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Synthesis, Identification and Biological Activities Of a New Series of Heterocyclic Amide

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تحضير وتشخيص سلسلة جديدة من ألامايدات غير المتجانسة مع دراسة فعاليتهم البايولجية

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الملخص

تم تحضير سلسلة جديدة من الامايدات غير المتجانسة من تفاعل phenyl acetyl chloride مع سلسلة من الامينات الحلقية غير المتجانسة المحتوية على وحدة 1,3,4-thiadiazol . وان التراكيب لهذه الامايدات المحضرة ثم تثبيتها بواسطة الطرق الطيفيةFTIR وطيف^{1}H-NMR وطيف ^{13}C-NMR لمركب واحد فقط، بالاضافة الى الفعالية البايولوجية للمركبات المحضرة ضد اربعة انواع من البكتريا *Staphylococcus aureus(+ve), Escherichia coli(-ve), Enterobactria Cloacae (-ve) & Klebsiella(-ve).*

Abstract

A new series of heterocyclic amides have been prepared (2a-k) from the reaction of phenyl acetyl chloride with a series of heterocyclic amines containing a 1,3,4- dithiazaol unit. The structures of these new compounds were confirmed on the basis of IR, and one of them by ^{1}H-NMR, ^{13}C-NMR. The synthesized products were tested for antimicrobial activity against a variety of test organisms: *Staphylococcus aureus(+ve), Escherichia coli(-ve), Enterobactria Cloacae (-ve) & Klebsiella(-ve).*

Keywords: amides, heterocyclic amides, spectroscopy

Introduction

Amides are compounds that contain the functional group of -CONH- .The amide functional group is also called peptide linkage because it links amino acids in proteins [1]. Amides are important commercial and biological compounds, because amides constitute the backbone of protein molecules, and their chemistry is of extreme importance [2]. Amides can also be made from Beckmann rearrangements [3] of ketoximes and conversion of thioamides into amides [4]. In 1999, Kubicova et.al [5] synthesized N, N'-diaryl alkane diamides, with the exception of N, N'-diaryl ethane diamides, which were prepared from the

corresponding pyridines by treatment with the appropriate acyl chlorides in pyridine at 0°C, and they studied the antimicrobacterial & antialgal activity of all prepared amides. The anilides of 2-alkylthio-4-pyridine carboxylic acid were synthesized as described previously [6] by subsequent treatment with thionyl chloride and substituted aniline. In 2006[6], it was reported condensation of the corresponding chlorides of some subtituted pyrazine-2-carboxylic acid, or 6-chloro-5-tert-butyl pyrazine-2-carboxylic acid with various ring-substituted amino-thiazoles or anilines yielded a series of amides. All the compounds were evaluated for their antimicrobacterial activity and inhibition against *E.coli.*

Experimental part:

1. Synthesis of 2-amino-[1,3,4]thiadiazole-5-thiol (1a) : [7-8]

A mixture of thiosemicarbazide (9.1g, 0.1mol) in absolute ethanol (35ml), anhydrous sodium carbonate (5.3g), and carbon disulfide (9.12g, 0.12mol) was refluxed with continuous stirring for (1hour),then warmed in a water bath for about 6 hours until the mixture becomes yellow. Ethanol was evaporated and 40ml of distill water was added to the residue, and acidification of the solution by concentrated HCI led to formation of yellow solid material, which was washed for several times with distilled water, collected and dried to obtain good yield (80%) and recryestallized by absolute ethanol. mp.: 230-232°C R_f: 0.82 (Diethyl ether / ethyl acetate 2:1)

2. Synthesis of 5-alkylsulfanyl-[1,3,4]thiadiazol-2-yl amine (1b-k): [8]

Solution of potassium hydroxide (0.56g, 0.01mol) in ethanol (10ml) was added to a stirred solution of 2-amino-(1,3,4)thiadiazol-5-thiol (1a) (1.33g, 0.01mol) in absolute ethanol (20ml). Then, alkyl bromide (0.011mol) was added drop wise to the reaction. The mixture, was refluxed for (3hour) in water bath then cooled to room temperature, filtered and the filtrate was poured into cold distilled water, (100ml), a yellow precipitate separated out. Solvents used for recrystallization, percentage of yield, mp., R_f and color of the synthesized heterocyclic amines (1b-k) were shown in table (1).

