

# **Eco-Friendly Synthesis, Characterization and Biological Evaluation of Some Novel Pyrazolines Containing Thiazole Moiety as Potential Anticancer and Antimicrobial Agents**

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## **General method for the evaluation of Cytotoxic Effects of certain Chemical compound**

Mammalian cell lines: MCF-7 cells (human breast cancer cell line), HepG-2 cells (human Hepatocellular carcinoma) and HCT-116 (colon carcinoma) were obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH<sub>2</sub>O and filtered through a Whatmann No.1 filter paper.

### Cell line Propagation:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were subcultured two times a week.

Cytotoxicity evaluation using viability assay [36,37]: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of  $1 \times 10^4$  cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> for a

period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells at 37°C, for 24 h, the viable cells yield was determined by a colorimetric method.

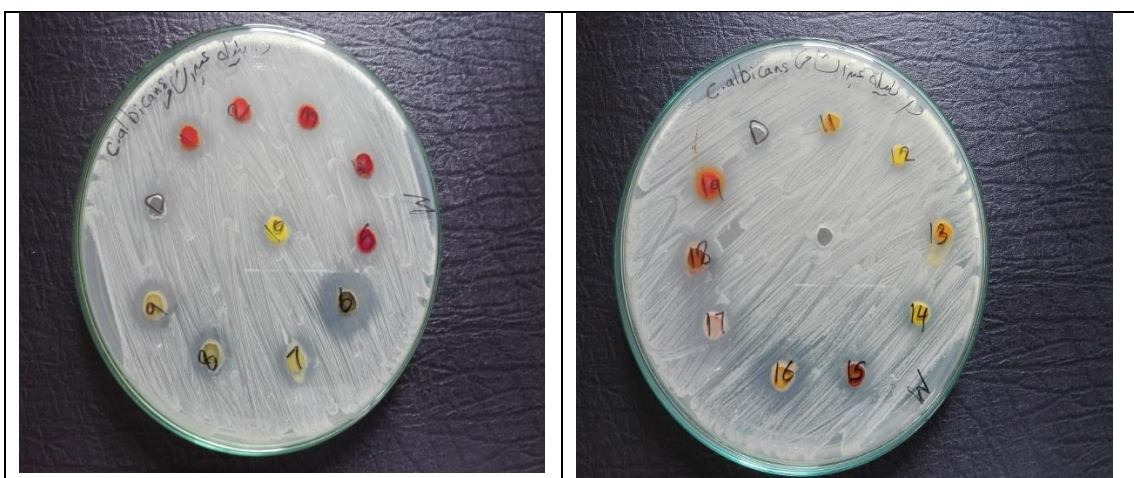
In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as  $[(OD_t/OD_c)] \times 100\%$  where  $OD_t$  is the mean optical density of wells treated with the tested sample and  $OD_c$  is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration ( $IC_{50}$ ), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA).

#### **Method of antimicrobial evaluation.**

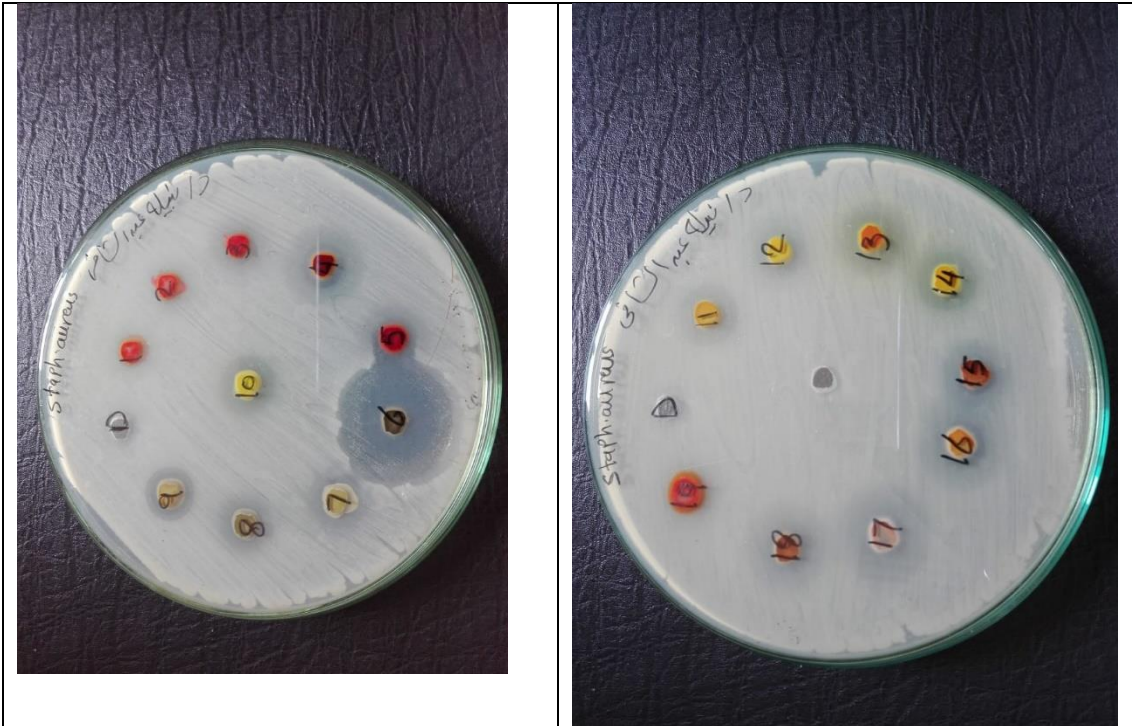
All microbial strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antimicrobial activity was investigated on a newly synthesized compound in order to increase the selectivity of these derivatives towards test microorganisms using the agar diffusion method using Mueller-Hinton agar medium for bacteria and Sabouraud's agar medium for fungi. Briefly, 100  $\mu$ l of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately  $10^8$

cells/mL for bacteria or  $10^5$  cells/mL for fungi. All the newly synthesized compounds were weighed and dissolved in dimethyl sulfoxide to prepare extract stock solution. One hundred  $\mu\text{L}$  of each sample at 5 mg/mL was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 h at 37 °C (for bacteria and yeast) and for 48 h at 28 °C (for filamentous fungi). After incubation, the microorganism's growth was observed. The resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. The size of this clear zone is proportional to the inhibitory action of the compound under investigation. DMSO was used for dissolving the tested compounds thus used as solvent control and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms. Positive controls were also performed using gentamycin as standard antibacterial drugs and ketoconazole as standard antifungal drug [38,39].

