



Synthesis and Antimicrobial Studies of 5-N-alkyl-1,3,4-oxadiazole-2-thiol Derivatives from Fatty Acids

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The novel 5-N-alkyl-1,3,4-oxadiazole-2-thiol derivatives have been synthesized by esterification of fatty acid followed by reaction the ester with hydrazine hydrate. The acid hydrazide was converted to 1,3,4-oxadiazole by ring closure mechanism. The synthesized compounds have been characterized by physical (melting point and TLC) and spectral (IR, ¹H NMR and mass) data. All the compounds were screened for their antimicrobial activity. The compounds **3F₁** & **3F₄** showed good inhibition activity against all four types of bacteria; while compound **3F₂** & **3F₃** shown moderate activity.

Keywords: 1,3,4-Oxadiazole, Fatty acids, Acid hydrazides, Anti-microbial activity.

INTRODUCTION

The macronutrient groups are dietary fats, lipids that include fatty acids, triglycerides, cholesterol, essential fatty acids, linoleic and α -linoleic acid. Fats are a key factor for the maintenance caloric balance and to the body weight. Specific fatty acids are also serves as precursor for numerous biological functions. Fat soluble vitamins (*i.e.*, vitamin A, D, E, K) and carotenoids are engaged in pathways that influence inflammation, coagulation and gene expression which are transported by fats [1].

1,3,4-Oxadiazole are an important class of heterocyclic compounds with a wide range of pharmaceutical and biological activity [2-4]. Their synthesis and transformations have been of interest for a long time. This interesting group of compounds possesses diverse biological activity such as antimicrobial [5,6], anti-inflammatory [7], antitubercular [8], anticonvulsant [9], anti-cancer [10], anti-HIV [11], hypoglycemic [12] and genotoxic [13,14] activities.

In view of above mentioned pharmacological importance of fatty acids and 1,3,4-oxadiazole our ongoing efforts to synthesize a novel derivatives of 5-N-alkyl-1,3,4-oxadiazole-2-thiol (**3F₁-F₄**) and evaluated them for their potential as antimicrobial agents.

EXPERIMENTAL

Melting point taken in open capillary tubes using Arson digital melting point apparatus and were uncorrected. IR spectra were recorded on BRUKER Alpha FTIR spectrometer,

¹H NMR spectra were recorded on BRUKER (400 MHz) in DMSO-*d*₆ using TMS as an internal standard. Mass spectra were recorded in apex mass spectrophotometer. The purity of the compounds for checked in silica gel-G pre-coated plates by using n-hexane and ethyl acetate (1:1) as the eluent and observed in UV-chamber.

Synthesis of acid esters (1F₁-1F₄): Fatty acid (10 g), in 35 mL of methanol and concentrated sulphuric acid was added through sides of the walls. The resulting mixture was allowed to reflux for 5 h, the contents were allowed to cool on water bath. To this, 5 % sodium bicarbonate was added and the resulting mixture was filtered and recrystallized from methanol.

Synthesis of acid hydrazide (2F₁-2F₄): Dissolved the esters of substituted fatty acids (0.01 mol), in 4 mL of hydrazine hydrate was added drop wise with stirring. The resulting mixture was allowed to reflux for 4 h. The reaction mixture was cooled to room temperature and diluted with water. It was filtered, washed thoroughly with water. The product was purified by recrystallization from methanol.

Synthesis of 5-N-alkyl-1,3, 4-oxadiazole-2-thiol (3F₁-3F₄): Potassium hydroxide (0.01 mol) was dissolved in absolute ethanol (50 mL). To the above solution, acid hydrazide (**2**) and carbon disulfide (0.01 mol) was added. The reaction mixture was refluxed on water bath for 38 h until hydrogen sulphide ceased completely. The reaction mixture was cooled to room temperature. On acidification with dil. hydrochloric acid, the precipitate was obtained which was filtered, washed thoroughly with water and recrystallized from ethanol.

