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Dithiin diisoimides: Synthesis and their antimicrobial studies

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Abstract: Sixteen derivatives of dithiin diisoimide 2a-2p have been synthesized and screened for antibacterial and antifungal activity. Compounds 2a-2g and 2i-2p are almost same or more active than gentamicine against *Acinetobacter*. Whereby compound 2,6-didodecyl-1*H*,5*H*-pyrrolo[3',4',5,6][1,4]dithiino[2,3-c]pyrrole-1,3,5,7(2*H*,6*H*)-tetrone (2d) having zone of inhibition 20 mm against *Acinetobacter* is the most potent among all these compounds and can be used as lead compound for the treatment of *Acinetobacter* infection.

Keywords: Dithiin diisoimides, succinamic acid, succinamic anhydride, amines, antibacterial

INTRODUCTION

Dithiins are included in phyto chemicals assist to maintain good health, lowers the cholesterol and triglyceride level and high blood pressure, results secure cardiovascular health. It helps to fight with diseases caused by infection and also effective against certain types of cancer. Garlic contains sulfur, and sulfur is itself an element that showed antioxidant activity. It is useful as an antiseptic, poor digestion and cough (Corzo-Martinez *et. al*., 2007). 2-Vinyl-4*H*-1,3-dithiin present in garlic, it act as antithrombotic and platelet aggregation inhibitory activity (Corzo-Martinez *et al*., 2007).

The recent research revealed that there are a number of sulfur containing organic compounds are found in garlic (*Allium sativom* Linn) which are responsible for its activity. These compounds, include allin (an allicin precursor), allicin, ajoene, scordinin, dithiins and diallyl sulfides (Bruno *et. al*., 2009; Kim, *et. al*., 1995). Allicin, have antibiotic and antithrombogenic activities. It has been also found that garlic will preserve its activity if it is processed in a way which keep its sulfur compounds from degradation by stomach acids. From centuries it has been used for the treatment of asthama, respiratory ailments, diabetes, pneumonia, rheumatism and cardiovascular disorder etc. The typical aroma of freshly chopped of the genus *Allium* is due to dithiin (which have two sulfur atoms), it is found in traces. It is detected by using cryogenic techniques GC-MS and HPLC (Abu-lafi *et al*., 2004).

Dithiins contain divalent sulfur and are six-member heterocyclic cyclacene (Kornmayer *et al*., 2009; Finar *et al*., 2007). 1,4-Dithiin ring is non-planar and angle linking C-S-C is 137°. Dicoordinated sulfur undergoes ring inversion and calculated energy barrier to ring inversion (4↔5) is 6.4 Kcal/mol [fig. 1] (Moriarty *et. al*., 1973).

Dithiin diisoimide possesses dithiin 3 moiety which has redox potential and capable to generates stable radical cations. Thermally stable dithiin undergoes oxidation reaction with H_2O_2 to form mono or disulfone (Andreu *et. al*., 2001). Compounds having 1,4-dithiin moiety have an ability to donate electrons so it can easily undergo isomerization, *i.e.* 1,4,5,8-tetrathianaphthlene TTN isomerizes into tetrathiafulvalene TTF. Sulfur could be removed from dithiin that lead to the formation of *cis* configurated double bonds (Caputo *et al*., 1994). Dithiin diisoimide proved to be very important biologically active molecule. Dithiin diisoimide and succinamic acid derivatives can be prepared by many ways (Amelichev *et al*., 2006; Safavy *et al*., 1997; MacDonald *et al*., 1980; Tarko *et al*., 2006; Geo *et al*., 1964; Itagaki *et al*., 2003; Kunkel, and Holstad, 1996; Burdulene *et al*., 1999; Arrizabalaga *et al*., 1984; Rankin *et. al*., 2001; Huang, and Risley, 2000; Finar *et al*., 2007; Cesare *et al*., 2004; Parham *et al*., 1959; Yamamoto *et al*., 2004; Murru *et al*., 2007; Zaidi *et al*., 2006).

As dithiins have significant biological activity, therefore, we decided to synthesize the libraries of dithiin diisoimides derivatives with different substitution at nitrogen with aliphatic and aromatic groups in order to get an active compound which may act as lead molecule for antibacterial. Therefore, in search of potent biologically active compounds, we have synthesized sixteen dithiin diisoimide derivatives 2a-2p and evaluated their

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antibacterial activity against Gram positive and Gram negative bacteria and antifungal activity and found significance results. In future, further modifications in substituted group at nitrogen might be help full in getting a number of biologically active lead compounds as drug candidates.

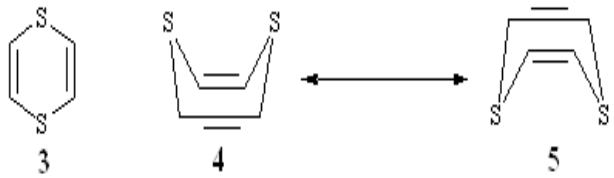


Fig. 1: Ring inversion of dicoordinated sulfur, calculated energy barrier (4↔5) is 6.4 Kcal/mol

MATERIALS AND METHODS

Reagents were purchased from Sigma-Aldrich, USA. Büchi 434 apparatus used to measured melting points. ^{1}H-NMR was performed on a Bruker AM 300 MHz. CHN analysis were done on a Carlo Erba Strumentazion-Mod-1106. Finnigan MAT-311A, Germany used for the EI MS. Thin layer chromatography (TLC) were carried out on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany).

General procedure for the synthesis of succinamic acid derivatives (1a-1p)

Succinamic acid derivatives 1a-1p were synthesized by treating succinic anhydride with different primary and secondary aliphatic and aromatic amines. In 3 mL of 1,4-dioxane, 5.0 mMol of succinic anhydride was added at room temperature then appropriate amine was added drop wise (4.99 mMol) with constant stirring, reaction mixture was refluxed for 30 minutes. After completion of reaction, white color solids were obtained (1a-1p), filtered and washed with hexane. Yields were obtained in the range of 59.0-87.8%.

General procedure for the synthesis of dithiin diisoimide derivatives (2a-2p)

Dithiin diisoimides were prepared by following the procedure, dropping funnel attached round bottom flask was placed in an ice bath with 2.1 mMol of succinamic acid derivative (1a-1p) and 13 mL (213.6 mMol) thionyl chloride was added drop wise with vigorous stirring at 0°C, then 5 mL of 1,4-dioxane was added to this suspension at room temperature with constant stirring and reaction continued for 16 hours. After completion of the reaction, solid that are glossy green in color were filtered, and washed with diethyl ether. Yields were obtained in the range of 20-78%. All compounds 2a-2p were found to be solid and reactions were monitored *via* TLC in (dichloromethane/hexane, 1:1), ^{1}H-NMR for all compounds 2b-2p were recorded in 300, 400 and 500 MHz.

2,6-Disopropyl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino [2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2a)

Solid; Yield: 76.8%; m.p.: 161°C R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.45 (t, J = 7.5 Hz, 4H, 2CH$_2$), 1.38-1.41(m, 4H, 2CH$_2$), 0.82 (t, J = 7.5 Hz, 6H, 2CH$_3$); EI MS: *m/z* (rel. abund. %) 338.407; Anal. Calcd for $C_{14}H_{14}N_2O_4S_2$; C = 49.69, H = 4.17, N = 8.28; Found: C = 49.64, H = 4.18, N = 8.27.

2,6-Di(tert-butyl)-1H,5H-pyrrolo[3',4':5,6][1,4]dithiin o[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2b)

Solid;Yield: 46.8%; m.p.: 165°C; R_f: 0.52 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.60 (s, 18H, 6CH$_3$); EI MS *m/z* = 366.461; Anal. Calcd for $C_{16}H_{18}N_2O_4S_2$; C = 52.44, H = 4.95, N = 7.64; Found: C = 52.34, H = 4.86, N = 7.65.

2,6-Dipentyl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2c)

Solid; Yield: 48.5%; m.p.:170°C; R_f: 0.55 (dichloromethane/hexane, 1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.46 (t, J = 7.5 Hz, 4H, 2CH$_2$), 1.18-1.32 (m, 12H, 6CH$_2$), 0.83 (t, J = 7.5 Hz, 6H, 2CH$_3$); EI MS *m/z* = 394.514; Anal. Calcd for $C_{18}H_{22}N_2O_4S_2$; C = 54.80, H = 5.62, N = 7.10; Found: C = 54.84, H = 5.60, N = 7.12.

2,6-Didodecyl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino [2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2d)

Solid; Yield: 76.8%; m.p.: 161°C; R_f: 0.59 (dichloromethane/hexane, 1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.45 (br.s, 4H, 2CH$_2$), 1.51 (br.s, 8H, 4CH$_2$), 1.23 (br.s, 32H, 16CH$_2$), 0.86 (s, 6H, 2CH$_3$); EI MS: *m/z* 590.891; Anal. Calcd for $C_{32}H_{50}N_2O_4S_2$; C = 65.05, H = 8.53, N = 4.74; Found: C = 65.04, H = 8.54, N = 4.75.