3.SynthesisofN-(5-alkylsulfanyl-[1,3,4]-thiadiazol-2-yl)-2-phenyl-acetamides(2a-k):[8]

The 2-amino-(1, 3, 4)thiadiazol-5-thio or 2-alkylsulfanyl-[1,3,4] thiadiazol-5-yl amine (1b-k) (0.01mol) were dissolved in a 2.5ml of dry benzene and mixed with (0.01mol) Et_3N. Phenyl acetyl chloride (0.01mol) was dissolved in 5ml of dry benzene and added drop wise to a stirred solution of heterocyclic amines at 0°C. The reaction mixture was allowed to stand for 24 hours, and then poured into water (100ml). All products were filtered off. Solvents used for recrystallization, percentage of yield, R_f and color of the synthesized amides (2a-k) were shown in Table (2)

4. Biological study: The sensitivity of heterocyclic amines and heterocyclic amides against four kinds of bacteria, (*Staphylococcus aureus, Escherichia coli, Enterobactria cloacae & Klebsiella)*

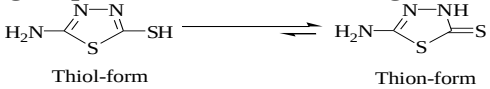
1. Muller-hinton medium was prepared using nutrient agar preservation of pure culture, then sterilized by autoclave, and poured in the petridish to a depth fo 4mm.
2. Activation of each type of bacteria, Staphylococcus aureus, Escherichia coli, Enterobactria cloacae & Klebsiella before culturing on the nutrient agar in nutrient broth (oxoid) which was used for dilution of bacterial and cultivation of culture isolated, for 24hr. in 37 ºc, then inoculation of the test plates.
3. Culturing the bacteria on nutrient agar.
4. Application of the heterocyclic amines and amides derivative disks, each disk was prepared by mixing a substance with KBr power (1:3).The mixture was pressed under pressure. KBr compound has been used as a blank disk: The prepared disk was placed on the surface of the cultured media with each of the above bacteria.
5. Incubation: The incubated plates were incubated for (24hour) at 37ºc.
6. Reading of zones of inhabitation: The diameter of each zone of inhibition was measured (including the diameter of the disk). Each zone size is interpreted by national committed for clinical laboratory stand into sensitive intermediate and resistant [9] larger zone of inhibition more (+Ve), while in the case of incurrence of clear zone the result will be (-Ve).

Results and discussions

1. Synthesis of 2-amino-[1,3,4]thiadiazole-5-thiol(1a) & 5-alkylsulfanyl-[1,3,4]thiadiazol-2-yl amine (1b-k): The main part of this research is synthesis of 2-alkylsulfanyl-[1,3,4]thiadiazol-5-yl amine compounds, where R ranges from CH_3 to $C_{10}H_{21}$, by alkylation of the 5-amino-[1,3,4]thiodiazole-2-thiol compound (1a) (which was prepared according to the method [7-8]) with alkyl bromides, using potassium hydroxide as catalyst.

The general feature of the IR, spectra of amine compounds (1a,1b-k) (table 3) consists of two bands at (3028-3272), (3184-3406)cm^{-1} assigned to υ NH_2 stretching

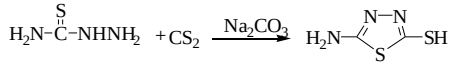
except compound (1a)(fig1) which showed three bands at 3398,3274 & 3088 cm^{-1}, which belongs to amine groups of the two resonating structures as bellow:



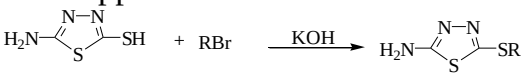
Thiol-form Thion-form

The symmetric and asymmetric stretching of C-H bands of CH_3 and CH_2 groups are between 2849-2973 cm^{-1}. The ^{1}H-NMR spectrum in DMSO of compounds (1a) (Fig. 2), showed a signal for the proton of the NH_2 group at 7.1 ppm[10-11], a signal for one proton of the NH group appears at 13.2 ppm[12-13] .The signals at 2.5 and 3.3 ppm belong to the solvent peaks. The ^{13}C-NMR of compound (1a)