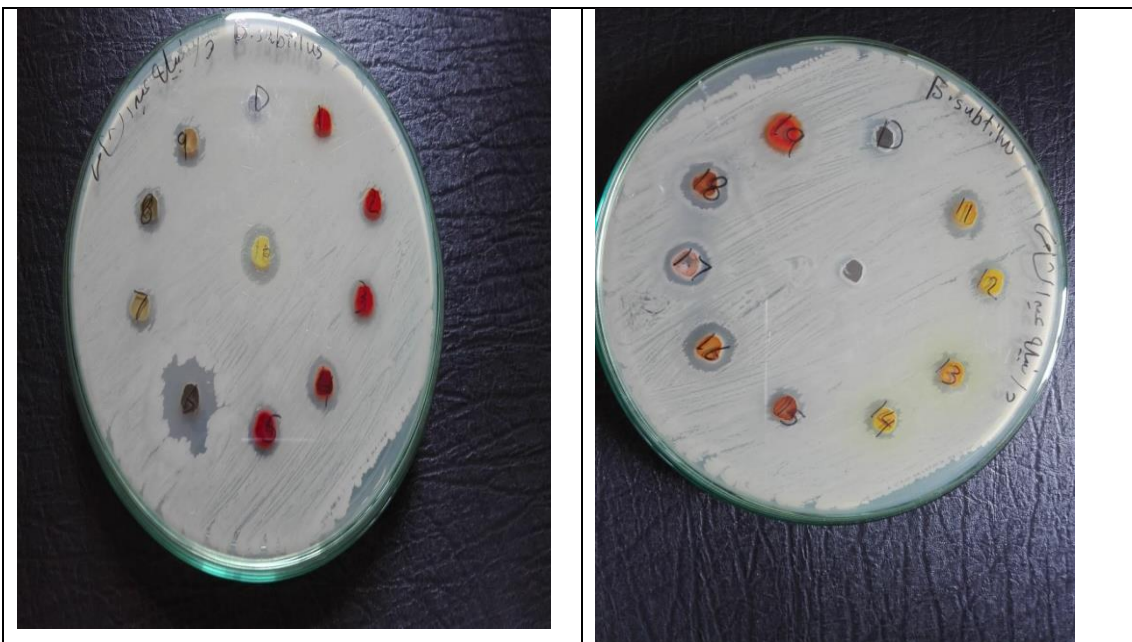
#### Figures of mean zone of inhibition of the newly synthesized pyrazolines



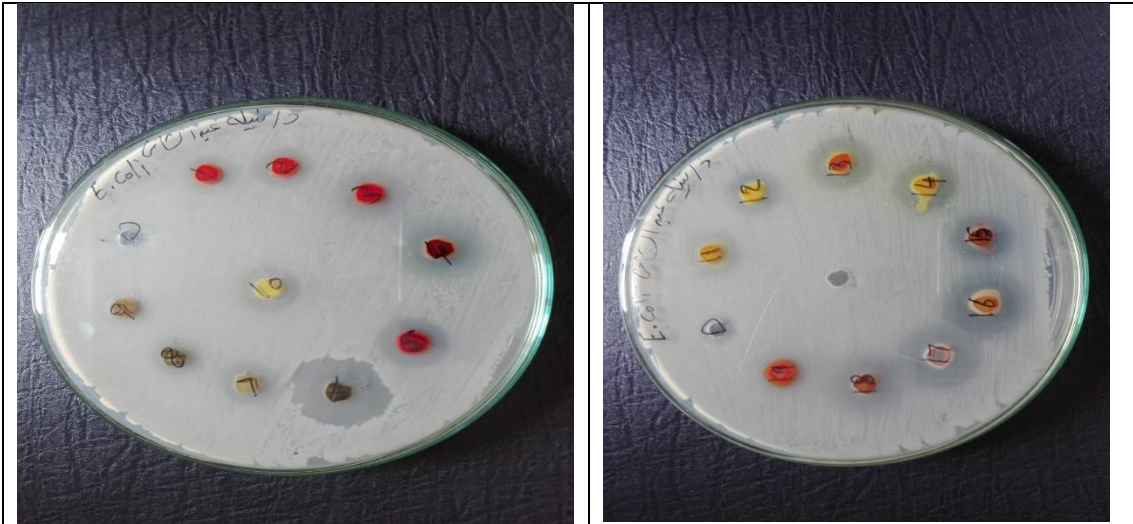
**Fig. S1** Mean zone of inhibition of the newly synthesized pyrazolines tested against *Candida albicans* (RCMB 05036).



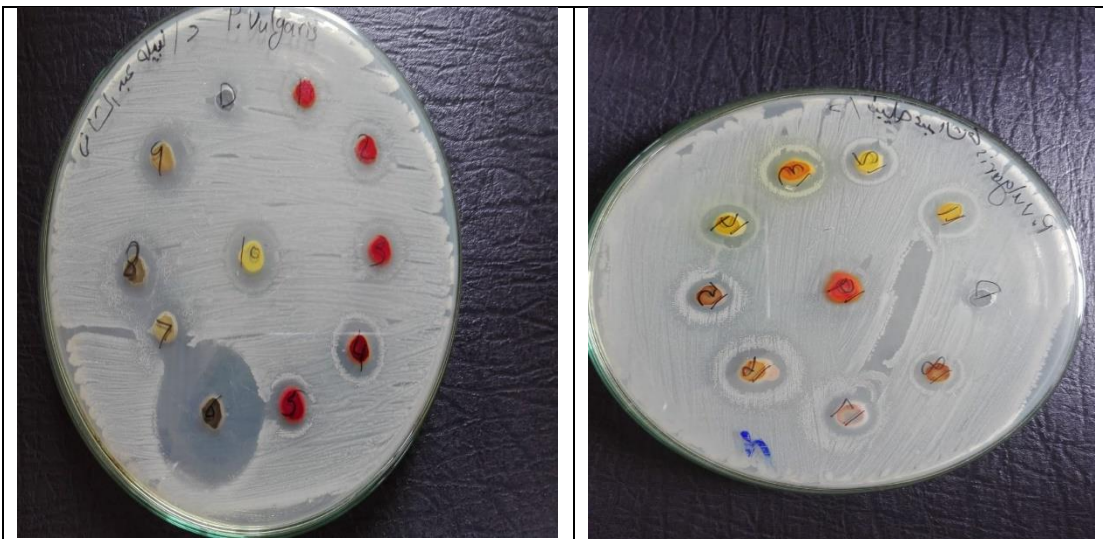
**Fig S2.** Mean zone of inhibition of the newly synthesized pyrazolines tested against *Staphylococcus aureus* (RCMB010010).



**Fig S3.** Mean zone of inhibition of the newly synthesized pyrazolines tested against *Bacillus subtilis* (RCMB 010067)



**Fig S4.** Mean zone of inhibition of the newly synthesized pyrazolines tested against *Escherichia coli* (RCMB 010052).



