S
1	$C_{17}H_{33}N_3S$	311.53	Benzene	95	130	65.54 65.30	10.68 10.54	13.49 13.13	10.29 10.00
2	$C_{19}H_{35}N_3SO$	353.57	Ethanol	90	135	64.54 64.22	9.98 10.21	11.88 11.65	9.07 8.78
3	$C_{24}H_{38}N_4SO$	430.65	Ethanol	70	140	66.94 66.78	8.89 8.66	13.01 12.79	7.45 7.18
4	$C_{24}H_{37}N_3SO$	415.64	Ethanol	85	140	69.35 69.17	8.97 9.18	10.11 9.86	7.71 7.41
5	$C_{24}H_{36}N_4SO_3$	460.63	n-butanol	85	147-150	62.58 62.33	7.88 8.02	12.16 12.43	6.96 7.22
6	$C_{19}H_{34}N_3SOCL$	388.01	Ethanol	85	150	58.81 59.27	8.83 8.99	10.83 11.43	8.26 7.89
7	$C_{19}H_{37}N_5SO$	383.60	Methanol	80	155	59.49 59.71	9.72 9.56	18.26 18.06	8.36 8.27
8	$C_{24}H_{37}N_3S$	399.64	Ethanol	70	143-145	72.13 72.44	9.33 9.51	10.51 10.23	8.02 7.86
9	$C_{25}H_{39}N_3S$	430.66	Ethanol	75	155	69.88 69.61	9.15 9.45	9.78 9.93	7.46 7.22
10	$C_{25}H_{35}N_3SO_2$	441.63	Ethanol	85	152-155	67.99 68.22	7.99 7.71	9.51 9.20	7.26 7.54

Synthesis of 2-amino-5- pentadecyl-1,3,4-thiadiazole (1):

A one pot reaction mixture of palmetic acid (0.01mole),thiosemicarbazide (0.01mole) and 20 ml $POCl_3$ was refluxed for 4hrs. The reaction mixture was concentrated then poured on crushed ice with stirringtogive a solid mass. The resulting productwas filtered off, dried and crystallized from benzene in an excellent yield (see table 1).). IR (KBr), cm^{-1}: νNH_2in the region of 3311-3260, νC-H aliphatic at 2920- 2850,νC=N at 1638, 1554 cm^{-1} beside the characteristic bands for thiadiazole ring.MS (EI, 70 eV), m/z (Irel, %): molecular ion peak ($M^{\cdot+}$) at m/z = 460, 0.45% and the base peak at m/z= 160, 100%..

Synthesis of N-(5-Pentadecyl-1,3,4-thiadiazol-2-yl)-acetamide (2):

Compound **1** (0.01mole) was refluxed in 30ml of acetic anhydride for 3hrs then cooled to room temperature. The solid product that separated was filtered off, washed with water, dried and crystallized from appropriate solvent. IR (KBr), cm^{-1}: shows νNH at 3167, νC-H aliphatic at (2918, 2849), νC=O at 1692 cm-1.MS (EI, 70 eV), m/z (Irel, %): molecular ion peak (M.++1) at m/z =354, 1.01 % and the base peak at m/z= 157, 100%.

Synthesis of 1-(5- pentadecyl-1,3,4-thiadiazole-2-yl)-3-phenyl urea (3):

A mixture of an equimolar ratio of compound**1** and phenylisocyanate in 40ml dry acetone was refluxed till completion after 5hrs. The solid product was obtained on cooling at ambient temperature then filtered off under vacuum using Buckner funneland recrystallized from ethanol. IR (KBr), cm^{-1}: νC-H aromatic at 3062, νC=O at 1714 and νNH at 3373, 3220 in addition to the other bands characteristic for the nucleus. ^{1}H-NMR (DMSO- d6) δ, ppm:δ'S at 0.9 (t,3H,terminal CH_3), 1.3-1.6 (m, 26H, $13CH_2$), 1.76 (t, 2H,CH_2), 7.06-7.57 (m, 5H,Ar-H), and 8.7 (s,2H,2NH) which disappear in the presence of D_2O.

Synthesis of N-(5- pentadecyl-1,3,4-thiadiazole-2-yl) benzamide (4) and 4-Nitro-N-(5- pentadecyl-1,3,4-thiadiazole-2-yl)benzamide (5).