(Fig. 3) shows a signal for carbon attached to $-NH_2$ at 181 ppm, and the carbon of the C=S group appears at 161ppm



1a

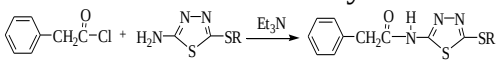


1(b-k)

R=CnH_2n+1
n=1-10

2.Synthesis of N-(5-alkylsulfanyl-[1,3,4]thiadiazol-2-yl)-2-phenyl-acetamide(2a-k):

The other part of this research is synthesis of new amides by reaction of phenyl acetyl chloride with substituted amines (1a-k) in the presence of Et_3N 1:1:1 moles respectively. The process is very rapid and exothermic which is controlled by decreasing the temperature to $0^{\circ}C$ and diluting the solution by adding dry benzene, the reaction was used mostly in the amides formation:



1(a-k)
R=H, CnH_2n+1

2(a-k)
R=H, CnH_2n+1

The presence of the substituted group in heterocyclic amines affects mainly the rate and the yield of the amides formation; its affected by the position and electronic environment of substituent in the heteroaromatic amines. The release of electrons of substituents like SCH_3 ,SC_2H_5,…to $SC_{10}H_{21}$ increases the electron density around the nitrogen atom which tends to increase the nucleophilicity of the nitrogen and thus increase the rate and the yield of products Table(1).

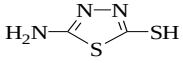
The IR spectra of the synthesized amides showed many bands due to the vibration of the different groups, all compounds(2b-k) showed a band between(3145-3163)
cm^{-1} which corresponds to the NH group of the amides [14], the signals of primary amines at 3028-3406 cm^{-1} disappeared (Table 4) . The ^{1}H-NMR spectrum of compound (2k) in $CDCl_3$ shows that there is a signal peak at 8.1 ppm for amide group[14], the signal of proton of SCH_2 group at 3.25 ppm, and signal of CH_2CO at 3.85 ppm. ,and signals of sixteen protons appear between 1.25-3.25 ppm. The ^{13}C-NMR of the compound (2k), showed the carbon of C=O group of amide which appeared at 177ppm (fig.6).

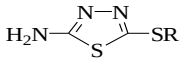
Determination of bacterial sensitivity:

The sensitivity of four kinds of bacteria *S-aureus*, *E.coli*, *Enterobacteiar* and *Klebsiella*, to different heterocyclic amine derivatives and aromatic amide compounds were carried out using compound discs of KBr (1:3). The effects of these compounds on four types of micro-organisms are represented in Table 5 & 6. There is a significant difference between the effects of the compounds used against various bacteria.

Table (1): The physical properties of different heterocyclic amines (1a-k) &

R_f. (diethyl ether / ethyl acetate 2:1)

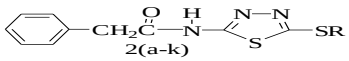

1a


1(b-k)