**Fig S5.** Mean zone of inhibition of the newly synthesized pyrazolines tested against *Proteus vulgaris* RCMB 004 (1) ATCC 13315

Zienab AbdElAal\_C\_V1

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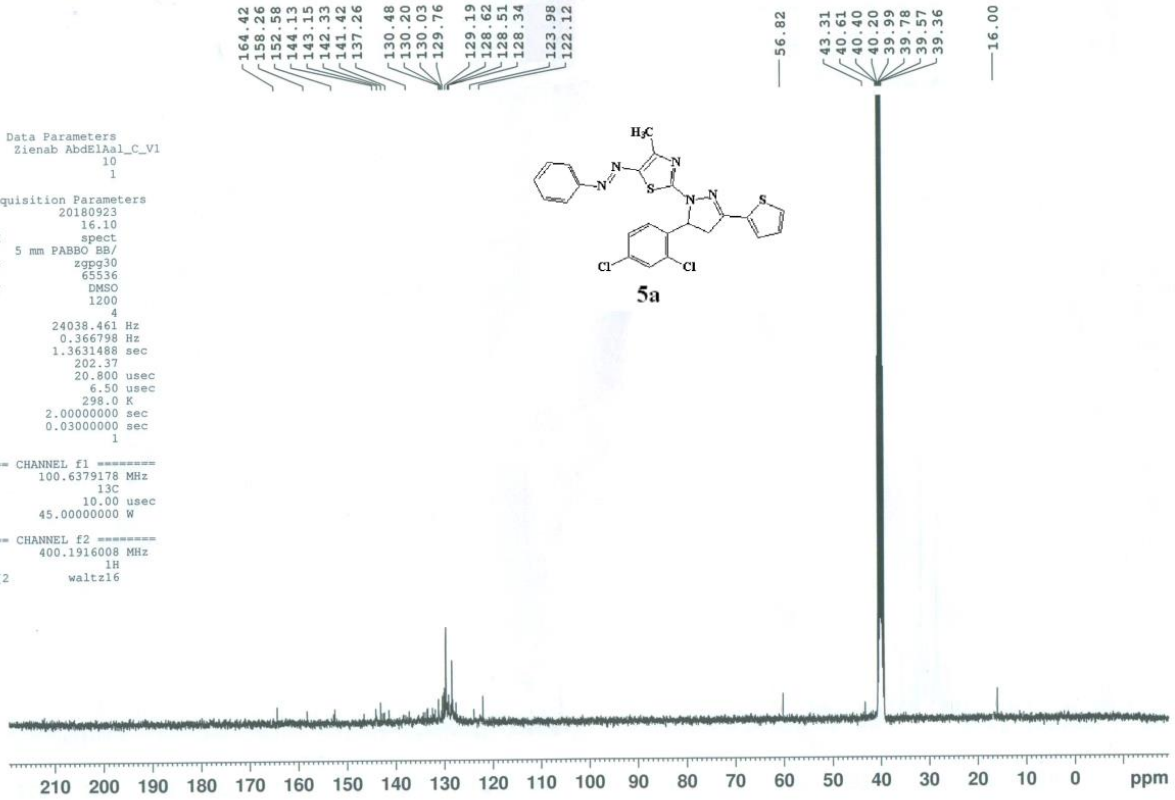
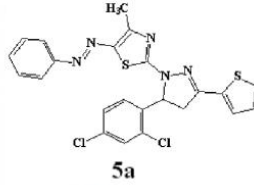


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<sup>13</sup>CNMR of compound 5a

Zienab AbdElAal\_H\_V3

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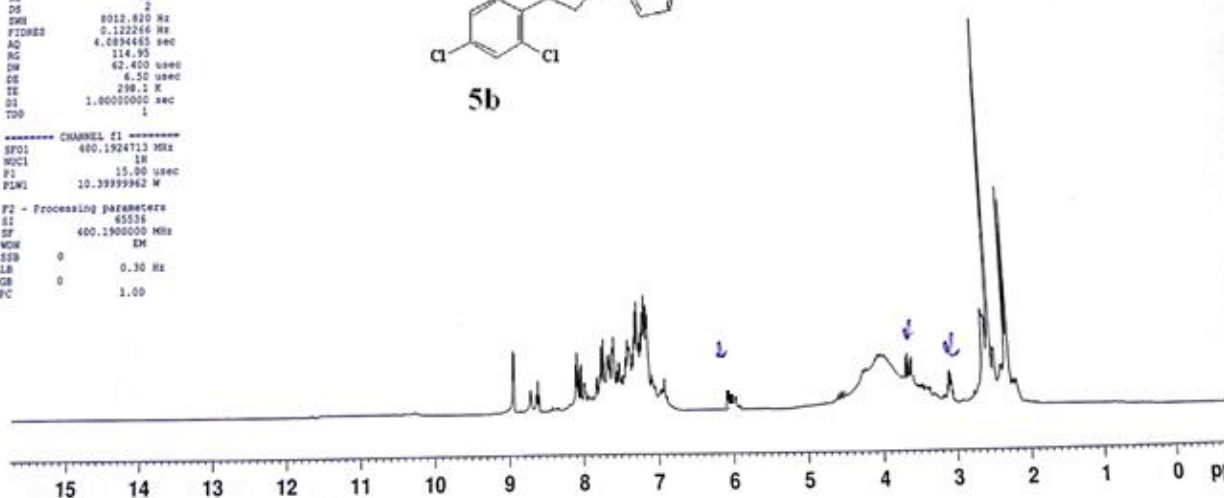
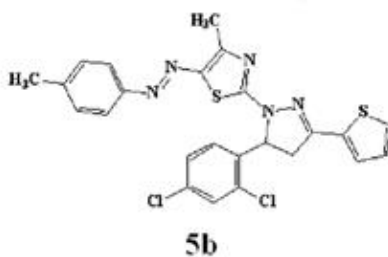
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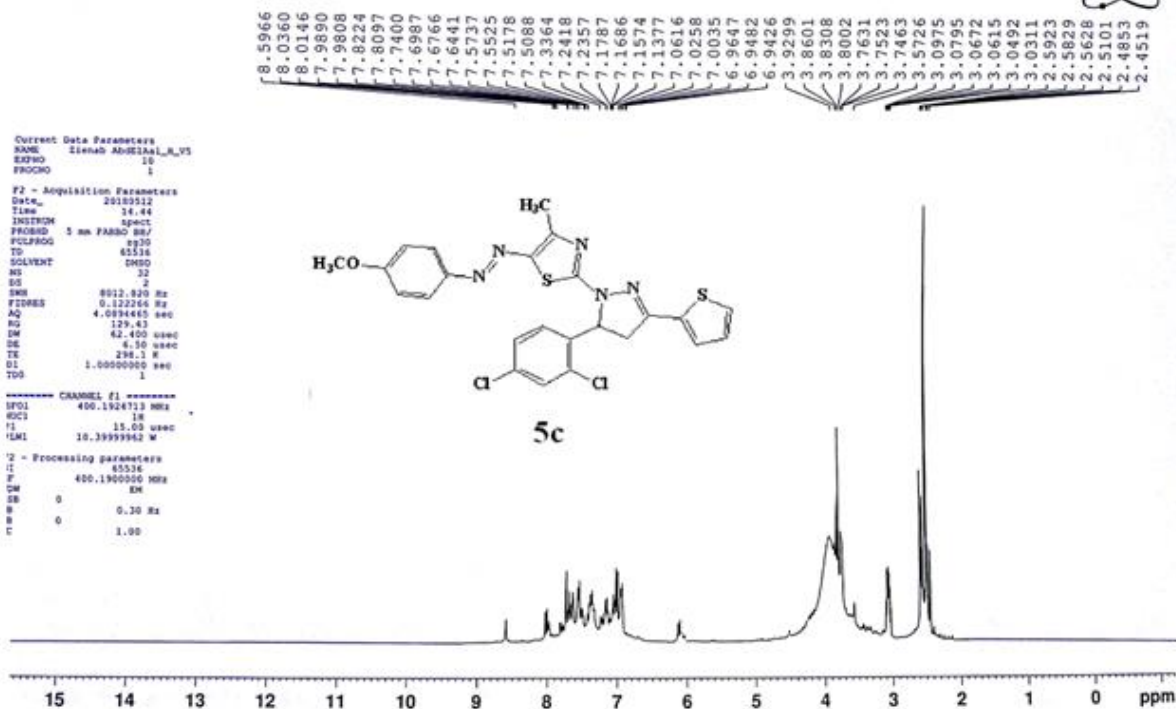
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<sup>1</sup>H NMR of compound 5b



