Synthesis of some dihydropyrimidine-based compounds bearing pyrazoline moiety and evaluation of their antiproliferative activity

Fadi M. Awadallah, Gary A. Piazza, Bernard D. Gary, Adam B. Keeton, Joshua C. Canzoneri

PII: S0223-5234(13)00644-2

DOI: 10.1016/j.ejmech.2013.10.003

Reference: EJMECH 6466

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 11 June 2013

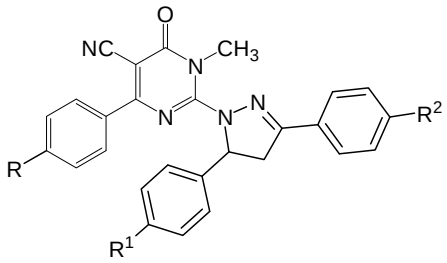
Revised Date: 24 September 2013

Accepted Date: 3 October 2013

Please cite this article as: F.M. Awadallah, G.A. Piazza, B.D. Gary, A.B. Keeton, J.C. Canzoneri, Synthesis of some dihydropyrimidine-based compounds bearing pyrazoline moiety and evaluation of their antiproliferative activity, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.10.003.

Graphical abstract:

Some pyrimidine-pyrazoline hybrids were synthesized via cyclocondensation of the precursor 2-hydrazonepyrimidines **3a,b** and the appropriate propenones **4a-h**. The compounds were screened for their antiproliferative activity against four human cell lines.



5a-h, 6a-h

Synthesis of some dihydropyrimidine-based compounds bearing pyrazoline moiety and evaluation of their antiproliferative activity

Fadi M. Awadallah,[a,1] Gary A. Piazza,[b] Bernard D. Gary,[b] Adam B. Keeton[b] and Joshua C. Canzoneri[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Eini 11562, Cairo Egypt.*
[b]*Department of Oncologic Sciences and Pharmacology, Drug Discovery Research Center, Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Avenue, Mobile, AL 36604-1405, USA*

Abstract.

Two series of 2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles **5a-h** and 4-(4-chlorophenyl)-2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitriles **6a-h** were synthesized via a cyclocondensation reaction of the corresponding 2-hydrazinopyrimidines **3a,b** with the appropriate 2-propen-1-ones **4a-h**. The target compounds were screened for their antiproliferative activity against A 549 (lung), HT 29 (colon), MCF 7 and MDA-MB 231 (breast) cell lines. The two most susceptible cell lines were the colon (HT 29) and breast (MDA-MB 231). Generally, the 4-unsubstitutedphenylpyrimidine derivatives **5a-h** were more active than their 4-chlorophenylpyrimidine analogs **6a-h**. Compounds **5e** and **5g**, showed high activity against three of the cell lines. The most active compound **5c** possessed IC_{50}= 1.76 µM against A 549 cell line.

Keywords: Pyrimidine; Pyrazoline; Antiproliferative activity; Human colon (HT 29) cell line, Human breast (MDA-MB 231) cell line.

1. Introduction.

Cancer is a major worldwide health problem. Although there has been a progress in the development of treatment and prevention of cancer, this disease remains the second major cause of death in the world. Still, the successful treatment of cancer remains a challenge in the 21st century, and there is a need to search for newer and safer anticancer agents that have a broader spectrum of cytotoxicity to tumor cells [1]. With the discovery of multicomponent reaction, the dihydropyrimidinones (DHPM's) were reported for the first time by Biginelli over a century ago [2]. The multifunctionalized dihydropyrimidinones scaffold represents a class of heterocyclic compounds with significant pharmacological efficiency and are receiving considerable amount of interest. They exhibit a diverse pharmacological profile like calcium channel blockade, α_{1a}-adrenoreceptor antagonism, antibacterial, antifungal and other related properties [3-6]. From natural marine sources, several alkaloids containing the dihydropyrimidine core unit were isolated such as batzelladine alkaloids, which were found to be potent HIV gp-120-CD4 inhibitors [7]. With the advent of combinatorial synthesis, which is particularly useful for multicomponent reactions like Biginelli condensation, diverse DHMPs libraries have been synthesized and subjected to high throughput screening for biological activity [8]. By the end of the last century a structurally simple compound, monastrol **I** has been identified on screening a large library of diverse small molecules, as a novel cell permeable molecule that causes mitotic arrest by blocking bipolar mitotic spindle in mammalian cells [9]. Moreover, many diversely substituted