compound	*R*	*Nomenclature heterocyclic amines*	*M.F. of Heterocyclic amines*	*Solvent used for Recrystallization*	*Yield %*	*Color &shape*	*MP. (°C)*	R_f
1a	-H	2-amino-[1,3,4]thiadiazol-5-thiol	$C_2H_3N_3S_2$	ethanol	80	yellow pow.	230-232	0.821
1b	$-CH_3$	5-methylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_3H_5N_3S_2$	ethanol	46.3	yellow pow.	85-87	0.653
1c	$-C_2H_5$	5-ethylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_4H_7N_3S_2$	ethanol -hexane	55	Pale yellow pow.	90-92	0.577
1d	$-C_3H_7$	5-propylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_5H_9N_3S_2$	ethanol	44.5	Pale brown pow.	95-96	0.880
1e	$-C_4H_9$	5-butylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_6H_{11}N_3S_2$	ethanol	52	yellow pow.	100-102	0.655
1f	$-C_5H_{11}$	5-pentylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_7H_{13}N_3S_2$	ethanol	49	Pale yellow pow.	105-106	0.609
1g	$-C_6H_{13}$	5-hexylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_8H_{15}N_3S_2$	dioxan	57.5	yellow pow.	109-111	0.712
1h	$-C_7H_{15}$	5-heptylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_9H_{17}N_3S_2$	hexane	61.5	Pale yellow pow.	98-100	0.680
1i	$-C_8H_{17}$	5-octylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_{10}H_{19}N_3S_2$	ethanol	60	Pale yellow pow.	117-119	0.804
1j	$-C_9H_{19}$	5-nonylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_{11}H_{21}N_3S_2$	benzene	63	Pale yellow pow.	120-122	0.644
1k	$-C_{10}H_{21}$	5-decaylsulfanyl-[1,3,4]thiadiazol-2-yl amine	$C_{12}H_{23}N_3S_2$	ethanol	68	Pale green pow.	126-127	0.775

Pow=powder

Table (2): The physical properties of different heterocyclic amides (2a-k) & R_f (hexane / diethyl ether 5:1)

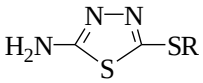

2(a-k)

R=H, CnH_{2n+1}
n=1-10

compound	*R*	*Nomenclature heteocyclic amines*	*M.F. of amides*	*Reaction time(hr)*	*Solvent used for Recrystalization*	*Yield %*	*Color & shape*	*M.P. (°C)*	*Rf*
2a	-H	N-(2-amino-[1,3,4]thiadiazol)-5-benzyl-thioesther	$C_8H_9N_3OS_2$	immediatly	toluene	77	yellow pow.	185-188	0.771
2b	$-CH_3$	N-(5-methylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{11}H_{12}N_3OS_2$	24	Ethanol	52	yellow pow.	93-94	0.721
2c	$-C_2H_5$	N-(5-ethylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{12}H_{14}N_3OS_2$	24	ethanol -hexane	57	Pale yellow pow.	103-104	0.811
2d	$-C_3H_7$	N-(5-propylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{13}H_{16}N_3OS_2$	24	Ethanol	55.5	Pale brown pow.	107-108	0.624
2e	$-C_4H_9$	N-(5-butylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{14}H_{18}N_3OS_2$	24	dioxan	63	yellow pow.	123-124	0.600
2f	$-C_5H_{11}$	N-(5-pentylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{15}H_{20}N_3OS_2$	24	Ethanol	59	Pale yellow pow.	139-140	0.730
2g	$-C_6H_{13}$	N-(5-hexylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{16}H_{22}N_3OS_2$	24	dioxan	71.5	yellow pow.	145-146	0.677
2h	$-C_7H_{15}$	N-(5-heptylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{17}H_{24}N_3OS_2$	24	Ethanol	71	Pale yellow pow.	131-133	0.591
2i	$-C_8H_{17}$	N-(5-octylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{18}H_{26}N_3OS_2$	24	Ethanol	72.5	Pale yellow pow.	151-153	0.703
2j	$-C_9H_{19}$	N-(5-nonylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{19}H_{28}N_3OS_2$	24	Benzene	75	Pale yellow pow.	167-168	0.711
2k	$-C_{10}H_{21}$	N-(5-decaylsulfanyl-[1,3,4]thiadiazol)-2-phenyl-acetamaide	$C_{20}H_{30}N_3OS_2$	24	Ethanol	72.5	Pale yellow pow.	172-174	0.621

Pow=powder

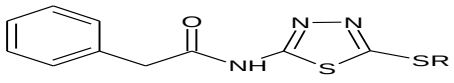
Table (3): Characteristic i.r bands (cm^{-1}) of the prepared heterocyclic amines (1a-k)



1(a-k)