<sup>1</sup>HNMR of compound 5c



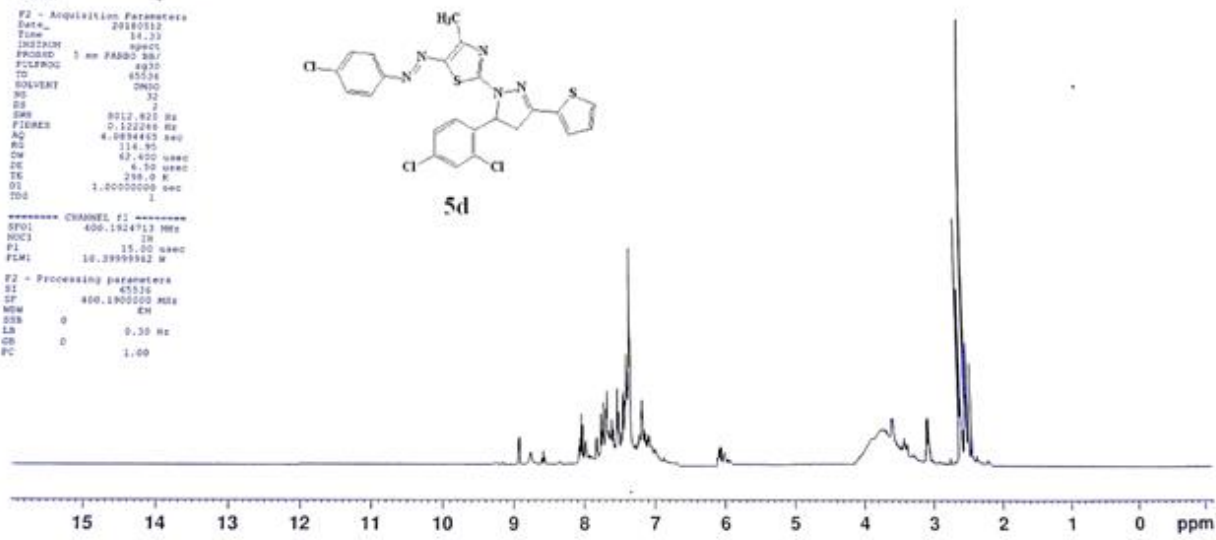
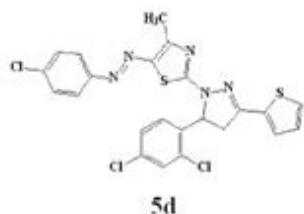


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<sup>1</sup>H NMR of compound 5d



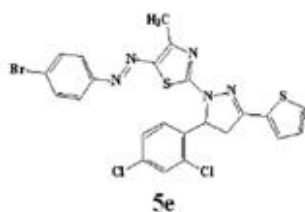
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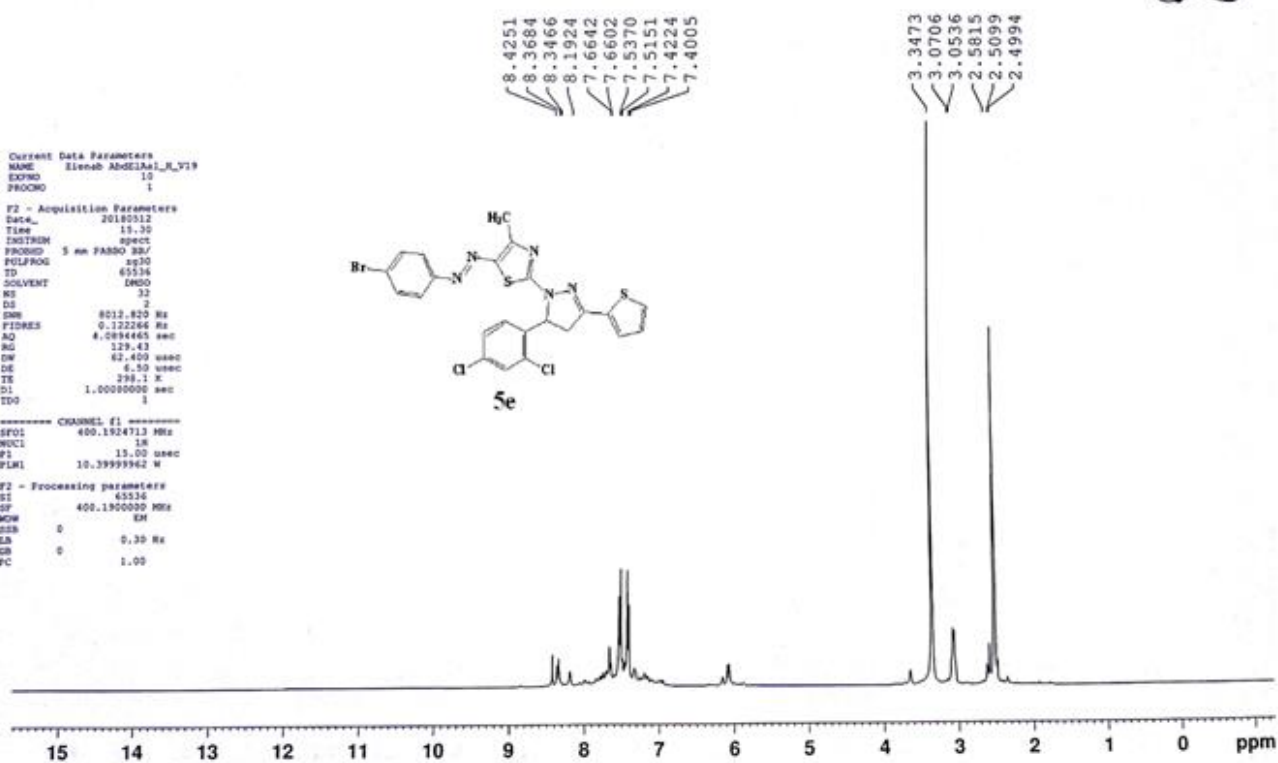
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<sup>1</sup>H NMR of compound **5e**