[1] Corresponding author: Tel.: 2 01223483941; fax: 20 2 2362 8426.
E-mail address: fadi2mae@gmail.com (F.M. Awadallah).

dihydropyrimidinones have been synthesized showing promising antitumor activity among which the ethanehydrazonoylpyrimidinone **II** is an example [10] (Fig. 1).

On the other hand, many pyrazoline derivatives are acknowledged to possess a wide range of bioactivities. The pyrazoline motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibit antiviral/antitumor [11-13], antibacterial [14,15], anti-inflammatory [16], analgesic [17], fungistatic [18], and anti-hyperglycemic activities [19]. A series of novel 3,5-diarylpyrazolines **III** [20] and thiazolylpyrazoline derivatives **IV** were recently reported as potent anticancer agents [21] (Fig. 1).

Figure 1

In view of these observations it was thought of interest to study the influence of pyrazoline moiety and dihydropyrimidinone scaffold combination, so that the two combined substructures, might exhibit synergistic antitumor effect. Therefore, two series of new pyrazolinyl-dihydropyrimidine derivatives, **5a-h** and **6a-h**, were synthesized and screened for their antitumor activity. On the molecular design level, the substitutions on the two phenyl rings on the pyrazoline moiety (R^1 and R^2) were subject to modifications regarding their electronic and lipophilic nature (Fig. 2). The impact of these molecular manipulations was studied from the results obtained from the cytotoxic biological assessment of all the synthesized compounds against the human lung cell line A 549, colon cancer cell line HT 29 and the breast cancer cell lines MCF 7 and MDA-MB231.

Figure 2

2. Results and discussion.

2.1. Chemistry

The starting 1-methyl-2-(methylthio)-6-oxo-4-pheny/(4-chlorophenyl)-1,6-dihydropyrimidine-5-carbonitriles **2a,b** were synthesized in a one pot reaction from the corresponding aldehyde, ethyl cyanoacetate and thiourea followed by methylation. The corresponding 2-hydrazino-1-methyl-6-oxo-4-pheny/(4-chlorophenyl)-1,6-dihydropyrimidine-5-carbonitriles **3a,b** were obtained through hydrazinolysis of the precursor methylthio derivatives (Scheme 1). The propen-1-one derivatives **4a-h** were synthesized via a base-catalyzed Claisen-Schmidt condensation of the appropriate benzaldehyde and acetophenone derivatives (Scheme 2).

Scheme1, Scheme 2

The target compounds **5a-h** and **6a-h** were obtained through a cyclocondensation reaction of the corresponding hydrazinopyrimidine derivatives **3a,b** and the appropriate propenones **4a-h** in absolute ethanol in the presence of sodium hydroxide (Scheme 3). Postulated structures of the newly synthesized compounds were in good agreement with their IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analyses data. The ^{1}H NMR spectra of the target compounds showed a prominent AMX system for the protons at C-4 and C-5 of the pyrazoline ring. Proton at C-4 (H_A) appeared as doublet of doublet at δ 2.65-3.23 ppm, proton at C-4 (H_M) appeared as doublet of doublet at δ 3.11-3.92 ppm, and proton at C-5 (H_X) appeared as doublet of doublet at δ 4.10-5.30 ppm. There are three coupling constants: J_{AX} = 6.3-6.9 Hz, J_{AM} = 17.1-18.2 Hz, and J_{MX} = 11.6-12.3 Hz. Other protons (N-CH_3, OCH_3, aromatic Hs) were shown in their usual range. ^{13}C NMR

showed the characteristic C-4 and C-5 carbon signals of the pyrazoline ring at δ 40.3-44.3 and 52.5-60.6 ppm, respectively, in addition to the other signals attributed to the carbon skeleton of the target compounds (c.f. the experimental section).