General procedure:

To a solution of 0.01mol of the amino thiadiazole**1** in 40mldry benzene containing triethylamine (3drops) as a catalyst, 0.01mol of either benzoyl chlorideor p-Nitro benzoyl chloride (0.01mole) was added. The reaction mixture was refluxed, monitored by TLC until completion after 3hrs,then cooled at room temperature. The separated solid was filtered off and crystallized from appropriate solvent (see table 1) to give **4** or**5**respectively.

For compound **4**: IR (KBr), cm^{-1}: νNH at (3227) and νC=O at 1691 cm^{-1}beside the characteristic bands of the product. MS (EI, 70 eV), m/z (Irel, %): MS (EI, 70 eV), m/z (Irel, %): molecular ion peak ($M^{\cdot+}$ + 1) atm/z = 416 , 11.1 % and the base peak at m/z = 105 , 100 %.

For compound **5**: IR (KBr), cm^{-1}: νNH at (3227) and νC=O at 1691 cm^{-1}. MS (EI, 70 eV), m/z (Irel, %): molecular ion peak ($M^{\cdot+}$) at m/z = 460, 0.45% and the base peak at m/z= 160, 100%.

Synthesis of 2-chloro-N-(5-pentadecyl-1,3,4-thiadiazol-2-yl)acetamide (6):
To a solution of 0.01mol of compound**1**in 40ml dry benzene containing triethyl amine (3 drops) as a ctalyst, 0.01mol of chloroacetyl chloride in 5ml dry benzene was added. The reaction mixture was refluxed for 5hrs and then cooled at ambient temperature to afford the solid product which was filered off, dried and recrystallized (for physical properties, see table 1).IR (KBr), cm^{-1}:νNH at 3230 and νC=O at 1691 cm^{-1} beside the CH aliphatic bands at 2918, 2849. MS (EI, 70 eV), m/z (Irel, %): molecular ion peak ($M^{\cdot+}$) at =388 , 18.7 % and the base peak at m/z = 191 , 100 %.

Synthesis of 2-hydrazo-N-(5- pentadecyl-1,3,4-thiadiazole-2-yl) acetamide (7):
A mixture of 0.01 mole hydrazine hydrate and 0.01mol of compound **6** in 40ml ethanol was refluxed for two hours. The reaction mixture was left to cool down at room temperature to afford the solid product in a very good yield (80%). The resulting product was filtered off, dried and recrystallized from the appropriate solvent. IR (KBr), cm^{-1}: νNH_2 at 3222 and νC=O at 1693 cm^{-1}beside the νCH aliphatic absorption bands at 2918, 2849.MS (EI, 70 eV), m/z (Irel, %):molecular ion peak ($M^{\cdot+}$1) at m/z =382 , 8.21 % and the base peak at m/z = 85 , 100 %.

Synthesis of 2-[(benzylidene)-amino]-5-pentadecyl-1,3,4-thiadiazole (8) and 2-[(4-methoxybenzylidene)-amino] 5-pentadecyl- 1,3.4-thiadiazole (9).
General procedure:
An equimolar mixture of compound **1** (0.01mole) and 0.01 mole of either benzaldehyde or p-methoxybenzaldehydein 30ml ethanol was heated under refluxand monitored by TLC till completion after 3hrs, then cooled to the ambient temperature. The separated solid product was filtered off and crystallized from ethanol to give pure crystals of the corresponding arylidene-thiadiazole**8**or **9** respectively.