enab AbdElAal\_H\_V12

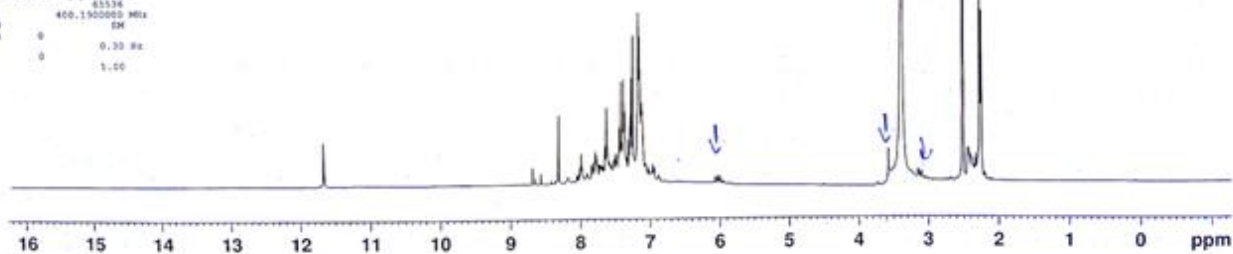
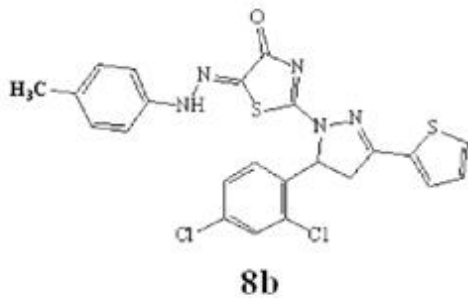
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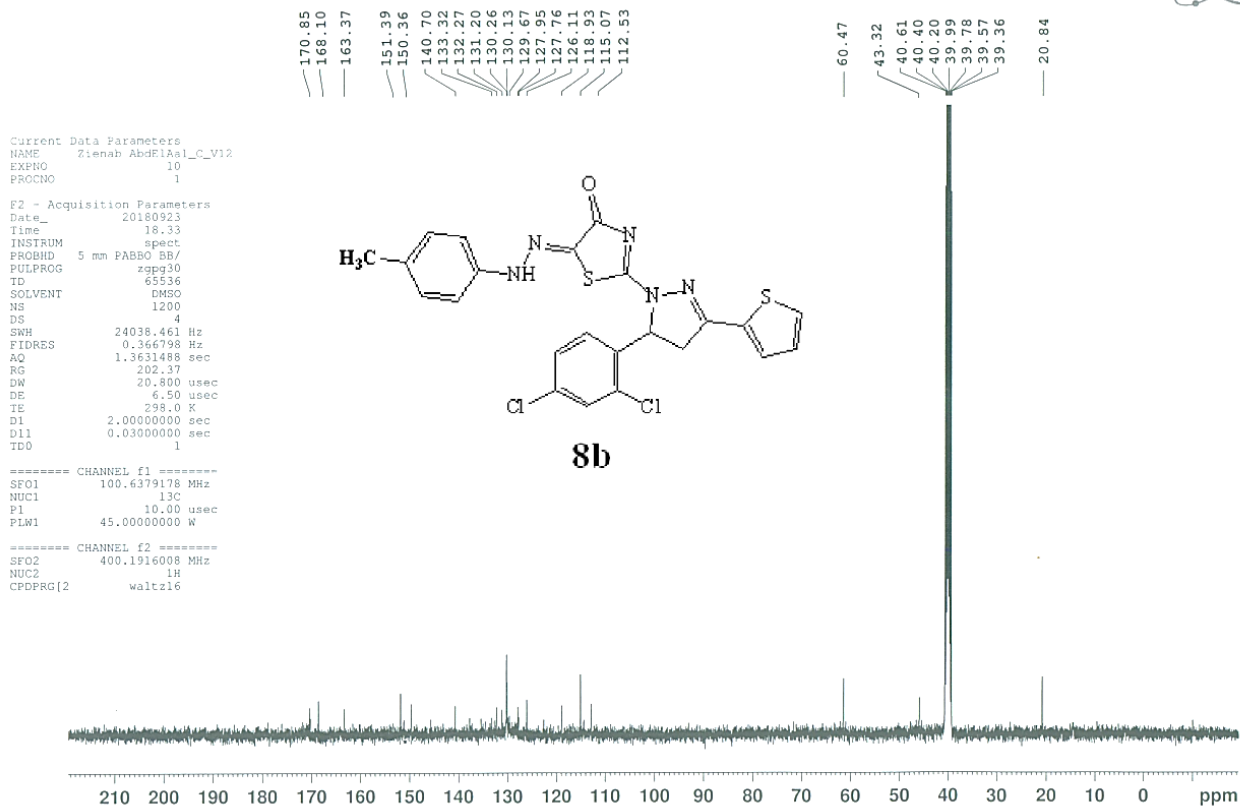
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<sup>1</sup>H NMR of compound **8b**