2,6-Dihexadecyl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino [2,3-c]pyrrole-1,3,5,7(2H,6H)-etrone (2e)

Solid; Yield: 76.8%; m.p.: 165°C; R_f: 0.67 (dichloromethane/hexane, 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.47 (t, J = 7.2 Hz, 4H, 2CH$_2$), 1.23 (br.s, 56H, 28CH$_2$), 0.87 (br.t, J = 6.0 Hz, 6H, 2CH$_3$); EI MS: *m/z* 703.106; Anal. Calcd for $C_{40}H_{66}N_2O_4S_2$;C = 68.33, H = 9.46, N = 3.98; Found: C = 68.34, H = 9.47, N = 3.95.

2,6-Dibenzyl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2f)

Solid; Yield: 76.8%; m.p.: 161°C R_f: 0.43 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.29 (br.s, 10H, Ar-H), 4.60 (s, 4H, 2CH$_2$); EI MS: *m/z* 434.495; Anal. Calcd for $C_{22}H_{14}N_2O_4S_2$; C = 0.82, H = 3.25, N = 6.45; Found: C = 60.81, H = 3.24, N = 6.46.

2,6-Bis(3-phenylpropyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2g)

Solid; Yield: 46.5%; m.p.:145°C; R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz,

$CDCl_3$): δ 7.29 (br.s, 10H, Ar-H), 4.28-4.31 (m, 12H, $6CH_2$); EI MS *m/z* = 490.603; Anal. Calcd for $C_{26}H_{22}N_2O_4S_2$; C = 63.65, H = 4.52, N = 5.71; Found: C = 63.64, H = 4.56, N = 5.72.

2,6-Bis(1,1-diphenylethyl)-1H,5H-pyrrolo[3',4':5,6] [1,4]dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2h)

Solid;Yield: 76.8%; m.p.: 142°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.89-7.83 (m, 20H, 4Ar-H), 6.40 (s, 6H, $2CH_3$); EI MS *m/z* = 614.744; Anal. Calcd for $C_{36}H_{26}N_2O_4S_2$; C = 70.34, H = 4.26, N = 4.56; Found: C = 70.35, H = 4.27, N = 4.55.

2,6-Dibenzhydryl-1H,5H-pyrrolo[3',4':5,6][1,4]dithiino [2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2i)

Solid;Yield: 75.3%; m.p.: 161°C; R_f: 0.39 (dichloromethane/hexane, 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.31 (m, 20H, 4Ar-H), 6.40 (s, 2H, 2CH); EI MS: *m/z* 586.691; Anal. Calcd for $C_{34}H_{22}N_2O_4S_2$; C = 69.61, H = 3.78, N = 4.77; Found: C = 69.64, H = 4.74, N = 4.73.

2,6-Bis(4-chlorophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2j)

Solid; Yield: 76.2%; m.p.: 161°C; R_f: 0.35 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.45 (br.d, $J_{2,3} = J_{6,5} = J_{2',3'} = J_{6',5'}$ =7.5 Hz, 4H, H-2,2',6,6'), 7.26 (br.d, $J_{3,2} = J_{5,6} = J_{3',2'} = J_{5',6'}$ = 7.5 Hz, 4H, H-3,3',5,5'); EI MS *m/z* = 475.332; Anal. Calcd for $C_{20}H_8Cl_2N_2O_4S_2$; C = 50.54, H = 1.70, N = 5.89; Found: C = 50.54, H = 1.71, N = 5.88.

2,6-Bis(2-chlorophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2k)

Solid; Yield: 45.5%; m.p.: 168°C; R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.36 (d, $J_{3,4} = J_{3',4'}$ = 8.2 Hz, 2H, H-3,3'), 7.16 (d, $J_{6,5} = J_{6',5'}$ = 8.1 Hz, 2H, H-6,6'), 7.02-7.06 (m, 4H, H-4,4',5,5'); EI MS *m/z* = 475.332; Anal. Calcd for $C_{20}H_8Cl_2N_2O_4S_2$; C = 50.54, H = 1.70, N = 5.89; Found: C = 50.54, H = 1.71, N = 5.88.

2,6-Bis(3-chlorophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2l)

Solid; Yield: 46.8% m.p: 162°C; R_f: 0.54 (dichloromethane/hexane, 1:1);^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.32-7.39 (m, 4H, H-4,4',6,6'), 7.07 (s, 2H, H-2,2'), 6.74-6.77 (m, 2H, H-5,5'); EI MS *m/z* = 475.332; Anal. Calcd for $C_{20}H_8Cl_2N_2O_4S_2$; C = 50.54, H = 1.70, N = 5.89; Found: C = 50.54, H = 1.71, N = 5.88.

2,6-Bis(4-bromophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2m)

Solid; Yield: 48.8%; m.p: 146°C; R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.29 (d, $J_{3,2} = J_{5,6} = J_{3',2'} = J_{5',6'}$ = 7.0 Hz., 4H, H-3,3',5,5'), 6.87 (d, $J_{2,3} = J_{6,5} = J_{2',3'} = J_{6',5'}$ = 7.0 Hz., 4H, H-2,2',6,6'); EI MS *m/z* = 564.233; Anal. Calcd for $C_{20}H_8N_2Br_2O_4S_2$; C = 42.57, H = 1.43, N = 4.96; Found: C = 42.54, H = 1.46, N = 4.94.

2,6-Bis(3-bromophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2n)

Solid; Yield: 42.8%, m.p: 143°C; R_f: 0.51 (dichloromethane/hexane, 1:1);^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.29 (s, 2H, H-2,2'), 7.14-7.19 (m, 4H, H-4,4',6,6'), 6.98-7.02 (m, 2H, H-5,5'); EI MS *m/z* = 564.233; Anal. Calcd for $C_{20}H_8N_2Br_2O_4S_2$; C = 42.57, H = 1.43, N = 4.96; Found: C = 42.54, H = 1.46, N = 4.94.

2,6-Bis(2-bromophenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2o)

Solid; Yield: 44.5%; m.p.: 148°C; R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.29-7.32 (m, 8H, H-3,3',4,4',5,5',6,6'); EI MS *m/z* = 564.233; Anal. Calcd for $C_{20}H_8N_2Br_2O_4S_2$; C = 42.57, H = 1.43, N = 4.96; Found: C = 42.54, H = 1.46, N = 4.94.

2,6-Bis(2-methylphenyl)-1H,5H-pyrrolo[3',4':5,6][1,4] dithiino[2,3-c]pyrrole-1,3,5,7(2H,6H)-tetrone (2p)

Solid; Yield: 55.5%; m.p.: 140°C; R_f: 0.51 (dichloromethane/hexane, 1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.34 (d, $J_{3,4} = J_{3',4'}$ = 8.0 Hz, 2H, H-3,3'), 7.19 (d, $J_{6,5} = J_{6',5'}$ = 8.1 Hz, 2H, H-6,6'), 7.03-7.07 (m, 4H, H-4,4',5,5'), 3.22 (s, 6H, $2CH_3$); EI MS *m/z* = 434.495; Anal. Calcd for $C_{22}H_{14}N_2O_4S_2$; C = 60.82, H = 3.25, N = 6.45; Found: C = 60.24, H = 3.26, N = 6.47.

Antibacterial bioassay

Sixteen derivatives of dithiin diisoimides 2a-2p were screened for antibacterial activity against Gram-positive and Gram-negative bacterial strains by using agar well diffusion method. The bacterial inoculums of two to eight hours old approximately 10^4-10^6 colony forming units (cfu)/mL were inoculated in an autoclave. The melted inoculums were cooled at 55°C, transferred into nutrient agar plate and set to solidify. With the help of a sterile metallic borer the wells were dug in the media about 24 mm. 100 μL of the test sample having concentration (1 mg/mL in DMSO) was added in respective wells. Other wells were complemented with DMSO and reference antibacterial drug *i.e.* gentamicin serving as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the zone of inhibition in (mm). Growth inhibition was calculated with reference to positive control (Atta-ur-Rahman *et al*., 2001).

Antifungal bioassay

Antifungal activities of all compounds were studied against eight fungal cultures. 10^5(cfu)/mL fungal spore suspensions was seeded in sabouraud dextrose agar (Oxoid, Hampshire, England) in a Petri dish, (Helfand, *et*.

al., 1982). Discs of all compounds were soaked in 20 mL having concentration (10 mg/mL in DMSO) and were placed at different positions on the agar plates. The plates were incubated at 32°C for seven days. The results were recorded as zone of inhibition in mm (Atta-ur-Rahman *et. al*., 2001).

RESULTS

Chemistry

Succinamic acid derivatives 1a-1p were synthesized by treating succinic anhydride with different primary and secondary aliphatic and aromatic amines (Arrizabalaga *et. al*., 1984; Burdulene *et al*., 1999; Rankin *et al*., 2001; Huang *et. al*., 2000; Serrano *et. al*., 2007; Cesare *et. al*., 2004; Finar *et. al*., 2007). Dithiin diisoimide derivatives 2a-2p have been synthesized from succinamic acid derivatives 1a-1p by reaction with excess of thionyl chloride using 1,4-dioxane as solvent (fig. 2) (table 1).