Compound	*C–H aliphatic*	*N–H Stretching*	*C=N(m) stretching*	*C=S stretching*
1a		**3398,3274,3088**	**1600**	**1363**
1b	**2940**	**3184,3028**	**1621**	
1c	**2973,2855**	**3262,3107**	**1922**	
1d	**2860,2962**	**3274,3111**	**1602**	
1e	**2956,2880**	**3276,3095**	**1632**	
1f	**2955,2854**	**3327,3210**	**1596**	
1g	**2955,2845**	**3276,3095**	**1623**	
1h	**2951,2852**	**3405,3272**	**1593**	
1i	**2950,2849**	**3406,3272**	**1563**	
1j	**2952,2852**	**3273,3111**	**1603**	
1k	**2919,2852**	**3273,3113**	**1600**	

Table (4): Assignment of characteristic frequencies υ (cm-1) of FTIR spectra for the prepared heterocyclic amides (2a-k)



2(a-k)

R=H,C_nH_{2n+1}

Compound	*N-H Stretching*	*C-H Aromatic*	*C=O(s) Amide*	*N-H(s) Deformation*	*C=C Aromatic*	*C-H Aromatic (oop)def.*
2a	***3150.3261***	***3029***	***1697***		***1556***	***703***
2b	***3150***	***3032***	***1690***	***1572***	***1561***	***720,693***
2c	***3158***	***3035***	***1695***	***1572***	***1559***	***726,689***
2d	***3150***	***3034***	***1689***	***1569***	***1556***	***723,688***
2e	***3163***	***3059***	***1691***		***1560***	***722,698***
2f	***3145***	***3052***	***1689***	***1566***	***1556***	***723,694***
2g	***3162***	***3030***	***1688***	***1575***	***1564***	***716,691***
2h	***1352***	***3039***	***1694***	***1569***	***1557***	***715,691***
2i	***3153***	***3039***	***1694***		***1558***	***715,690***
2j	***3149***	***3028***	***1690***	***1597***	***1555***	***718,694***
2k	***3145***	***3034***	***1694***	***1565***	***1550***	***715,692***

Table (5): Results of S-aureus, E.coli, Enterobacteria cloacae & Klebsiella sensitivity against heterocyclic amines (1a-j)

Compound	Microorganism			
	S-aureus G(+ve)	*E.coli G(-ve)*	*Enterobacteria G(-ve)*	*Klebsiella G(-ve)*
1a	++	-	+	-
1b	-	-	+	-
1c	++	-	+++	-
1d	-	-	+++	-
1e	-	-	+++	-
1f	-	-	++	-
1g	-	-	+++	-
1h	+	-	+	-
1i	-	-	++	-
1j	+	-	-	-

Table (6): Results of S-aureus, E.coli, Enterobacteria cloacae & Klebsiella sensitivity against heterocyclic amide compounds (2a-j)

Compound	Microorganism			
	S-aureus G(+ve)	*E.coli G(-ve)*	*Enterobacteria G(-ve)*	*Klebsiella G(-ve)*
2a	++++	++	++++	-
2b	+	-	++	-
2c	+	-	+++	-
2d	+++	+++	++	-
2e	++	++	+	-
2f	+++	++	++++	-
2g	-	-	+	-
2h	+	-	++	-
2i	+++	++	++	-
2j	++	++	+	-

The key to the symbols: highly active ++++ (inhibition zone >34mm); active +++ (inhibition zone 25-34mm); moderately active ++ (inhibition zone 19-25mm); slightly active + (inhibition zone 12-19mm); inactive- (inhibition zone < 12mm).