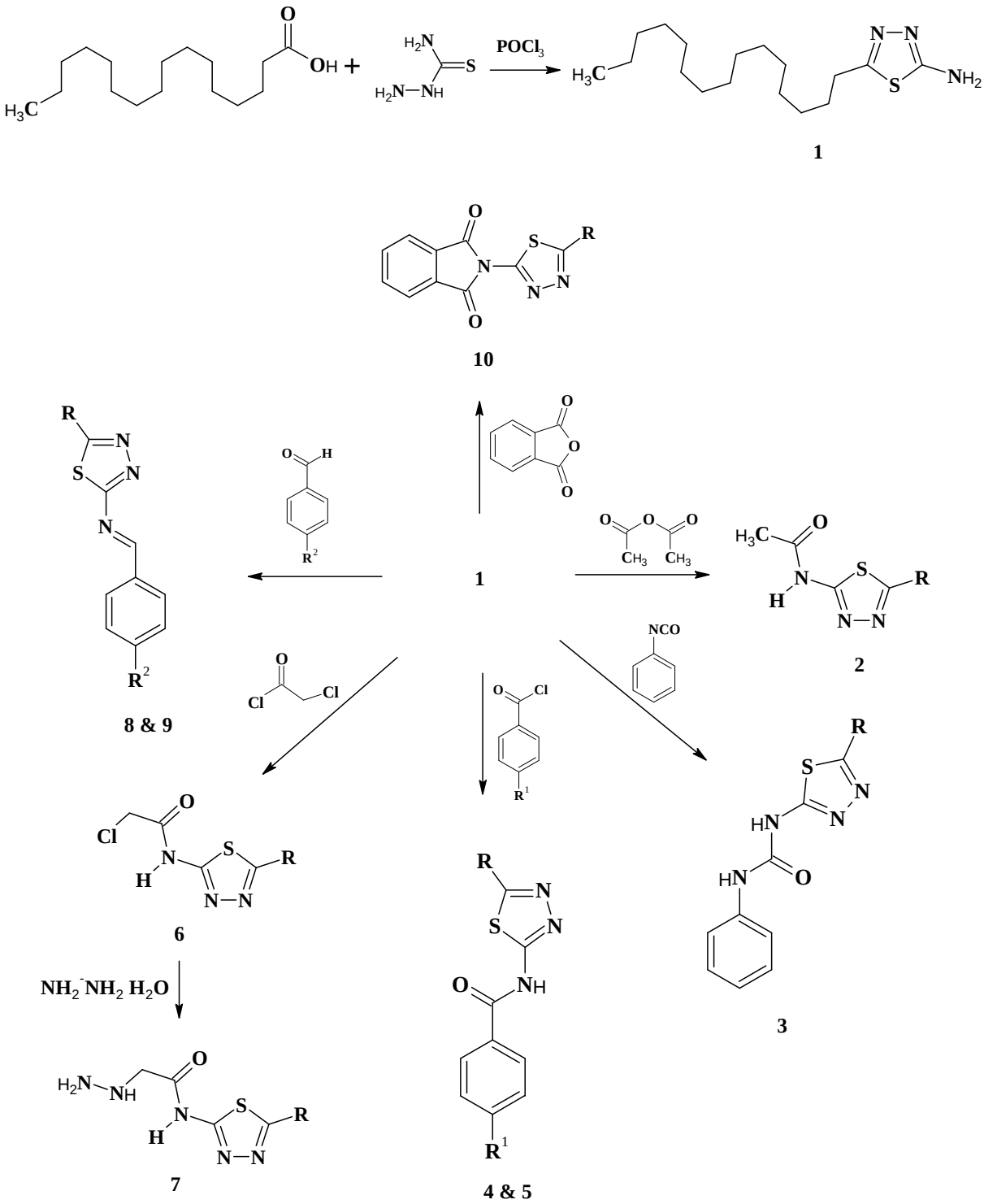
For compound **8**: IR (KBr), cm^{-1}: νC=N at 1586 cm^{-1}, aromatic and aliphatic bands at 3040 and 2910 respectively.MS (EI, 70 eV), m/z (Irel, %):molecular ion peak ($M^{\cdot+}$-1) at m/z = 399, 92.86% and the base peak at m/z= 112 , 100%.

For compound **9**: IR (KBr), cm^{-1}: νC=N at 1586 cm^{-1}, νC-O at 1100 and ν ofp-substituted benzene ring at 961 cm^{-1} beside aromatic and aliphatic bands at 3080 and 2875 respectively.MS (EI, 70 eV), m/z (Irel, %):molecular ion peak ($M^{\cdot+}$+3) at m/z = 434, 8.94% and the base peak at m/z= 137, 100%.