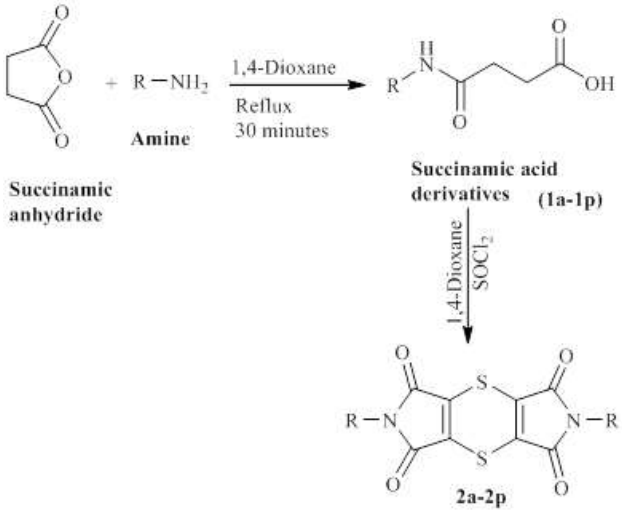


Fig. 2: Synthesis of dithiin diisoimide derivatives (2a-2p)

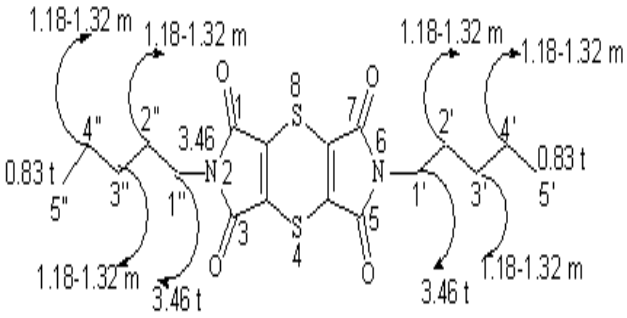


Fig. 3: ^{1}H-NMR chemical shifts of representative compound 2c

Fig. 4, 6-16 are represented the NMR spectra of compounds (2a, 2c-2g, 2i, 2k, 2l, 2n-2p). Fig. 5 is represented the Mass spectrum of compound 2b.

The results of antibacterial activities are shown in table 2 & 3.

DISCUSSION

Antibacterial and antifungal activities

Dithiin diisoimide derivatives 2a-2p have been synthesized and all sixteen compounds were screened for antimicrobial (antibacterial and antifungal) (Atta-ur-Rahman *et. al*., pp.60, 2001; Atta-ur-Rahman *et. al*., pp.16, 2001; Atta-ur-Rahman *et. al*., pp.22, 2001) activity most of them (2a-2p) exhibited good activity. Compounds 2a-2p were tested against Gram negative bacteria table 2, it was found that all compounds were less or inactive as compared to the standard gentamicine against *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi, Salmonella typhi para A, Salmonella typhi para B, Shigella flexneri, Shigella dysenteriae and Aeromonas*. Gentamicine itself was inactive against *Escherichia coli* MDR, however, compounds 2a-2d and 2f-2i were found to be active against *E. coli* MDR. Compounds 2a-2g and 2i-2p have comparable or more activity than gentamicine against *Acinetobacter*. The compound 2,6-didodecyl-1*H*,5*H*-pyrrolo[3',4':5,6][1,4]dithiino[2,3-c]pyrrole-1,3,5,7 (2*H*,6*H*)-tetrone (2d) having zone of inhibition 20 mm against *Acinetobacter* was the most potent among all these compounds, this may be due to presences of *n*-dodecyl group (C12 chain) as compared to compound 2c (*n*-pentyl C5 chain) which may has less lipophilic character. However, compound 2e (*n*-hexadecyl C16 chain) found to be less active than 2d that may be due to flipping of long chain around the single bond thus producing hindrance in the moiety and less activity of compound 2h may also be due to same reason. Due to higher activity of 2d compound, this can be used as lead molecule for development of effective drug for *Acinetobacter* infection.

Compounds 2a-2p were also tested against Gram positive bacteria (table 3), it was found that compounds 2a, 2k, 2l, 2o and 2p have comparable zone of inhibition as the standard gentamicine against *Bacillus subtilis*. The activity of compounds 2a, 2c-2e, 2g, and 2i were equivalent to gentamicine against Methicillin-Resistant *Staphylococcus aureus* (MRSA). The activity of compound 2d was almost similar to gentamicine against *Corynebacterium xerosis*, while the potency of compound 2i and gentamicine against *Staphylococcus aureus* were equal. Compounds 2a-2p were found to be less to inactive against *Streptococcus pyogenes, Staphylococcus epidermidis, Streptococcus faecalis, Corynebacterium diphtheria*. All compounds were found to be inactive against *Staphylococcus saprophyticus*. However, compounds 2a-2p were inactive against all tested fungal strains *i.e., Rhizopus sp., Penicillium sp., Mucor, Saccharomyces, Aspergillus niger, Aspergillus fiavus, Candida tropicalis and Candida albicans*.

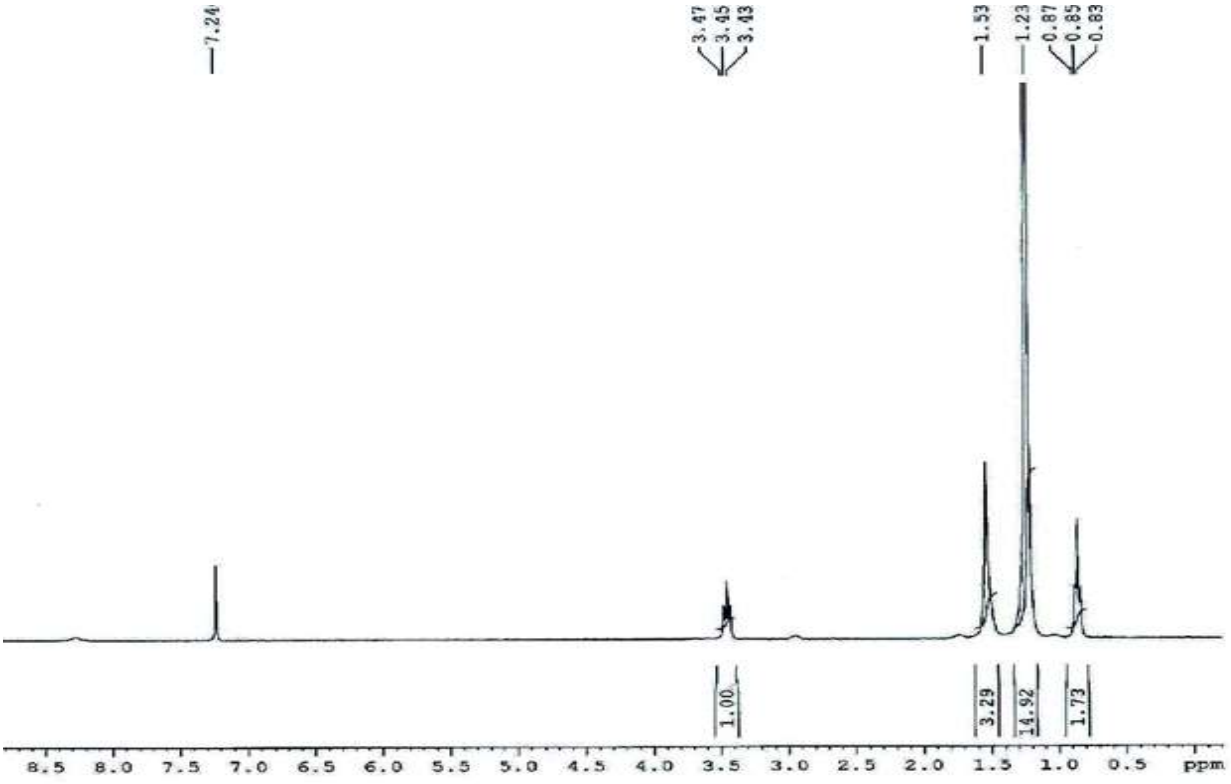


Fig. 4: ^{1}H-NMR spectrum of compound (2a)