 $^{13}\text{C}$ -NMR of compound **8b**

Zienab AbdElAal\_H\_V11

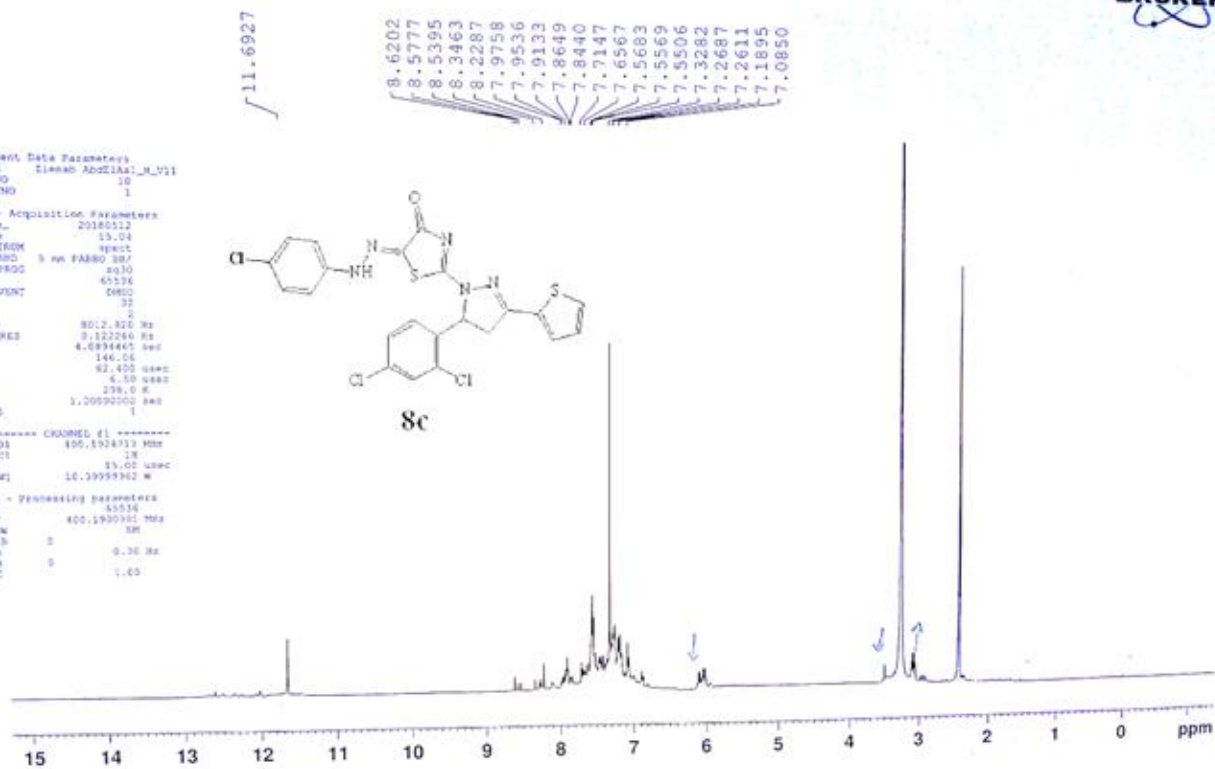
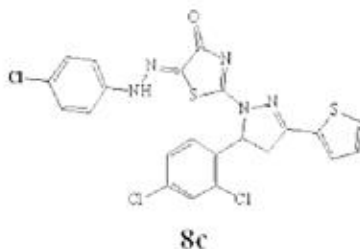
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<sup>1</sup>H NMR of compound 8c



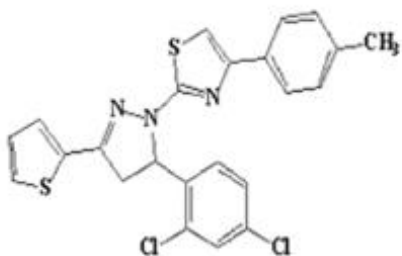
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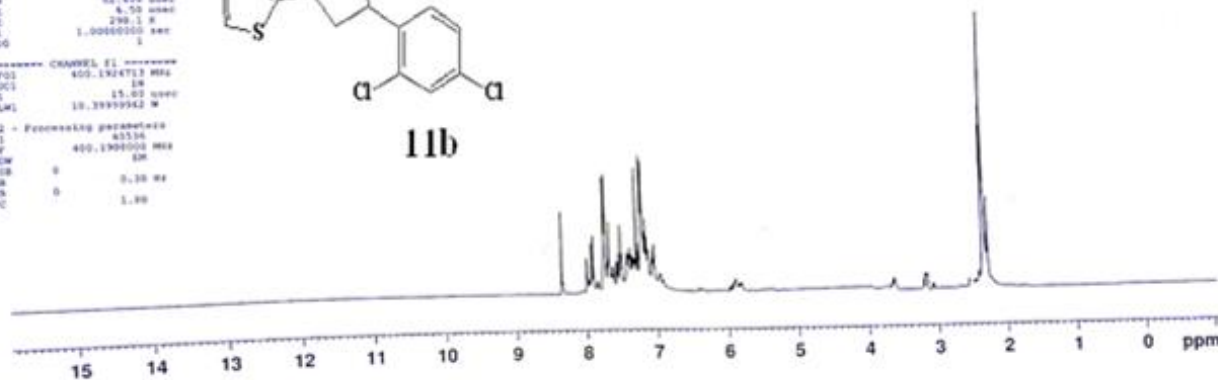
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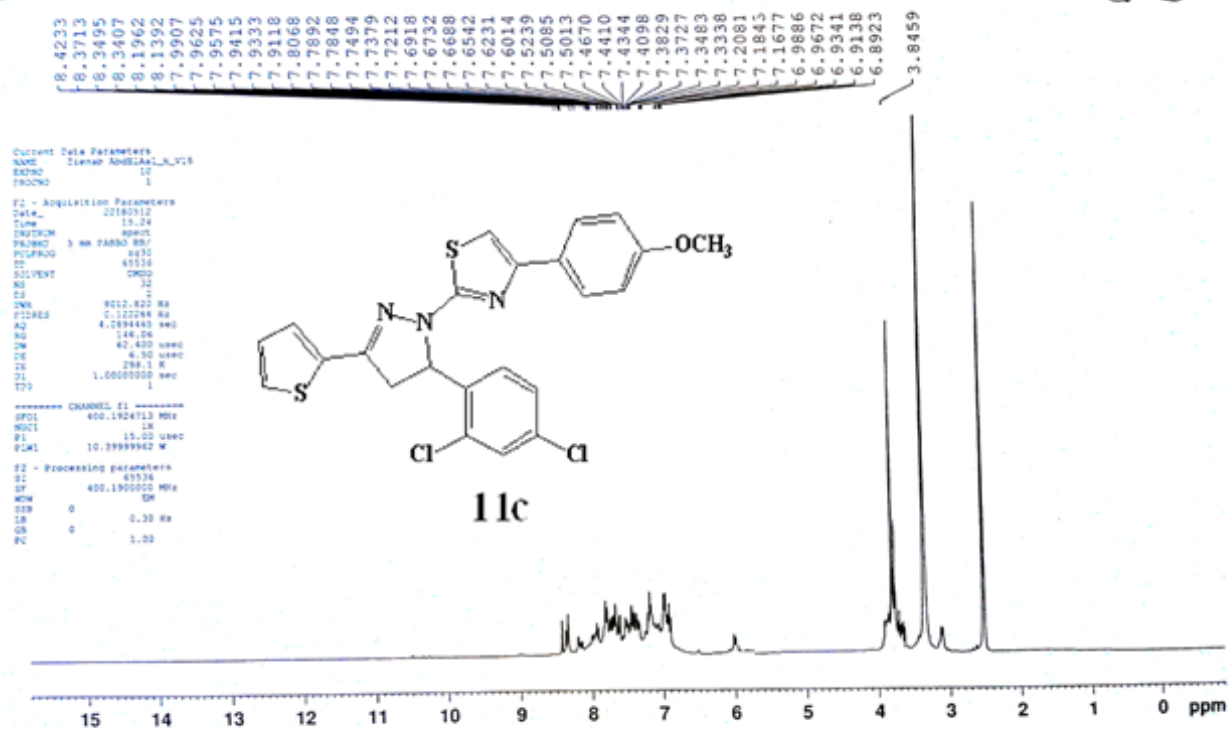
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11b