Scheme 3

2.2. Antiproliferative activity

The antiproliferative activity of all synthesized compounds were investigated against four human cell lines, namely, lung (A 549), colon (HT 29) and breast (MCF 7 and MDA-MB 231) using doxorubicin (Dox) as positive control. Compounds were first evaluated in triplicate for their percent proliferation inhibition. All the tested compounds revealed percentage inhibition above 60% and subsequently, their IC_{50} values were calculated in µM from a graph displaying the dose-survival percentage curve obtained after testing 8 concentrations for each tested compound with four replicates per concentration (Table 1**).** Generally, results showed that the two most susceptible cell lines were the colon (HT 29) and the breast (MDA-MB 231) cell lines that were inhibited by the tested compounds at IC_{50} values ranging from 2.49-19.51 µM and 3.99-29.14 µM, respectively. Apart from some exceptions, inhibition of the other two cell lines required higher concentrations of the tested compounds. Also, it could be noticed that the 4-chlorophenylpyrimidine derivatives **6a-h** were generally less potent than their corresponding 4-unsubstitutedphenylpyrimidine analogs **5a-h**. Regarding the activity of the tested compounds against HT 29 and MDA-MB 231 cell lines, the most active compound against both cell lines was **5a** which had no substitutions on any of the phenyl rings (IC_{50}= 2.49 and 3.99 µM, respectively). Activity of **5a** against colon HT 29 cell line was slightly higher than doxorubicin (IC_{50} Dox= 2.75 µM). Substitution on the phenyl groups located on positions 3 and 5 of the pyrazoline ring led to decrease in activity. Compounds substituted with (R^1) only such as compound **5f** or having another substitution (R^2) such as **5e**, **5g** and **5h** were more effective against both HT 29 and MDA-MB 231 cell lines than those unsubstituted with R^1 and which had only R^2 substituent as in **5b-5d**. The pattern of activity of compounds **6a-h** against HT 29 and MDA-MB 231 cell lines was different from that of compounds **5a-h**. The promising compounds emerging in this series were those substituted with R^1 such as **6e** and **6f**, in addition to the derivatives substituted with R^2 only as in **6c** and **6d**.

Regarding activity against lung (A 549) and breast (MCF 7) cell lines, the 4-unsubstitutedphenylpyrimidine analogs **5a-h** were more effective than their 4-chlorophenyl analogs. Regarding the activity against the lung (A 549) cell line, the most active compound was **5c** (IC_{50}= 1.76 µM). Compounds **5b**, **5c** and **5e** showed activity against lung A 549 cell line comparable to or higher than doxorubicin (IC_{50}= 3.25, 1.76 and 2.00 µM, respectively, c.f. IC_{50} Dox= 3.13 µM). Good activity was also demonstrated by compound **5g** (IC_{50}= 6.07 µM, respectively). Lower activity was observed with the other tested compounds. Considering the activity against the breast (MCF 7) cell line, only two compounds exhibited promising activity; namely **5b** and **5g** (IC_{50}= 3.28 and 4.80 µM, respectively).

Concerning the nature of substituent groups R^1 and R^2, there was no consistent relation that could be established between the lipophilicity and/or electronic property of these groups and the antiproliferative activity.

From this SAR analysis, it was noteworthy that some compounds exhibited promising activity against three of the cell lines such as compounds **5e** (IC_{50}= 2.00, 4.62 and 5.10 µM against A 549, HT 29 and MDA-MB 231, respectively) and **5g** (IC_{50}= 6.07, 9.12 and 4.80 µM against A 549, HT 29 and MCF 7, respectively). These compounds may be candidates for future lead optimization.