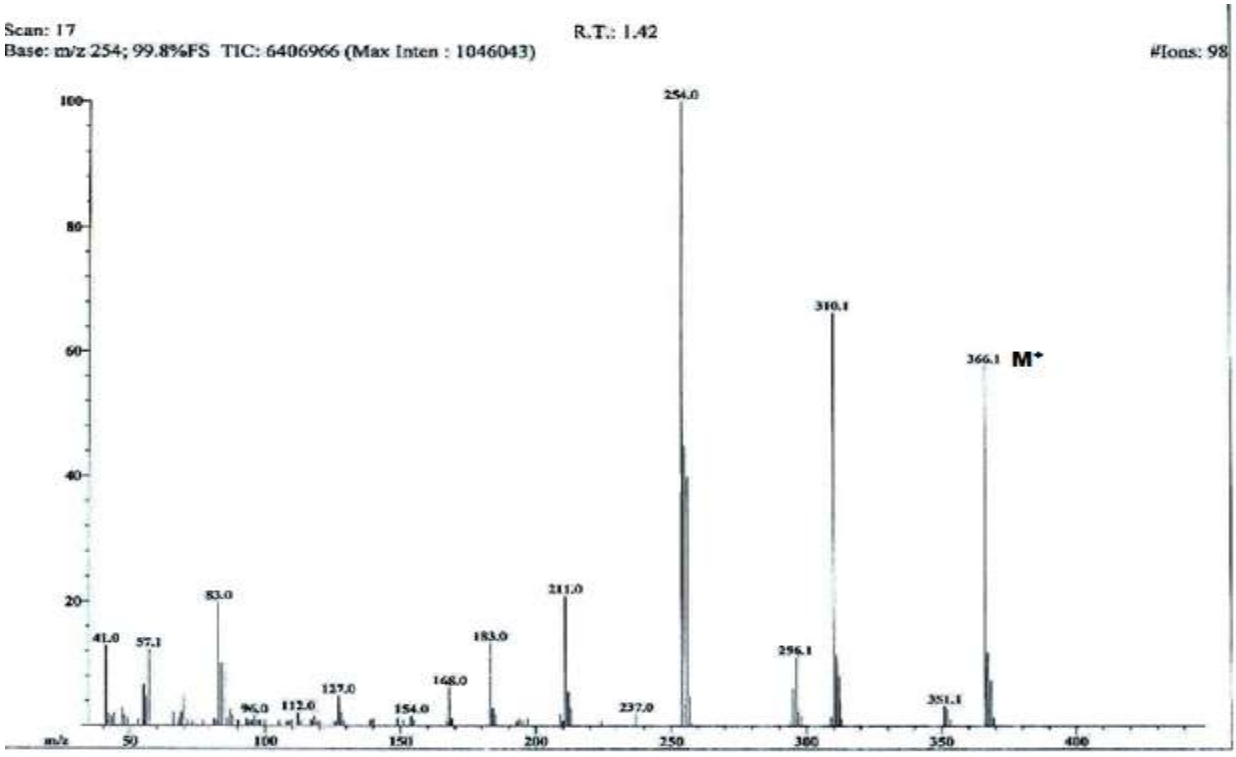


Fig. 5: Mass spectrum of compound (2b)

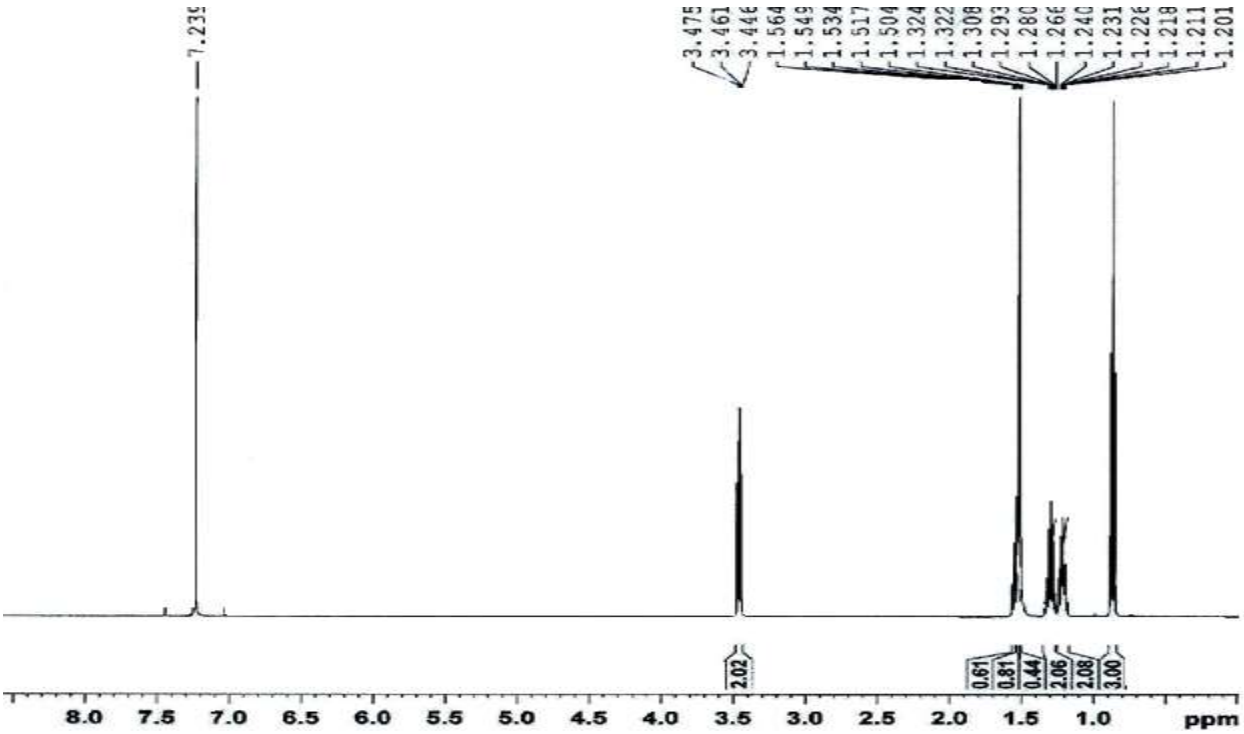


Fig. 6: ^{1}H-NMR spectrum of compound (2c)

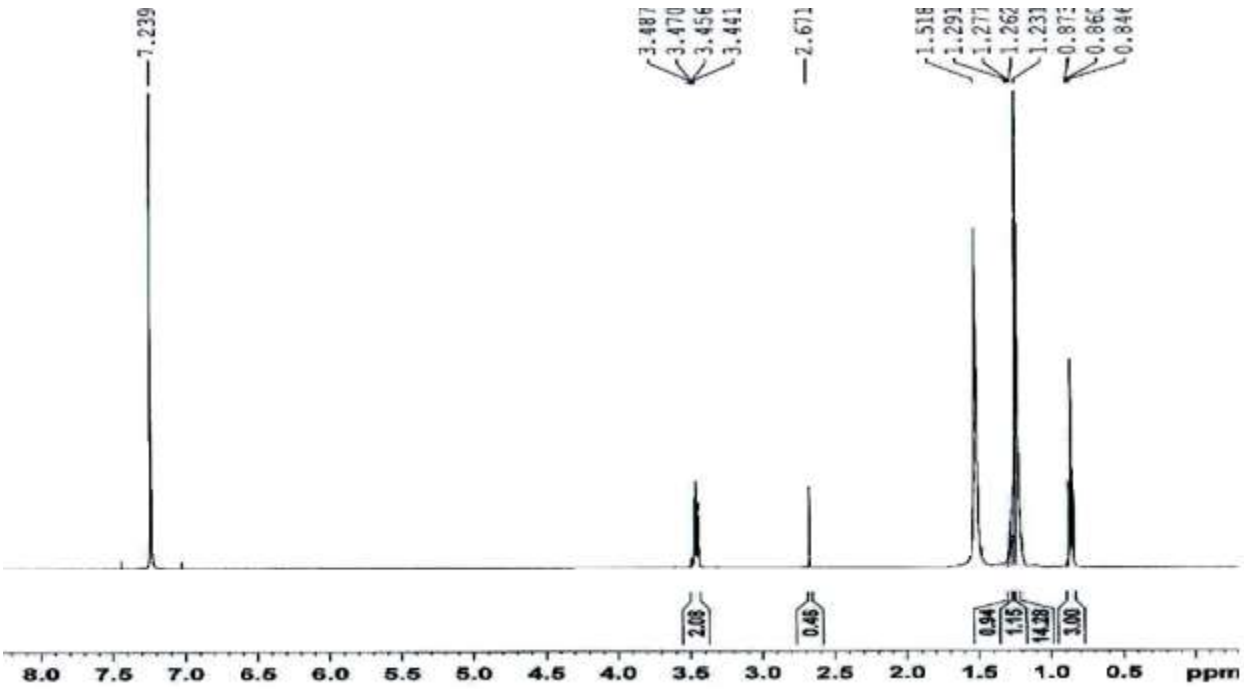


Fig. 7: ^{1}H-NMR spectrum of compound (2d)