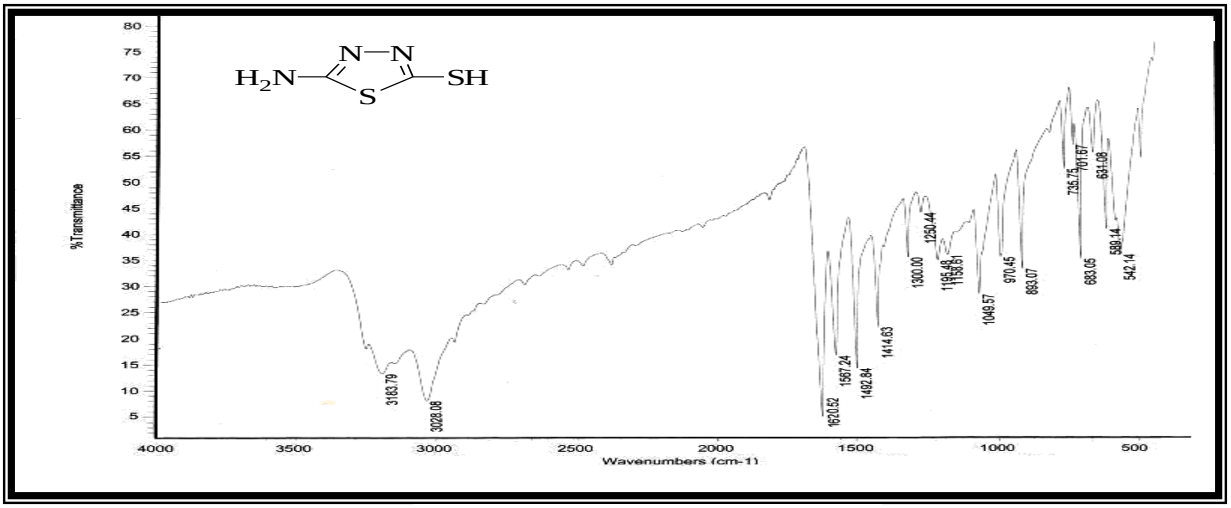


Fig (1): FTIR spectrum of compound (1a)

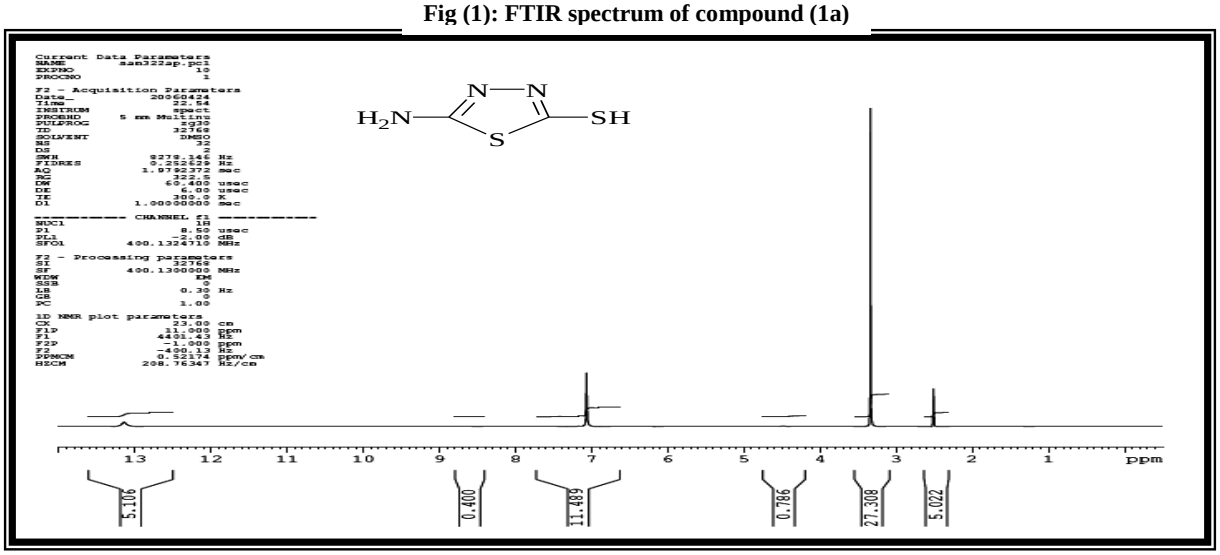


Fig (2):^{1}H-NMR spectrum of Compound (1a)