Table 1

3. Conclusion

Two series of pyrimidine-pyrazoline hybrids, **5a-h** and **6a-h**, were synthesized via a cyclocondensation reaction of the precursor 2-hydrazonepyrimidines **3a,b** and the appropriate propenones **4a-h**. The target compounds were screened for their antiproliferative activity against four human cell lines: lung (A 549), colon (HT 29), breast (MCF 7 and MDA-MB 231) cell lines. The two most susceptible cell lines were the colon (HT 29) and breast (MDA-MB 231). Generally the 4-unsubstitutedphenylpyrimidine analogs **5a-h** were more effective than their chloro analogs **6a-h**. Compounds **5e** and **5g**, showing high activity against three of the cell lines, were considered promising lead compounds for future optimization.

4. Experimental.

4.1. Chemistry

Melting points were determined with Stuart SMP3 version 5 apparatus and were uncorrected. FT-IR spectra were recorded on Bruker FT-IR 8400S spectrophotometer using KBr cell. Unless otherwise noted, ^{1}H NMR spectra were recorded in DMSO-d_6 on Varian mercury 300BB at 300 MHz. ^{13}C NMR spectra were run at 75.46 MHz. Chemical Shifts were given in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 ev) mass spectrometer. Elemental microanalysis was performed at the Regional Center for Mycology and Biotechnology, Azhar University. TLC were monitored on FLUKA silica gel TLC aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm using chloroform/methanol (9.5:0.5) as eluent to follow the course of the reactions and to check the purity of the products. All reagents and solvents were purified and dried by standard techniques.

*4.1.1. 6-oxo-4-pheny/(4-chlorophenyl)-2-sulfanyl-1,6-dihydropyrimidine-5-carbonitriles (**1a,b**):*

The titled compounds **1a** and **1b** were synthesized according to the reported methods [22].

*4.1.2. 1-Methyl-2-(methylthio)-6-oxo-4-pheny/(4-chlorophenyl)-1,6-dihydropyrimidine-5-carbonitriles (**2a,b**) and 2-hydrazino-1-methyl-6-oxo-4-pheny/(4-chlorophenyl)-1,6-dihydropyrimidine-5-carbonitriles (**3a,b**):*

The titled compounds **2a, 2b**, **3a** and 3**b** were synthesized according to the reported methods [23].

*4.1.3. (E)-1-(un)substitutedphenyl-3-(un)substitutedphenylprop-2-en-1-ones (**4a-h**):*

Compounds **4a**, **4c**, **4f**, **4g** [24], **4b** [25], **4d**, **4e** [26] and **4h** [27] were synthesized according to the literature procedures.

*4.1.4. General procedure for the preparation of compounds (**5a-h**) and (**6a-h**):*

A mixture of compound **3a/3b** (4 mmol), the appropriate propenone **4a-h** (4 mmol) and sodium hydroxide (0.2g, 5mmol) in absolute ethanol (30 ml) was refluxed for 72 h. The reaction mixture was poured on water, neutralized with 2N hydrochloric acid and the residue was filtered off. The crude product obtained was crystallized from isopropanol.

*4.1.4.1. 2-(3,5-Diphenyl-4,5-dihydropyrazol-1-yl)-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5a**):*

M.p. 160-162^{o}C, yield: 75%. IR υ_{max}/cm^{-1}: 3057 (CH aromatic), 2950 (CH aliphatic), 2222 (CN), 1681 (C=O), 1614 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.55 (s, 3H, N-CH$_3$), 3.23 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.6 Hz, J_{AM}= 17.9 Hz), 3.58 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.8 Hz, J_{MA}= 17.9 Hz), 4.75 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.6 Hz, J_{XM}= 11.9 Hz), 7.27-7.80 (m, 15H, aromatic H). Anal. Calcd. for $C_{27}H_{21}N_5O$ (431.39): C, 75.16; H, 4.91; N, 16.23. Found: C, 75.22; H, 4.95; N, 16.35. correct in all