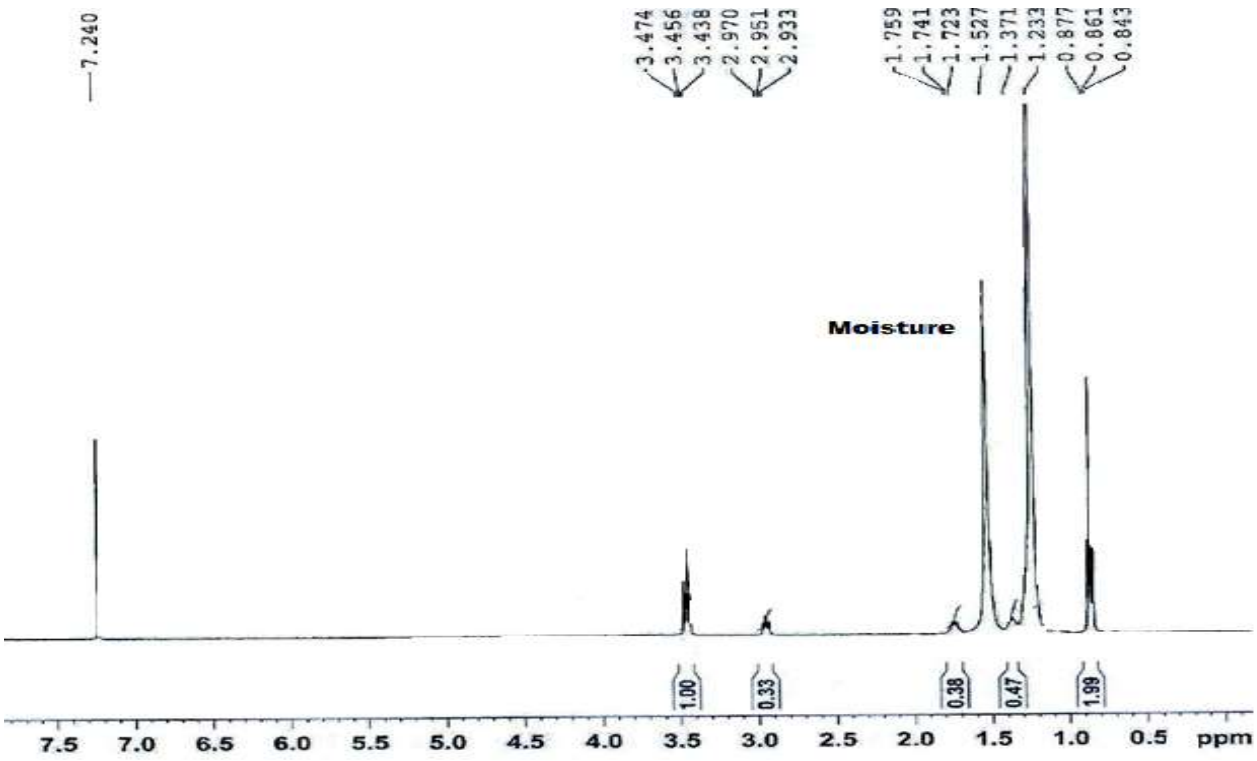


Fig. 8: ^{1}H-NMR spectrum of compound (2e)

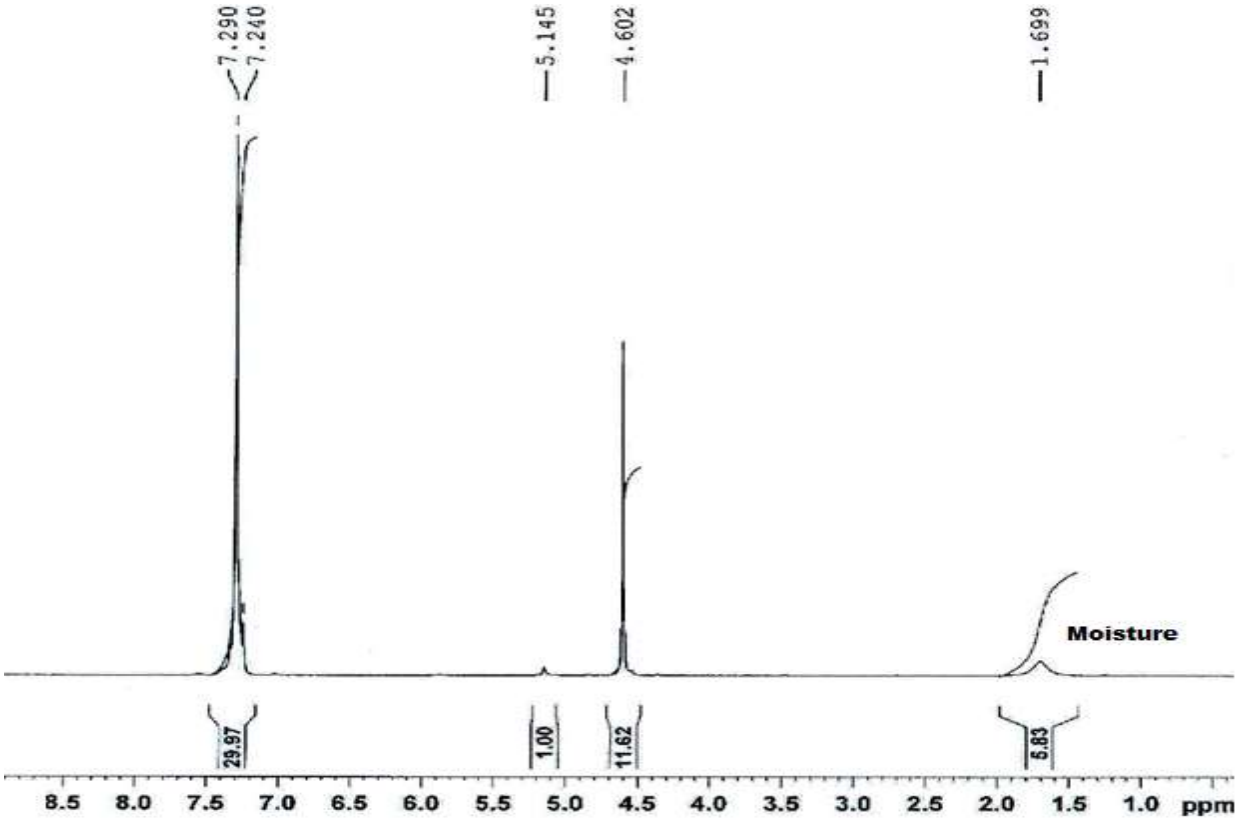


Fig. 9: ^{1}H-NMR spectrum of compound (2f)

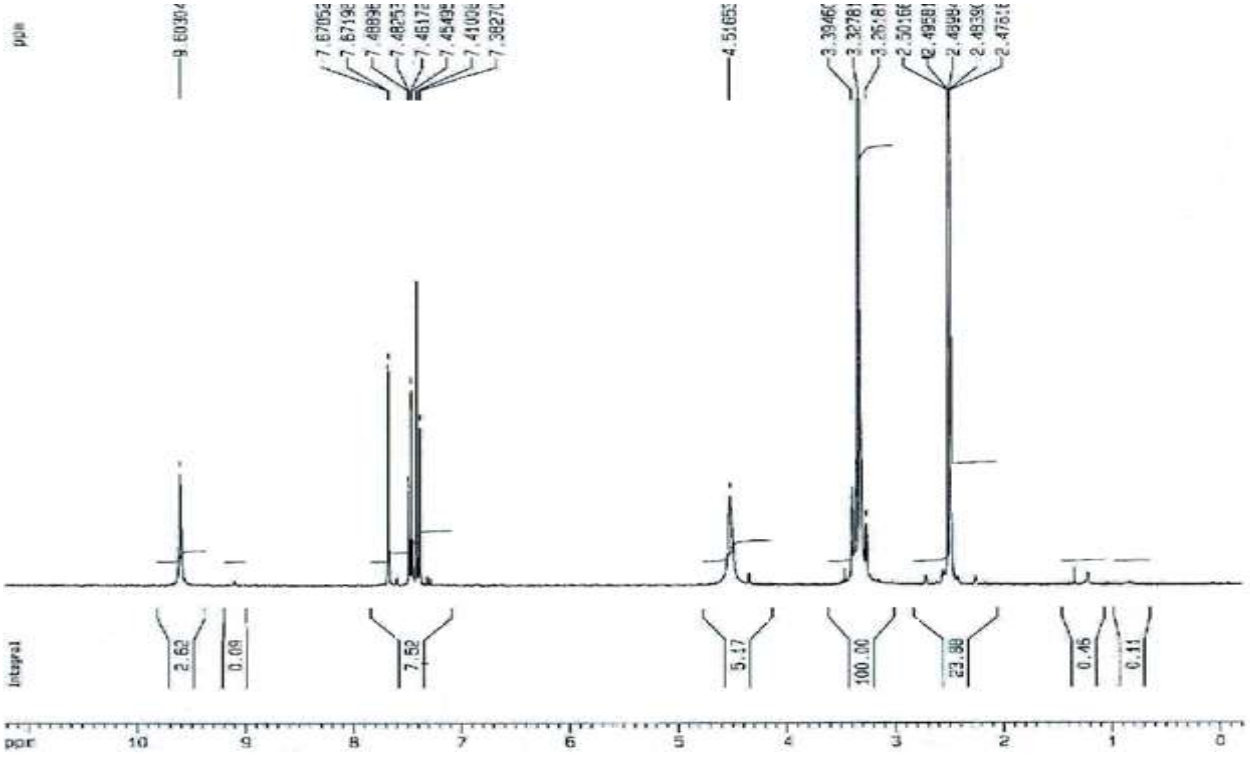


Fig. 10: ^{1}H-NMR spectrum of compound (2g)