Synthesis of 2-(5- pentadecyl-1,3,4-thiadiazole-2-yl)isoindoline-1,3-dione (10):
A mixture of0.01 mole of compound **1** was fused with 0.03 mole phthalicanhydrideon sand bath for 2hrs, then cooled at room temperature. The melted product was triturated with waterto afford the solid product which was filtered off, washed with water, dried and recrystallized from ethanol.IR (KBr), cm^{-1}: two νC=O at 1735 and 1787 cm^{-1}, and 2918, 2849 cm^{-1} for νCH aliphatic.^{1}H-NMR (DMSO- d6) δ, ppm:δ at 0.8 (t,3H,terminal CH_3), 1.3-1.6 (m, 26H,13CH_2), 1.84 (t, 2H,CH_2), and 7.8-8.2 (m, 4H,Ar-H). MS (EI, 70 eV), m/z (Irel, %): molecular ion peak ($M^{\cdot+}$) at m/z = 441, 39 % and the base peak at m/z = 244 , 100 %.

Antibacterial, antifungal and antiyeast activation of the synthesized compounds:
The antimicrobial activities of the synthesized thiadiazoles in this study were determined in *vitro* using the hole plate and filter paper disc method (Rosen, 1989) which considered the most commonly used technique for determining sensitivity of chemotherapeutic agents. Compounds were dissolved in 10% acetone at different concentrations (125, 250, 500 µg/ml). Agar plates were inoculated uniformly from fresh broth culture of Gram +ve bacteria (*Escherichia coli*), Gram –ve bacteria (*Bacillus subtilis*), Fungi (*Pencicilliumchrysogenum*), and yeast (*Candida albicans*). The disks were incubated at 28˚C for 24hr, and the formed inhibition zones were diffused into the agar from the disk (this refers to the organism was inhibited by material) and were measured in mm [24 - 26].

Bacterial media: Nutrient agar and broth (pH 7.0), Peptone (0.5g), Beef extract (0.3g), Agar (15.0g) and distilled water (1000.0)



R = $C_{15}H_{31}$- , 4; R^1 = H; 5;R^1 = No_2, 8; R^2 = H, 9; R^2 = OCH_3

(Scheme 1)

Fungal media: $MgSO_4$(0.5g); KCl(0.5g); Sucrose (30.0g); $FeSO_4$ (0.01g); $NaNO_3$ (3.0g); K_2HPO_4 (1.0g); Agar (15.0g) and distilled water (1000.0 ml).

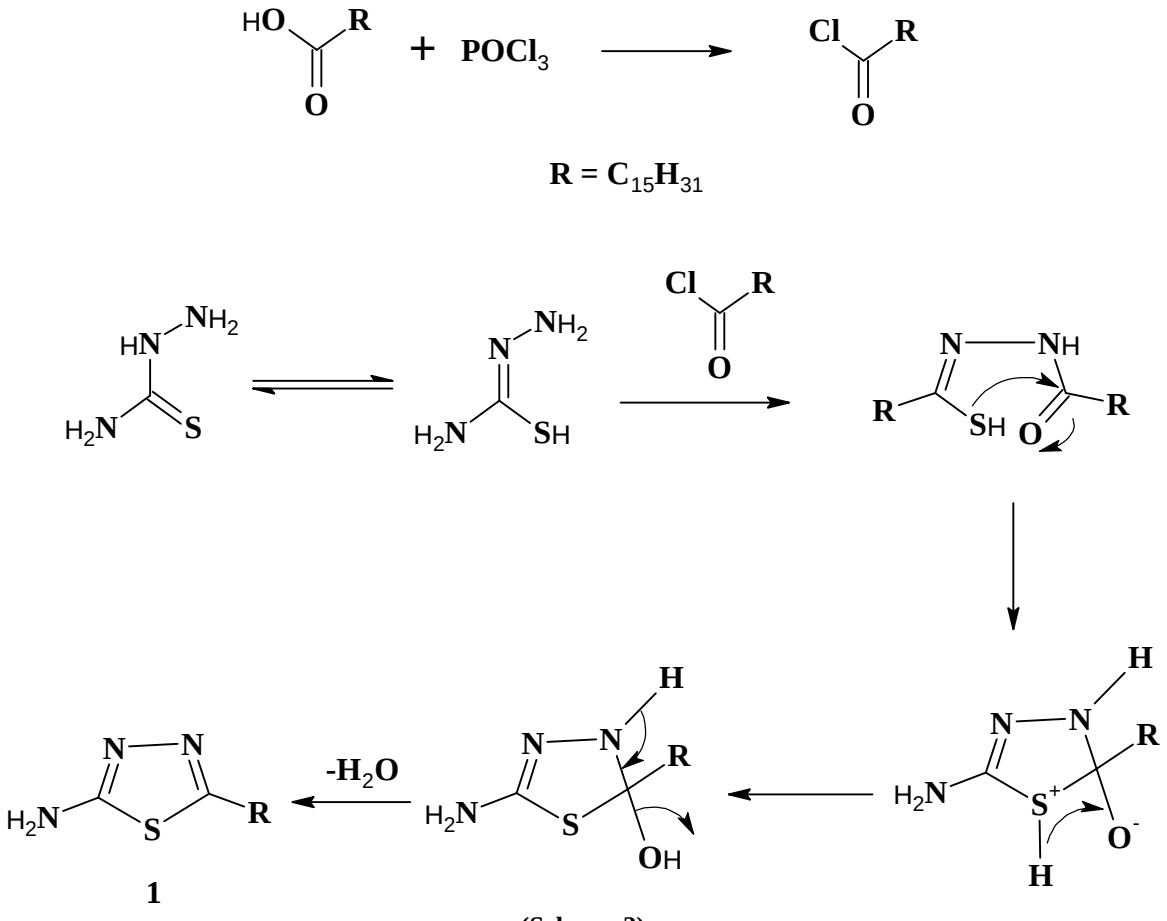
Table 2: Antimicrobial activity of some synthesized thiadiazole compounds.