*4.1.4.2. 2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5b**):*

M.p. 173-174^{o}C, yield: 71%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2920, 2850 (CH aliphatic), 2223 (CN), 1681 (C=O), 1622 (C=N). ^{1}H NMR: δ 2.51 (s, 3H, N-CH$_3$), 2.65 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.4 Hz, J_{AM}= 17.8 Hz), 3.11 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.8 Hz), 4.38 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.4 Hz, J_{XM}= 11.9 Hz), 7.40-7.67 (m, 14H, aromatic H). MS, m/z (%): 465.40 (M^{+}, 0.28); 80.00 (100). Anal. Calcd. for $C_{27}H_{20}ClN_5O$ (465.93): C, 69.60; H, 4.33; N, 15.03. Found: C, 69.58; H, 4.37; N, 15.11.

*4.1.4.3. 2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5c**):*

M.p. 92-94^{o}C, yield: 65%. IR υ_{max}/cm^{-1}: 3059 (CH aromatic), 2922, 2848 (CH aliphatic), 2222 (CN), 1681 (C=O), 1608 (C=N). ^{1}H NMR: δ 2.47 (s, 3H, N-CH$_3$), 2.95 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.5 Hz, J_{AM}= 17.9 Hz), 3.28 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.9 Hz), 3.78 (s, 3H, OCH$_3$), 4.24 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.5 Hz, J_{XM}= 11.9 Hz), 7.33-7.87 (m, 14H, aromatic H). ^{13}C NMR: δ 27.9 (N-CH$_3$), 40.3 (C-4 pyrazoline), 54.7 (C-5 pyrazoline), 55.9 (OCH3), 112.9-143.8 (aromatic Cs), 150.5 (C-2 pyrazoline), 160.2 (C-OCH$_3$), 161.0 (C-2 pyrimidine), 161.3 (C=O). Anal. Calcd. for $C_{28}H_{23}N_5O_2$ (461.51): C, 72.87; H, 5.02; N, 15.17. Found: C, 72.89; H, 5.11; N, 15.26.

*4.1.4.4. 2-[5-(4-Fluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5d**):*

M.p. 130-131°C, yield: 68%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2920, 2848 (CH aliphatic), 2222 (CN), 1681 (C=O), 1602 (C=N). ^{1}H NMR: δ 2.57 (s, 3H, N-CH$_3$), 2.95 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.4 Hz, J_{AM}= 17.9 Hz), 3.20 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.9 Hz), 4.25 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.4 Hz, J_{XM}= 11.9 Hz), 7.15-7.95 (m, 14H, aromatic H). ^{13}C NMR: δ 26.5 (N-CH$_3$), 44.3 (C-4 pyrazoline), 60.6 (C-5 pyrazoline), 114.5-142.6 (aromatic Cs), 150.3 (C-2 pyrazoline), 158.3 (C-2 pyrimidine), 160.2 (C=O). Anal. Calcd. for $C_{27}H_{20}FN_5O$ (449.48): C, 72.15; H, 4.48; N, 15.58. Found: C, 72.21; H, 4.53; N, 15.69.