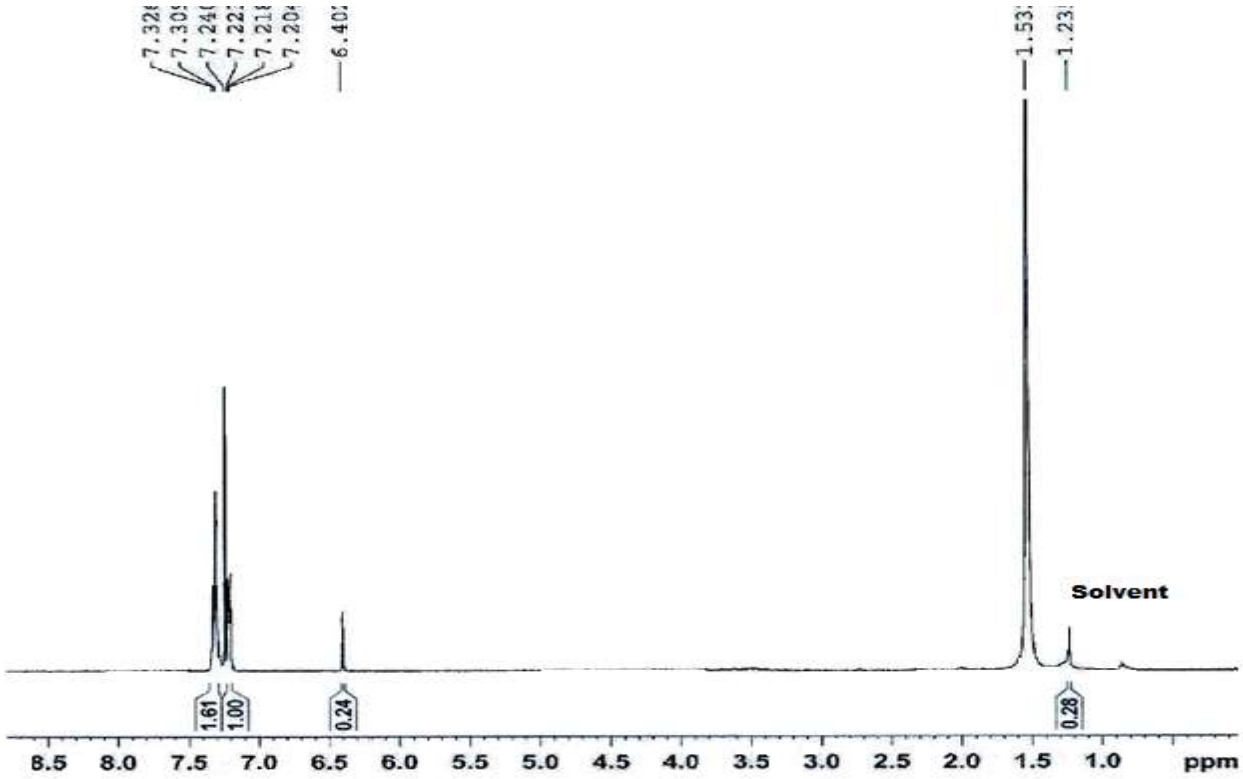


Fig. 11: ^{1}H-NMR spectrum of compound (2i)

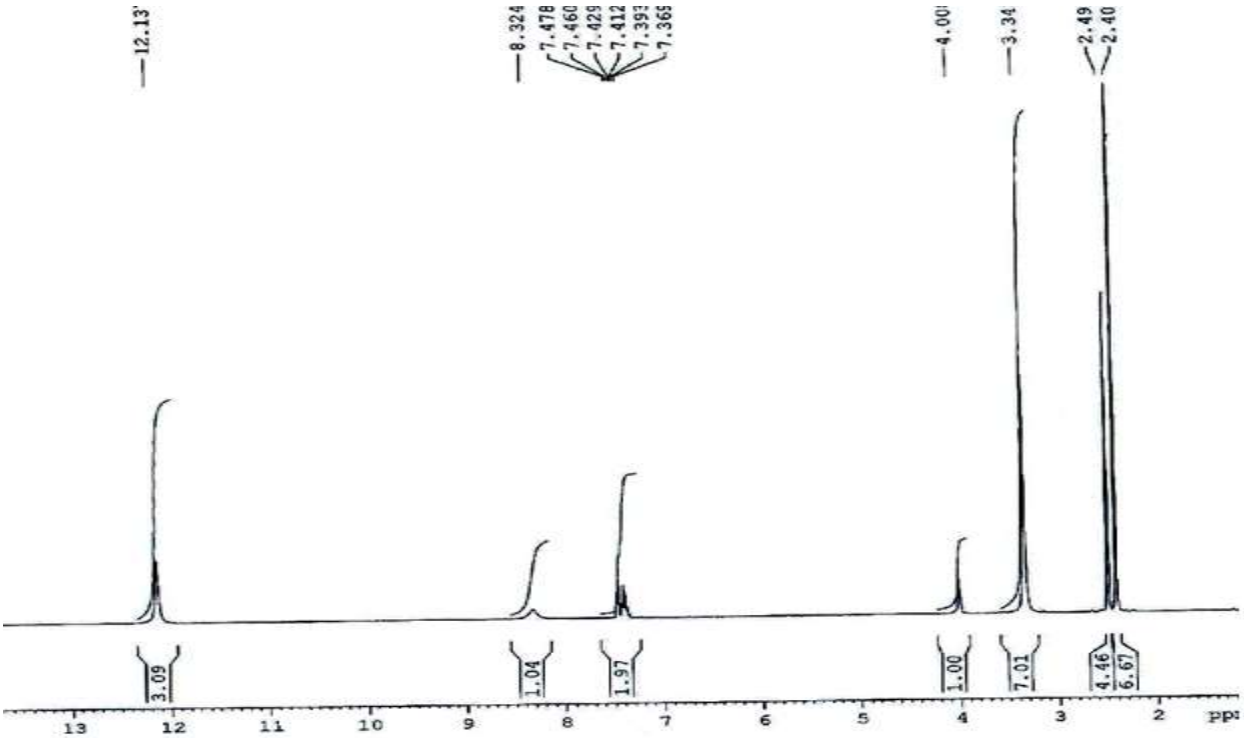


Fig. 12: ^{1}H-NMR spectrum of compound (2k)

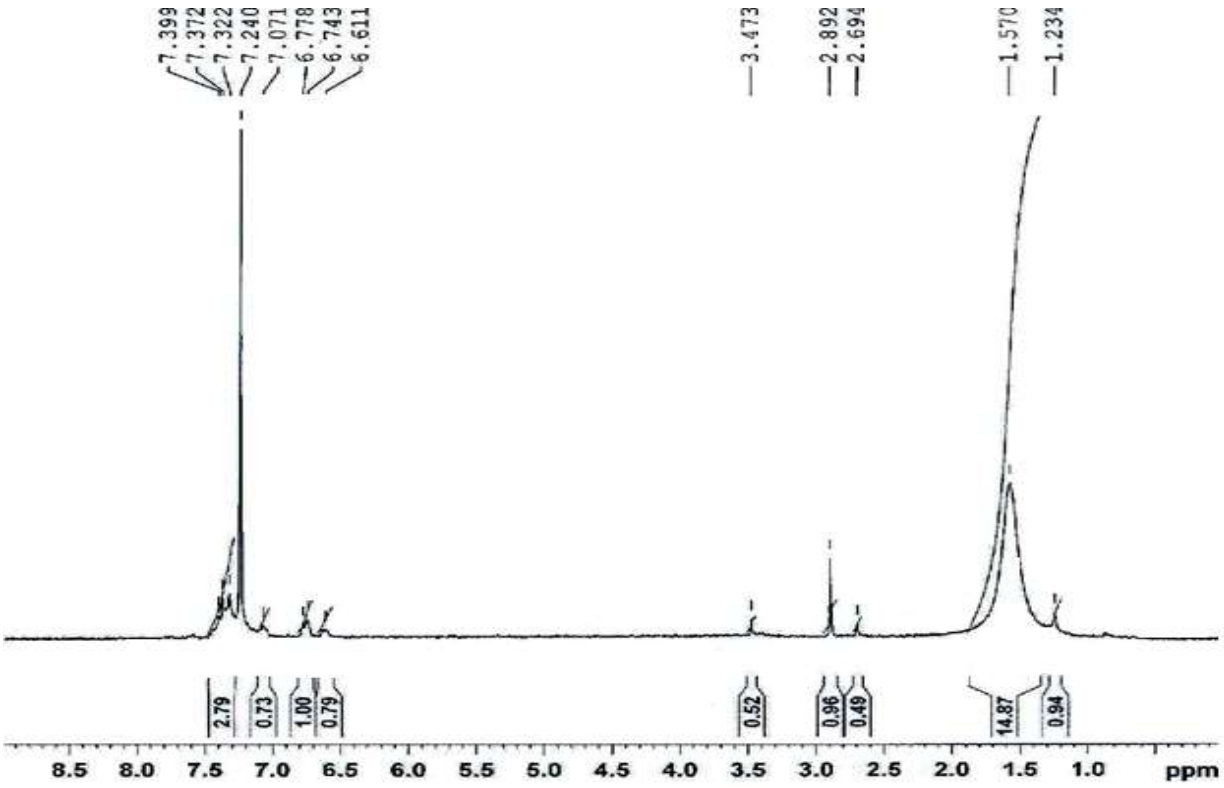


Fig. 13: ^{1}H-NMR spectrum of compound (2l)