Compds	Bacteria				Fungi		Yeast	
	E. coli (-ve)		*B.subtilis(+ve)*		*P.chrysogenum*		*C. albicans*	
	A	**MIC**	**A**	**MIC**	**A**	**MIC**	**A**	**MIC**
1	+++	125	+++	125	+++	125	++	125
2	+++	125	++	250	+++	125	+++	125
3	++	125	+++	250	++	125	++	500
4	+	125	++	250	+++	125	+	500
6	++	500	++	500	+	250	+	500
7	++	125	++	125	++	250	+	250
8	++	125	+++	250	++	125	+++	250
10	++	125	++	250	++	250	+	250

A = Antimicrobial activity of tested compounds
MIC = Minimum inhibitory concentration
+ => 10 mm slightly active, ++ = >20 mm moderately active, +++=> 30 mm highly active.

DISCUSSION

In one pot reaction ofpalmitic acid with thiosemicarbazide in presence of phosphorus oxychloride afforded the formation2-amino-5-pentadecyl-1,3,4-thiadiazole**1.** The product was assumed to be constructed via formation of the in situ palmitoyl chloride which in turn reacted was cyclised with the thiosemicarbazide to give the corresponding thiazole (Scheme 2)**.**



(Scheme 2)

IR spectrum showed a clear peak at 3311-3260cm-1 for NH_2 group and absorption peak at 1638-1554 $cm^{-1}$for the cyclic C=N frequencies in addition to C-Haliphatic at 2920- 2850cm^{-1}. Mass-spectrum showed molecular ion peak ($M^{\cdot+}$- 1) at m/z = 310, 0.56 % and the base peak at m/z = 115 , 100 %.

Compound **1** has been acetylated with acetic anhydride to give the corresponding Also this compound N-(5-Pentadecyl-1,3,4-thiadiazol-2-yl)-acetamide**2**. IR-spectrum showed NH at 3167$cm^{-1}$and C=O at 1692 cm^{-1} and molecular ion peak ($M^{\cdot+}$+1) on Mass spectrum at m/z =354.

On the other hand reaction of **1** with phenylisocyanate was carried out in boiling acetone, the corresponding thiadiazolyl urea **3** was revealed. The product was formed via the nuleophilic addition of amino group in thiadizole on the the carbonyl group of isocyanat.IR-spectrum showed the existence of C-H aromatic at 3062, C=O at 1714 and 3373,3220 $cm^{-1}$for NH absorption group in addition to C-Haliphatic at 2920- 2850cm^{-1}. ^{1}H-NMR spectrum showed a multiple peak7.06-7.57ppm for the aromatic phenyl protons and singlet peak at 8.7ppm for the two NH groups in addition to a triplet peak at 0.9ppmfor the terminal CH_3 of the long fatty chain, and multiplet peak of 28 aliphatic protons at 1.3-1.6 ppmand 1.76.