*4.1.4.5. 2-[5-(4-Fluorophenyl)-3-(4-tolyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5e**):*

M.p. 145-147°C, yield: 62%. IR υ_{max}/cm^{-1}: 3059 (CH aromatic), 2920, 2850 (CH aliphatic), 2222 (CN), 1674 (C=O), 1622 (C=N). ^{1}H NMR: δ 2.31 (s, 3H, CH$_3$), 2.52 (s, 3H, N-CH$_3$), 3.06 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.5 Hz, J_{AM}= 17.9 Hz), 3.34 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.9 Hz), 4.23 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.5 Hz, J_{XM}= 11.9 Hz), 7.22-7.85 (m, 13H, aromatic H). ^{13}C NMR: δ 21.1 (CH$_3$), 26.4 (N-CH$_3$), 40.3 (C-4 pyrazoline), 57.6 (C-5 pyrazoline), 115.5-143.4 (aromatic Cs), 150.3 (C-2 pyrazoline), 159.6 (C-2 pyrimidine), 161.6 (C=O). Anal. Calcd. for $C_{28}H_{22}FN_5O$ (463.51): C, 72.56; H, 4.78; N, 15.11. Found: C, 72.58; H, 4.83; N, 15.25.

*4.1.4.6. 2-[3-(4-Chlorophenyl)-5-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5f**):*

M.p. 133-135°C, yield: 77%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2920, 2848 (CH aliphatic), 2223 (CN), 1681 (C=O), 1616 (C=N). ^{1}H NMR: δ 2.51 (s, 3H, N-CH$_3$), 3.05 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.6 Hz, J_{AM}= 18.0 Hz), 3.42 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 18.0 Hz), 4.25 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.6 Hz, J_{XM}= 11.9 Hz), 7.10-7.95 (m, 14H, aromatic H). Anal. Calcd. for $C_{27}H_{20}ClN_5O$ (465.93): C, 69.60; H, 4.33; N, 15.03. Found: C, 69.66; H, 4.37; N, 15.14.

*4.1.4.7. 2-[3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5g**):*

M.p. 124-126°C, yield: 65%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2922, 2872 (CH aliphatic), 2223 (CN), 1681 (C=O), 1621 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.30 (s, 3H, N-CH$_3$), 3.09 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.4 Hz, J_{AM}= 18.2 Hz), 3.84 (s, 3H, OCH$_3$), 3.87 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 18.2 Hz), 4.80 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.4 Hz, J_{XM}= 11.9 Hz), 6.84-7.59 (m, 13H, aromatic H). ^{13}C NMR: δ 27.5 (N-CH$_3$), 40.4 (C-4 pyrazoline), 52.5 (C-5 pyrazoline), 55.8 (OCH3), 120.7-142.4 (aromatic Cs), 151.5 (C-2 pyrazoline), 159.3 (C-OCH$_3$), 160.0 (C-2 pyrimidine), 162.3 (C=O). MS, m/z (%): 495.95 (M$^+$, 2.81); 105.00 (100). Anal. Calcd. for $C_{28}H_{22}ClN_5O_2$ (495.96): C, 67.81; H, 4.47; N, 14.12. Found: C, 68.02; H, 4.53; N, 14.31.

*4.1.4.8. 2-[3,5-Bis-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**5h**):*

M.p. 196-198°C, yield: 58%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2916, 2848 (CH aliphatic), 2222 (CN), 1681 (C=O), 1600 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.33 (s, 3H, N-CH$_3$), 3.15 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.4 Hz, J_{AM}= 17.9 Hz), 3.89 (s, 6H, 2 OCH$_3$), 3.92 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.8 Hz, J_{MA}= 17.9 Hz), 5.30 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.4 Hz, J_{XM}= 11.8 Hz), 6.91-7.67 (m, 13H, aromatic H). MS, m/z (%): 491.05 (M$^+$, 23.97); 343.00 (100). Anal. Calcd. for $C_{29}H_{25}N_5O_3$ (491.54): C, 70.86; H, 5.13; N, 14.25. Found: C, 70.89; H, 5.17; N, 14.33.