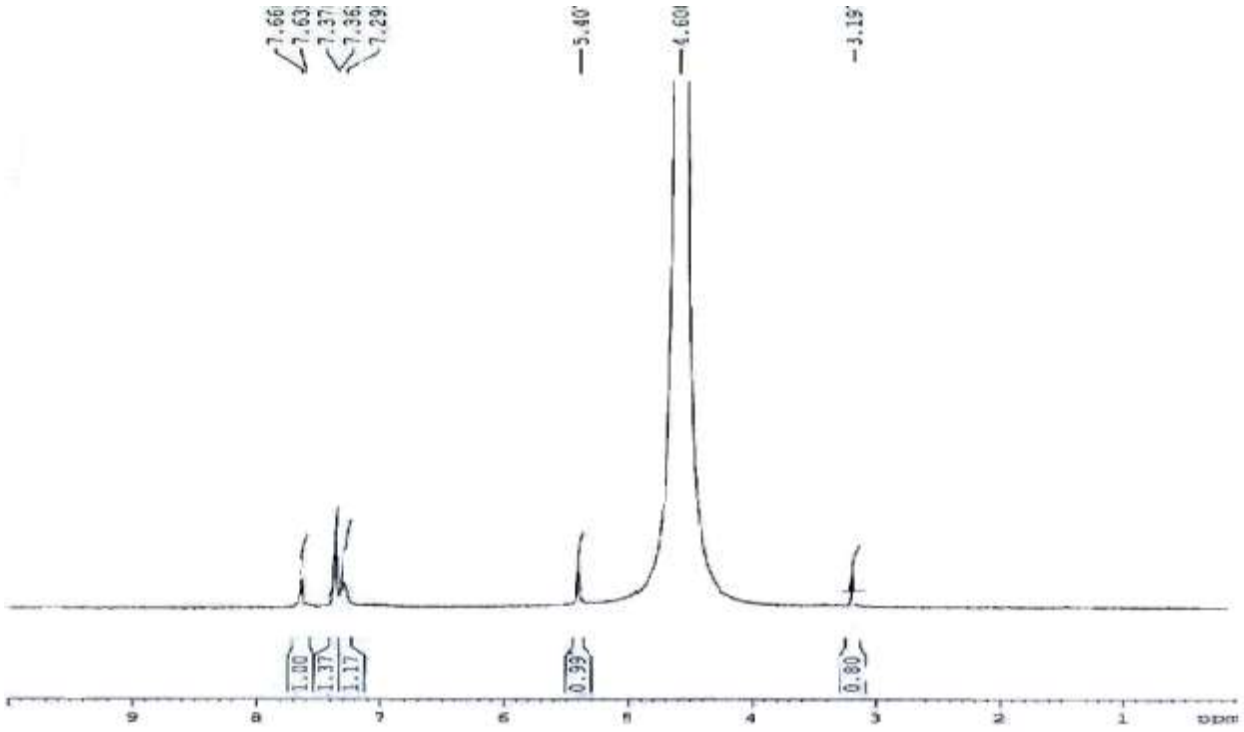


Fig. 14: ^{1}H-NMR spectrum of compound (2n)

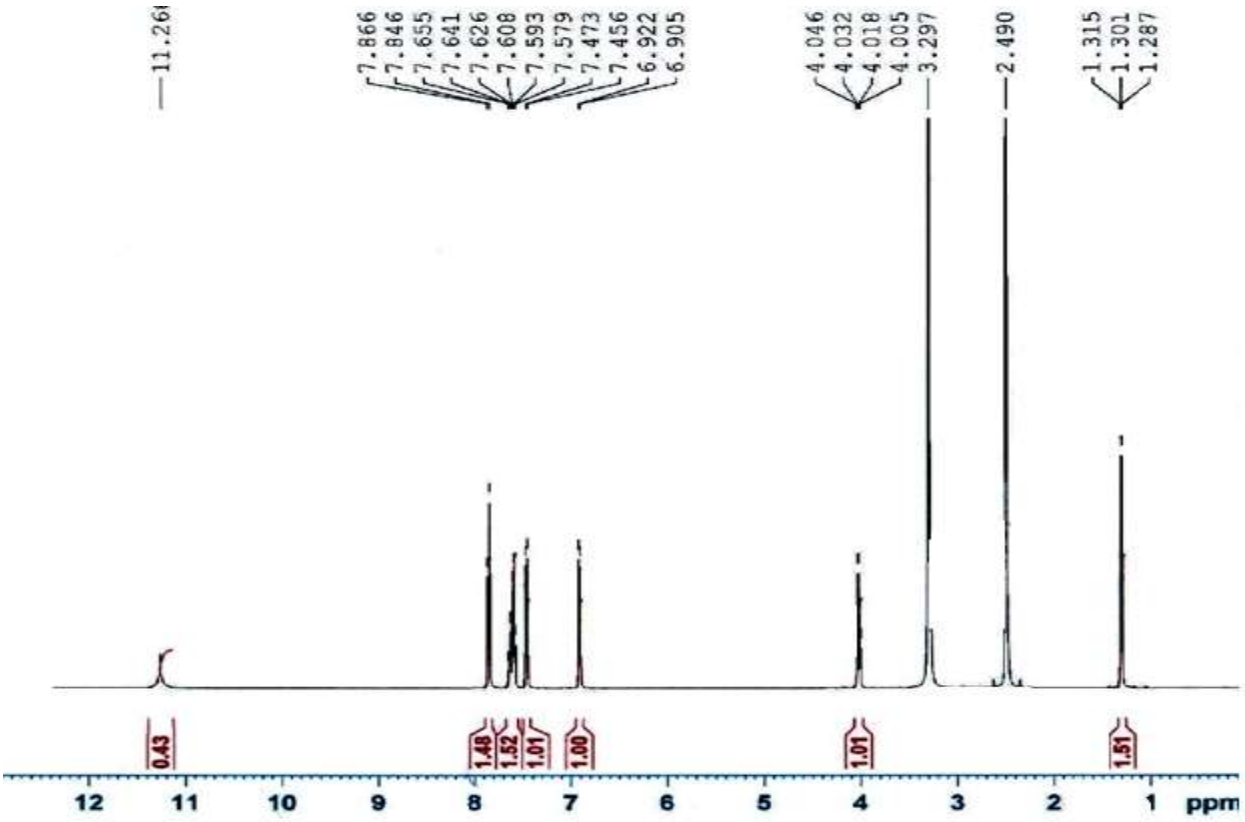


Fig. 15: ^{1}H-NMR spectrum of compound (2o)

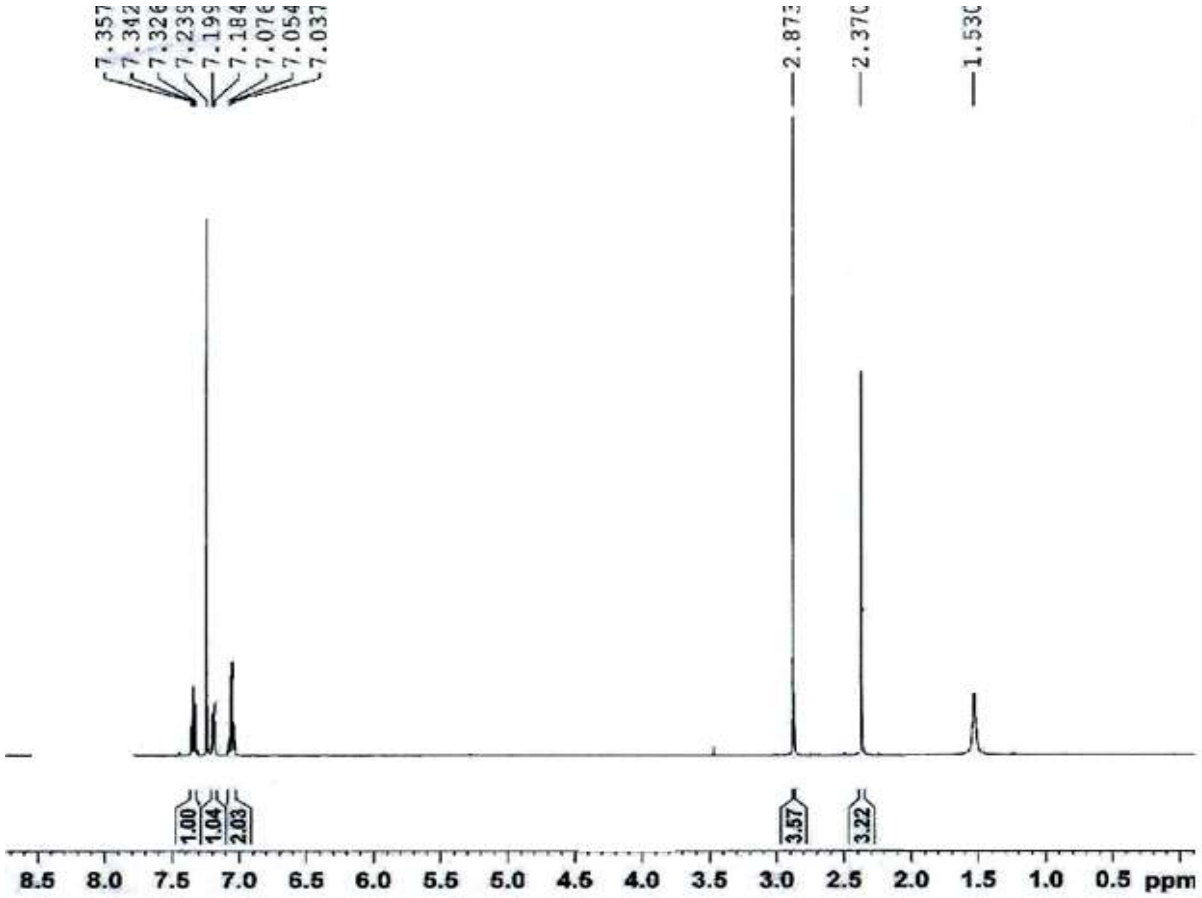
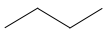
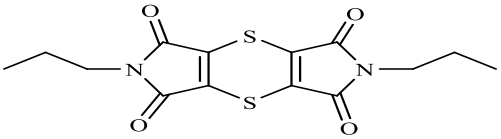
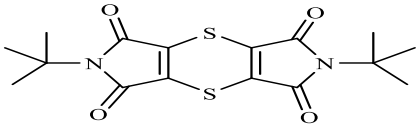
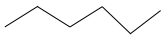
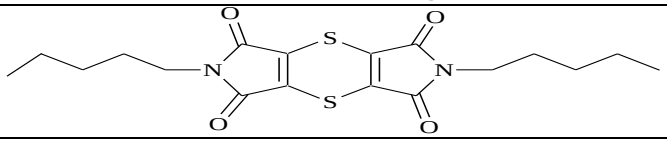
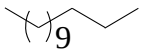
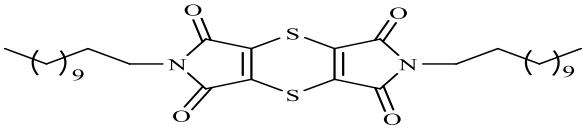
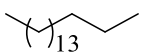
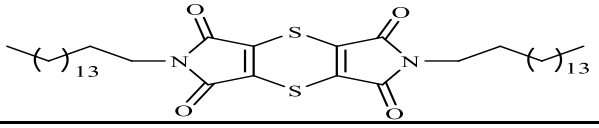


Fig. 16: ^{1}H-NMR spectrum of compound (2p).