5-(Heptadec-8-1-yl)-1,3,4-oxadiazole-2-thiol (3F₁): Yield: 30 %; m.p.: 174-179 °C; IR (KBr, ν_{max} , cm^{-1}): 2937 (C-H stretching long alkyl chain), 1477 (C-O-C stretching), 1595 (C=C stretching), 1716 (C=N stretching), 2384 (C-SH stretching); ^1H NMR δ : 0.87-0.88 (t, 3H, terminal CH_3), 1.20-1.41 (m, 20H, $-\text{CH}_2-$), 1.45-1.48 (d, 2H, $-\text{CH}_2-$), 1.94-1.97 (d, 4H, allylic protons), 2.14-2.18 (t, 2H, $-\text{CH}_2-$), 5.30-5.35 (d, 2H, $\text{CH}=\text{CH}$); MS m/z : 340 ($\text{M}+2$).

5-Pentadecyl-1,3,4-oxadiazole-2-thiol (3F₂): Yield (%): 45 %; m.p.: 174.17 °C; IR (KBr, ν_{max} , cm^{-1}): 2935 (C-H stretching, long alkyl chain), 1168 (C-O-C stretching), 1623 (C=N stretching), 1064 (C-O stretching), 2383 (C-SH stretching); ^1H NMR δ : 0.82-0.85 (t, 3H, terminal CH_3), 1.22-1.25 (m, 25H, $-\text{CH}_2-$), 1.57-1.64 (m, 2H, $-\text{CH}_2-$), 2.66-2.69 (t, 2H, $-\text{CH}_2-$), 14.25 (s, 1H, SH); MS m/z : 312 (M^+).

5-Heptadecyl-1,3,4-oxadiazole-2-thiol (3F₃): Yield (%): 52 %; m.p.: 80-85 °C; IR (KBr, ν_{max} , cm^{-1}): 2935 (C-H stretching), 1168 (C-O-C stretching), 1622 (C=N stretching), 1063 (C-O stretching), 2383 (C-SH stretching); ^1H NMR δ : 0.85 (t, 3H, terminal CH_3), 1.22-1.25 (m, 28H, $-\text{CH}_2-$), 1.57-1.64 (m, 2H, $-\text{CH}_2-$), 2.65-2.69 (t, 2H, $-\text{CH}_2-$), 14.25 (s, 1H, SH); MS m/z : 341 (M^+).

5-Undecyl-1,3,4-oxadiazole-2-thiol (3F₄): Yield (%): 48 %; m.p.: 79-82 °C; IR (KBr, ν_{max} , cm^{-1}): 2936 (C-H stretching), 1177 (C-O-C stretching), 1517 (C=N stretching), 1065 (C-O stretching), 2384 (C-SH stretching); ^1H NMR δ : 0.82-0.85 (t, 3H, terminal CH_3), 1.15-1.26 (m, 17H, $-\text{CH}_2-$), 1.57-1.64 (m, 2H, $-\text{CH}_2-$), 2.66-2.70 (t, 2H, $-\text{CH}_2-$), 14.25 (s, 1H, SH); MS m/z : 256 (M^+).

Antimicrobial activity: The antimicrobial activity of title compounds determined *in vitro* by using disc diffusion method

by using four types microorganisms like *E. coli*, *P. aeruginosa* (Gram-positive), *S. aureus*, *B. subtilis* (Gram-negative) at 50, 100 $\mu\text{g mL}^{-1}$ concentrations respectively, in the nutrient agar media by measuring the zone of inhibition in mm. The solution of required concentrations (50, 100 $\mu\text{g mL}^{-1}$) of test compounds were prepared by dissolving the compounds in DMSO. Streptomycin was used as standard.