Reaction of **1** with acid chlorides such as benzoyl chloride or p-nitro-benzoyl was allowed in dry benzene using triethylamine or pipridine as a catalyst, the corresponding aryl amide derivatives ofthiadiazoles**4** and **5** were afforded respectively via elimination of a hydrochloride molecule. IR- spectrum showed NHabsorption peak at 3227-3116 and C=O at 1668-1691 cm^{-1}. Mass spectrum of compound**4** showed molecular ion peak ($M^{\cdot+}$ +1) at m/z = 416, 11.1% and460, 0.45% for compound **5.**

Similarly,2-Amino-5-pentadecyl-1,3,4-thiadiazole **1**was reacted with chloroacetyl chloride in dry benzene containing of piperidine or triethyl amine to give the chloroacetamide derivative**6**. When the product 6 was allowed to react further with hydrazine in ethanol, the corresponding amido hydrazine **7** was obtained. Formation of compounds **6** and **7** was proceededVia elimination of hydrochloride molecule from reactants in each case.IR spectrum of **6** showed NH group at 3230 and C=O at 1691 cm^{-1} in addition to the aliphatic peaks.Mass-Spectrum revealed molecular ion peak ($M^{\cdot+}$) at =388 , 18.7%. IR spectrum of **7**which showed NH group at 3222 and C=O at 1693cm^{-1}.
When 2-amino-5-pentadecyl-1,3,4-thiadiazole was allowed to be fused with phthalic anhydride on sand bath above its melting point, 2-(5-pentadecyl-1,3,4- thiadiazol-2-yl)isoindoline-1,3-dione **10** was gained via the well-known condensation reaction process. IR spectrum of **10** showed two C=O at1735, 1787 cm^{-1} and disappearance of NH_2 peak in addition to the long alkyl chain at 2920- 2850cm^{-1}.

The Schiff's base5-pentadecyl-2-(benzylidene)-amino-1,3,4-thiadiazole**8** or5-pentadecyl-2-(4-methoxybenzylidene)-1,3.4-thiadiazole **9**has been obtained from reaction of **1** with either benzaldehyde or anisaldehyde respectively in boiling ethanol along with few drops of triethyl amine or piperidine. The reaction was proceeded via nucleophilic addition of amin group of thiadiazole**1** to the carbonyl group of the aldehyde followed by elimination elimination of a molecule of water to give the correspondingazomethines**8** and **9**. IR spectrum which showed C=N at 1586cm^{-1} with the disappearance of NH_2 band in both of 8 and 9 in addition to two absorption bandsat 1100 for C-O and 961cm^{-1}p-substituted benzene ring of compound 9. Mass spectra showed molecular ion peak ($M^{\cdot+}$-1) at m/z = 399, 92.86% for compound **8** and molecular ion peak ($M^{\cdot+}$+3) at m/z = 434, 8.94% for compound **9**.

Biological activities of thiadiazolescompounds 1-10:
The biological activity of all synthesized compounds containing thiadiazolenucleus have exhibited high to moderateinhibitory effect against all employed microorganisms. Thiadiazolylacetamide**2**has showed the highest antimicrobial effect of all microorganisms and moderate effect on gram positive bacteria. The benzamide derivative**4**showed the highest antifungal activity while was the lowest influence on gram negative*Escherichia coli*and *Candida albicans* yeast. Schiff's base **8** showed the highest inhibitory effect onboth of gram positive bacteria and *Candida albicans*yeast.

In general, the results have illustrated clearly that such thiadiazole compounds are effective and inhibited the growth of all tested microorganisms.

CONCLUSION

In this study we have performed the synthesis of newly thiadiazole derivatives having a long alkyl chain with molecular weight suitable for becoming an amphiphilic molecule with correct hydrophilic-lypophilic balance. This advantage could enhance their anti-microbial activities and lowering their toxicity. Compounds**1**and **2**have showed the highest inhibitory effect on all employed microorganisms.

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