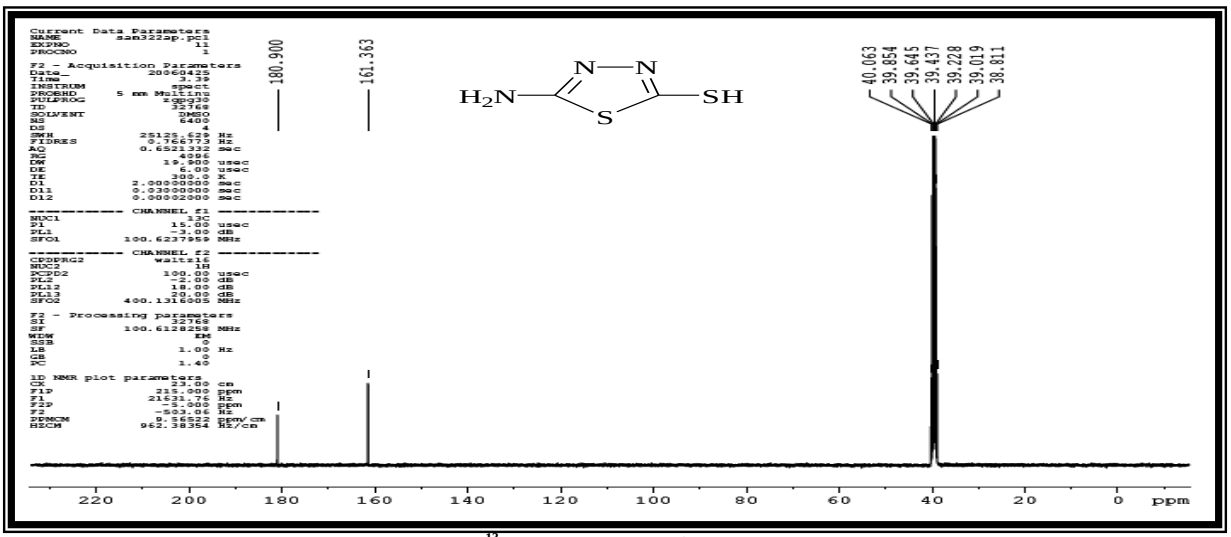


Fig (3): ^{13}C-NMR spectrum of Compound (1a)

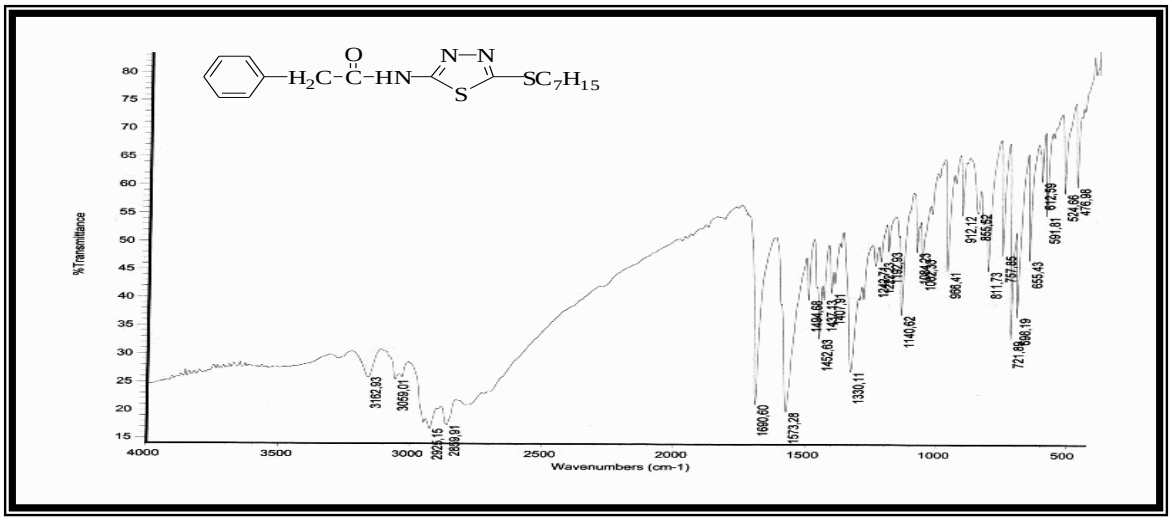


Fig (4): FTIR spectrum of Compound (2h)