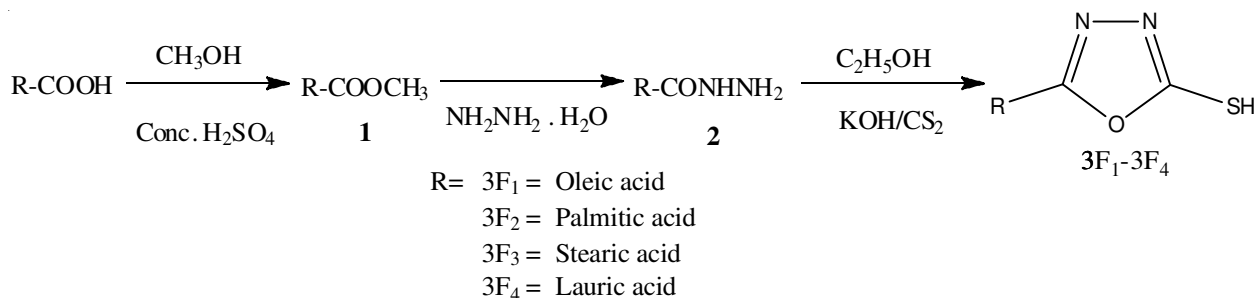
RESULTS AND DISCUSSION

As depicted in **Scheme-I**, in addition to 1,3,4-oxadiazoles we have synthesized 5-N-alkyl-1,3,4-oxadiazole-2-thiol (**3F₁-3F₄**) through a three step reaction and acid ester of fatty acid (**1**) has been synthesized from fatty acid and methanol in the presence of conc. H_2SO_4 by esterification. In step 2, the formed ester of fatty acid was hydrazinolyzed to give acid hydrazides (**2**), which later cyclize to form a title compounds *i.e.* 5-N-alkyl-1,3,4-oxadiazole-2-thiol (**3F₁-3F₄**) by reacting with CS_2 and KOH in ethanol. This procedure afforded various 1,3,4-oxadiazoles in moderate yields. The infrared spectra of the substituted 5-N-alkyl-1,3,4-oxadiazole-2-thiol (**3F₁**) showed characteristic absorption bands, one of the which appearing at 2936 cm^{-1} was strong alkyl chain C-H which are all final compounds (**3F₁-3F₄**) shown $1200\text{--}1100\text{ cm}^{-1}$ C-O-C, $1600\text{--}1500\text{ cm}^{-1}$ C=N and 2354 cm^{-1} SH Group. Similarly, ^1H NMR spectra of synthesized compounds (**3F₁-3F₄**) showed multiplet signals at δ 1.12-1.26 ppm due to methylene protons. The terminal CH_3 protons appeared as triplet at δ 0.82-0.85 ppm. The SH proton as a singlet at δ 14.22 ppm. A triplet at δ 5.30-5.35 ppm integrating for 2 olefinic proton ($\text{CH}=\text{CH}$) was attributed to the $\text{C}_9\text{-C}_{10}$ proton of **3F₁**, doublet at 1.94-2.01 ppm for four allylic protons was attributed to $\text{C}_8\text{-C}_{11}$ protons of **3F₁**.

TABLE-1
ANTIMICROBIAL ACTIVITY OF 5-N-ALKYL-1,3,4-OXADIAZOLE-2-THIOL (**3F₁-F₄**)

Compound code	Conc. ($\mu\text{g/mL}$)	Zone of inhibition			
		Gram-positive organisms		Gram-negative organisms	
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
3F₁	50	20	10	24	12
	100	25	15	27	18
3F₂	50	10	9	10	N
	100	17	14	12	9
3F₃	50	N	N	10	N
	100	9	N	15	12
3F₄	50	22	N	14	N
	100	27	N	20	8
Streptomycin	50	22	25	28	21
Control		–	–	–	–

N = No inhibition



Scheme-I: Conventional synthetic route for 5-N-alkyl-1,3,4-oxadiazole-2-thiol

The results of the antimicrobial study of the newly synthesized compounds was reported as zone of inhibition against *E. coli*, *P. aeruginosa* (Gram-positive), *S. aureus*, *B. subtilis* (Gram-negative) (Table-1). All the synthesized compounds have shown significant antimicrobial activity. The compound **3F₁** and **3F₄** showed maximum activity at both 50 and 100 µg/mL concentrations whereas compounds **3F₂** and **3F₃** showed moderate activity. The results revealed that fatty acid moiety is a satisfactory backbone for significant antimicrobial activity and the presence of unsaturated double bonds in the structure of fatty acid is also responsible for the good activity. Thus by hybridization of 1,3,4-oxadiazole ring enhance the activity.

Conclusion

In this communication, our aim has been verified by the synthesis of 5-N-alkyl-1,3,4-oxadiazole-2-thiol (**3F₁**-**3F₄**) derivatives in which 1,3,4-oxadiazole ring incorporated with fatty acid hydrazide moiety in a single frame work. Obtained results revealed that most of tested compounds showed promising activity as antimicrobial agents. After all the above findings it can be concluded that these molecule become lead molecule for further synthetic and biological evaluation.

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