<sup>1</sup>H NMR of compound 11b

<sup>1</sup>H NMR of compound 11c





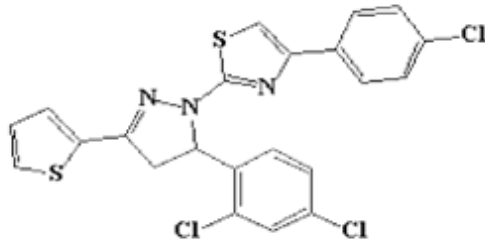
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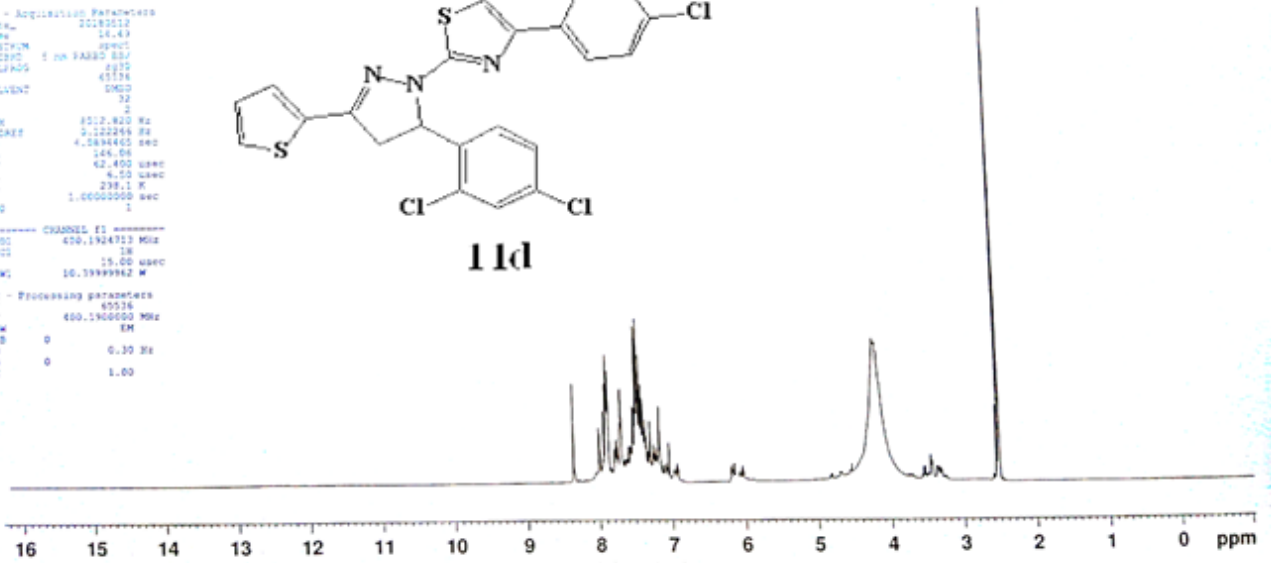
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11d

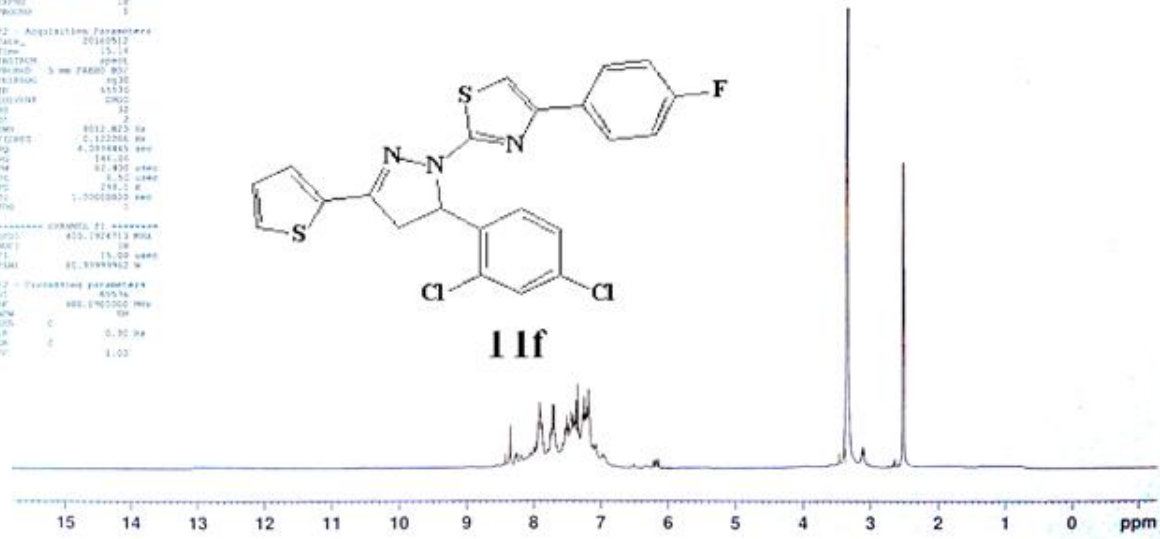
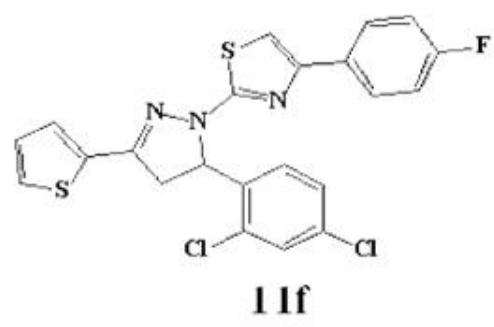