Table 1: Derivatives of dithiin diisoimides 2a-2p

Compound No.	R	Structure
2a		
2b		
2c		
2d		
2e		

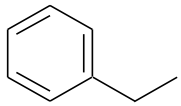
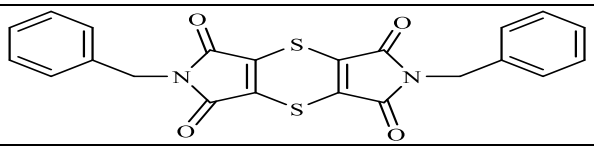
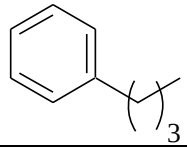
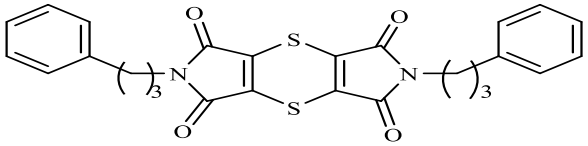
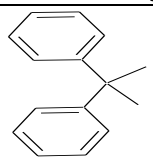
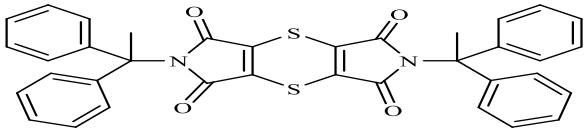
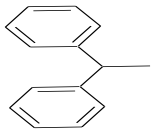
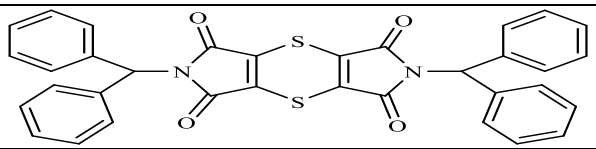
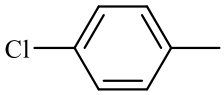
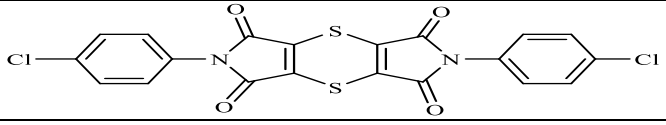
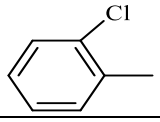
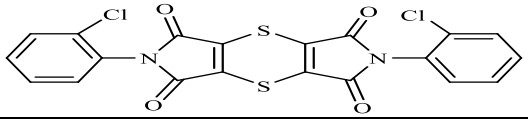
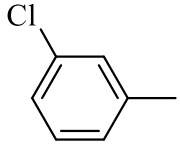
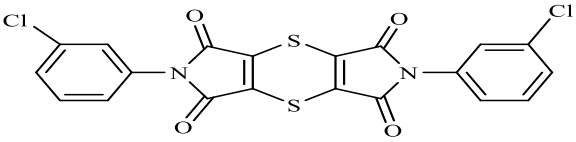
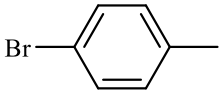
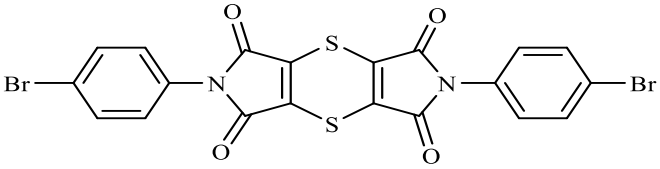
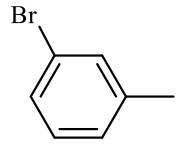
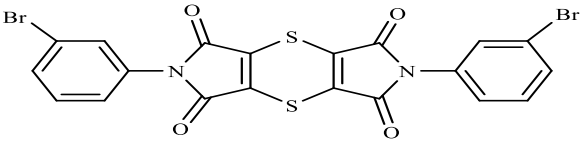
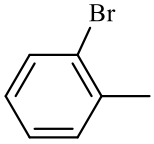
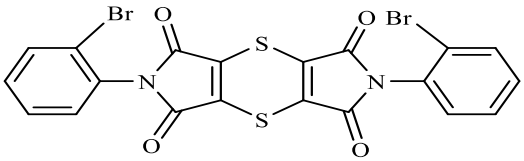
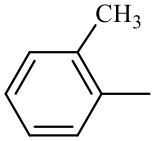
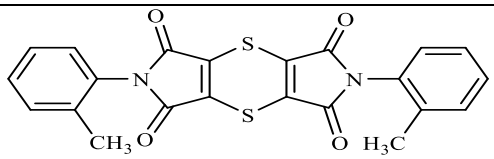
Compound No.	R	Structure
2f		
2g		
2h		
2i		
2j		
2k		
2l		
2m		
2n		
2o		
2p		

Table 2: *In vitro* antibacterial activity against Gram negative bacteria (Zone of Inhibition in mm)

S. No.	Organisms Name	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l	2m	2n	2o	2p	Gentamicine
1	*Escherichia coli*	17	19	22	22	21	18	18	23	18	19	-	-	-	-	-	-	29
2	*Escherichia coli* MDR	15	15	16	10	-	18	15	15	14	-	-	-	-	-	-	-	-
3	*Klebsiella pneumoniae*	17	15	15	15	15	16	13	16	16	15	15	15	15	15	10	-	23
4	*Proteus mirabilis*	15	20	16	18	21	16	14	20	17	19	-	-	-	-	-	-	32
5	*Salmonella typhi*	16	17	17	18	15	17	14	19	17	16	17	14	15	15	14	14	25
6	*Salmonella typhi para A*	18	17	19	16	17	16	15	19	17	17	16	17	-	-	-	-	25
7	*Salmonella typhi para* B	18	18	16	16	16	18	15	15	16	16	12	10	15	-	13	12	25
8	*Shigella flexneri*	15	16	14	17	17	17	13	16	15	15	18	14	17	15	15	19	23
9	*Shigella dysenteriae*	19	18	17	17	18	21	18	20	20	20	16	15	17	16	18	14	28
10	*Aeromonas*	18	14	14	14	13	-	-	15	13	14	15	17	10	15	17	12	27
11	*Acinetobacter*	15	15	16	20	15	15	15	10	14	15	14	14	14	16	15	13	14

Table 3: *In vitro* antibacterial activity against Gram positive bacteria (Zone of Inhibition in mm)

S. No.	Organisms name	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l	2m	2n	2o	2p	Gentamicine
1	*Bacillus subtilis*	20	15	16	17	16	17	17	16	16	17	19	20	17	17	20	19	22
2	Methicillin-Resistant *Staphylococcus aureus* (MRSA)	18	17	20	18	19	12	18	14	18	15	10	-	-	-	-	-	20
3	*Streptococcus pyogenes*	15	16	17	16	16	17	10	16	15	15	10	-	-	18	-	18	25
4	*Corynebacterium xerosis*	17	16	17	22	20	20	18	16	19	17	-	-	-	-	-	-	25
5	*Staphylococcus aureus*	20	19	19	20	20	17	19	20	23	18	16	15	17	14	16	-	25
6	*Staphylococcus epidermidis*	18	18	19	18	20	17	18	20	20	21	15	18	10	11	15	14	28
7	*Streptococcus faecalis*	16	17	16	12	14	16	11	13	12	16	-	-	13	15	15	11	25
8	*Corynebacterium diphtheria*	18	17	17	17	16	16	16	16	15	15	15	16	17	12	12	14	25
9	*Staphylococcus saprophyticus*	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	24

CONCLUSION

The activity of compounds 2a-2g and 2i-2p are almost same or more than the standard gentamicine against *Acinetobacter*. While the compound 2,6-didodecyl-1*H*,5*H*-pyrrolo[3',4',5,6][1,4]dithiino[2,3-c]pyrrole-1,3,5,7 (2*H*,6*H*)-tetrone (2d) is the most potent among all these compounds and can be used as lead compound for the treatment of *Acinetobacter* infection.

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