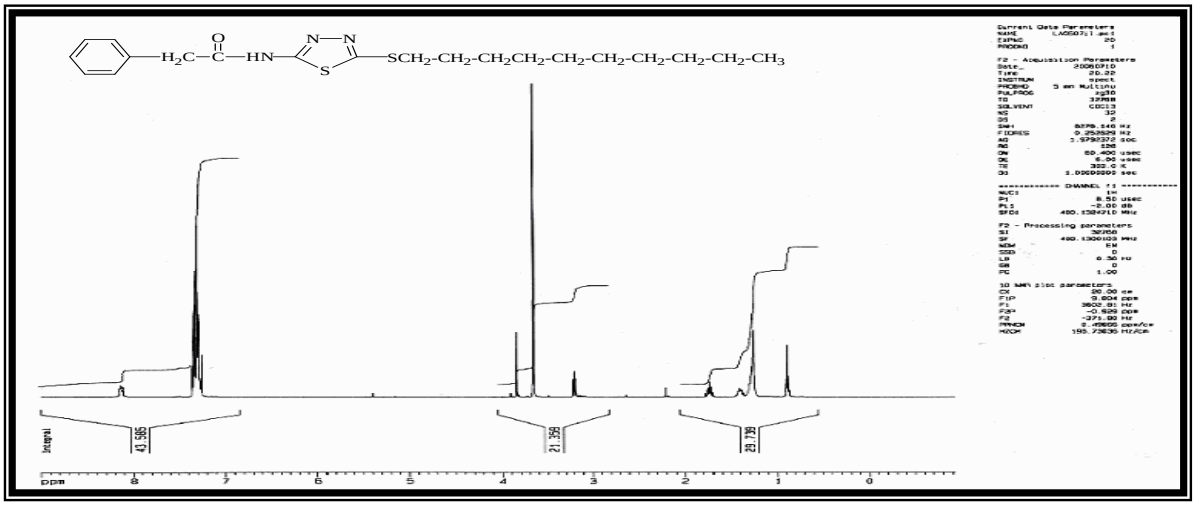


Fig (5):^{1}H-NMR spectrum of Compound (2k)

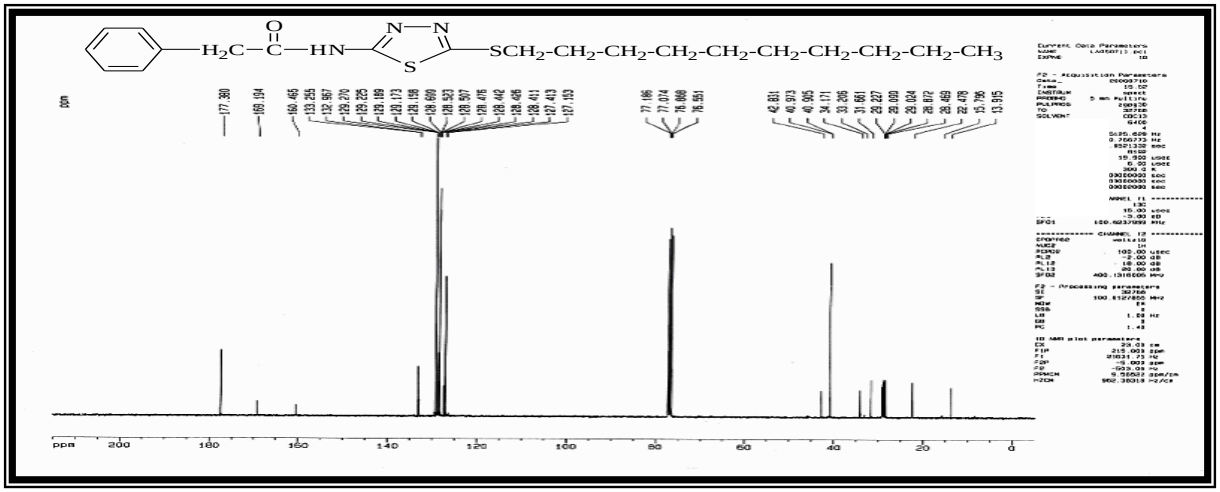


Fig (6):^{13}C-NMR spectrum of Compound (2k)

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