<sup>1</sup>H NMR of compound 11d



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7.7565  
7.7440  
7.7341  
7.7194  
7.6985  
7.5312  
7.5014  
7.4409  
7.4134  
7.3833  
7.3696  
7.3466  
7.2711  
7.2490  
7.2277  
7.2037  
7.1814

```
Current Data Parameters
NAME: Zienab ABDI AAL_H_V15
PROCNO: 1
F2 - Acquisition Parameters
Date_ 20160512
Time 15.14
INSTRUM spect
PROBHD 5 mm F4000 BBO
PULPROG zgpg30
TE 300.2
D1 0.05000000
d11 0.02000000
d12 0.02000000
d13 0.02000000
d14 0.02000000
d15 0.02000000
d16 0.02000000
d17 0.02000000
d18 0.02000000
d19 0.02000000
d20 0.02000000
d21 0.02000000
d22 0.02000000
d23 0.02000000
d24 0.02000000
d25 0.02000000
d26 0.02000000
d27 0.02000000
d28 0.02000000
d29 0.02000000
d30 0.02000000
d31 0.02000000
d32 0.02000000
d33 0.02000000
d34 0.02000000
d35 0.02000000
d36 0.02000000
d37 0.02000000
d38 0.02000000
d39 0.02000000
d40 0.02000000
d41 0.02000000
d42 0.02000000
d43 0.02000000
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d45 0.02000000
d46 0.02000000
d47 0.02000000
d48 0.02000000
d49 0.02000000
d50 0.02000000
d51 0.02000000
d52 0.02000000
d53 0.02000000
d54 0.02000000
d55 0.02000000
d56 0.02000000
d57 0.02000000
d58 0.02000000
d59 0.02000000
d60 0.02000000
d61 0.02000000
d62 0.02000000
d63 0.02000000
d64 0.02000000
d65 0.02000000
d66 0.02000000
d67 0.02000000
d68 0.02000000
d69 0.02000000
d70 0.02000000
d71 0.02000000
d72 0.02000000
d73 0.02000000
d74 0.02000000
d75 0.02000000
d76 0.02000000
d77 0.02000000
d78 0.02000000
d79 0.02000000
d80 0.02000000
d81 0.02000000
d82 0.02000000
d83 0.02000000
d84 0.02000000
d85 0.02000000
d86 0.02000000
d87 0.02000000
d88 0.02000000
d89 0.02000000
d90 0.02000000
d91 0.02000000
d92 0.02000000
d93 0.02000000
d94 0.02000000
d95 0.02000000
d96 0.02000000
d97 0.02000000
d98 0.02000000
d99 0.02000000
d100 0.02000000
===== CHANNEL f1 =====
NUC1 13C
PULPROG zgpg30
TE 300.2
D1 0.05000000
d11 0.02000000
d12 0.02000000
d13 0.02000000
d14 0.02000000
d15 0.02000000
d16 0.02000000
d17 0.02000000
d18 0.02000000
d19 0.02000000
d20 0.02000000
d21 0.02000000
d22 0.02000000
d23 0.02000000
d24 0.02000000
d25 0.02000000
d26 0.02000000
d27 0.02000000
d28 0.02000000
d29 0.02000000
d30 0.02000000
d31 0.02000000
d32 0.02000000
d33 0.02000000
d34 0.02000000
d35 0.02000000
d36 0.02000000
d37 0.02000000
d38 0.02000000
d39 0.02000000
d40 0.02000000
d41 0.02000000
d42 0.02000000
d43 0.02000000
d44 0.02000000
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d46 0.02000000
d47 0.02000000
d48 0.02000000
d49 0.02000000
d50 0.02000000
d51 0.02000000
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d53 0.02000000
d54 0.02000000
d55 0.02000000
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d57 0.02000000
d58 0.02000000
d59 0.02000000
d60 0.02000000
d61 0.02000000
d62 0.02000000
d63 0.02000000
d64 0.02000000
d65 0.02000000
d66 0.02000000
d67 0.02000000
d68 0.02000000
d69 0.02000000
d70 0.02000000
d71 0.02000000
d72 0.02000000
d73 0.02000000
d74 0.02000000
d75 0.02000000
d76 0.02000000
d77 0.02000000
d78 0.02000000
d79 0.02000000
d80 0.02000000
d81 0.02000000
d82 0.02000000
d83 0.02000000
d84 0.02000000
d85 0.02000000
d86 0.02000000
d87 0.02000000
d88 0.02000000
d89 0.02000000
d90 0.02000000
d91 0.02000000
d92 0.02000000
d93 0.02000000
d94 0.02000000
d95 0.02000000
d96 0.02000000
d97 0.02000000
d98 0.02000000
d99 0.02000000
d100 0.02000000
===== CHANNEL f2 =====
NUC1 1H
PULPROG zgpg30
TE 300.2
D1 0.05000000
d11 0.02000000
d12 0.02000000
d13 0.02000000
d14 0.02000000
d15 0.02000000
d16 0.02000000
d17 0.02000000
d18 0.02000000
d19 0.02000000
d20 0.02000000
d21 0.02000000
d22 0.02000000
d23 0.02000000
d24 0.02000000
d25 0.02000000
d26 0.02000000
d27 0.02000000
d28 0.02000000
d29 0.02000000
d30 0.02000000
d31 0.02000000
d32 0.02000000
d33 0.02000000
d34 0.02000000
d35 0.02000000
d36 0.02000000
d37 0.02000000
d38 0.02000000
d39 0.02000000
d40 0.02000000
d41 0.02000000
d42 0.02000000
d43 0.02000000
d44 0.02000000
d45 0.02000000
d46 0.02000000
d47 0.02000000
d48 0.02000000
d49 0.02000000
d50 0.02000000
d51 0.02000000
d52 0.02000000
d53 0.02000000
d54 0.02000000
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d56 0.02000000
d57 0.02000000
d58 0.02000000
d59 0.02000000
d60 0.02000000
d61 0.02000000
d62 0.02000000
d63 0.02000000
d64 0.02000000
d65 0.02000000
d66 0.02000000
d67 0.02000000
d68 0.02000000
d69 0.02000000
d70 0.02000000
d71 0.02000000
d72 0.02000000
d73 0.02000000
d74 0.02000000
d75 0.02000000
d76 0.02000000
d77 0.02000000
d78 0.02000000
d79 0.02000000
d80 0.02000000
d81 0.02000000
d82 0.02000000
d83 0.02000000
d84 0.02000000
d85 0.02000000
d86 0.02000000
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d88 0.02000000
d89 0.02000000
d90 0.02000000
d91 0.02000000
d92 0.02000000
d93 0.02000000
d94 0.02000000
d95 0.02000000
d96 0.02000000
d97 0.02000000
d98 0.02000000
d99 0.02000000
d100 0.02000000
```



<sup>1</sup>H NMR of compound **11f**