*4.1.4.9. 4-(4-Chlorophenyl)-2-(3,5-diphenyl-4,5-dihydropyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6a**):*

M.p. 270-272°C, yield: 68%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2918, 2848 (CH aliphatic), 2193 (CN), 1681 (C=O), 1614 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.34 (s, 3H, N-CH$_3$), 3.10 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.9 Hz, J_{AM}= 17.1 Hz), 3.91 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 12.3 Hz, J_{MA}= 17.1 Hz), 5.23 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.9 Hz, J_{XM}= 12.3 Hz), 6.77-7.65 (m, 14H, aromatic H). Anal. Calcd. for $C_{27}H_{20}ClN_5O$ (465.93): C, 69.60; H, 4.33; N, 15.03. Found: C, 68.68; H, 4.34; N, 15.12.

*4.1.4.10. 4-(4-Chlorophenyl)-2-[5-(4-chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6b**):*

M.p. 265-267°C IR υ_{max}/cm^{-1}: 3062 (CH aromatic), 2920, 2848 (CH aliphatic), 2193 (CN), 1662 (C=O), 1601 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.50 (s, 3H, N-CH$_3$), 3.05 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.3 Hz, J_{AM}= 17.7 Hz), 3.17 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.7 Hz), 3.86 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.3 Hz, J_{XM}= 11.9 Hz), 6.84-7.67 (m, 13H, aromatic H). ^{13}C NMR: δ 27.4 (N-CH$_3$), 40.4 (C-4 pyrazoline), 56.6 (C-5 pyrazoline), 114.4-142.2 (aromatic Cs), 153.3 (C-2 pyrazoline), 158.9 (C-2 pyrimidine), 164.2 (C=O). MS, m/z (%): 500.95 (M$^+$, 16.43); 120.00 (100). Anal. Calcd. for $C_{27}H_{19}Cl_2N_5O$ (500.38): C, 64.81; H, 3.83; N, 14.00. Found: C, 64.86; H, 3.81; N, 14.13.

*4.1.4.11. 4-(4-Chlorophenyl)-2-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6c**):*

M.p. 275-277°C, yield: 64%. IR υ_{max}/cm^{-1}: 3064 (CH aromatic), 2920, 2848 (CH aliphatic), 2193 (CN), 1666 (C=O), 1597 (C=N). ^{1}H NMR (C*D*Cl$_3$): δ 2.40 (s, 3H, N-CH$_3$), 3.15 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.6 Hz, J_{AM}= 17.9 Hz), 3.89 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.6 Hz, J_{MA}= 17.9 Hz), 4.80 (s, 3H, OCH$_3$), 4.23 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.6 Hz, J_{XM}= 11.6 Hz), 6.80-7.58 (m, 13H, aromatic H). ^{13}C NMR: δ 26.5 (N-CH$_3$), 44.0 (C-4 pyrazoline), 54.8 (C-5 pyrazoline), 55.3 (OCH3), 113.5-136.7 (aromatic Cs), 150.7 (C-2 pyrazoline), 159.2 (C-OCH$_3$), 162.8 (C-2 pyrimidine), 168.25 (C=O). Anal. Calcd. for $C_{28}H_{22}ClN_5O_2$ (495.96): C, 67.81; H, 4.47; N, 14.12. Found: C, 67.79; H, 4.51; N, 14.23.

*4.1.4.12. 4-(4-Chlorophenyl)-2-[5-(4-fluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6d**):*

M.p. 281-282°C, yield: 76%. IR υ_{max}/cm^{-1}: 3064 (CH aromatic), 2920, 2850 (CH aliphatic), 2194 (CN), 1662 (C=O), 1621 (C=N). ^{1}H NMR: δ 2.50 (s, 3H, N-CH$_3$), 3.18 (dd, 1H, C4-H$_A$ pyrazoline, J_{AX}= 6.5 Hz, J_{AM}= 17.9 Hz), 3.41 (dd, 1H, C4-H$_M$ pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.9 Hz), 4.10 (dd, 1H, C5-H$_X$ pyrazoline, J_{XA}= 6.5 Hz, J_{XM}= 11.9 Hz), 7.18-7.88 (m, 13H, aromatic H). MS, m/z (%): 483.10 (M$^+$, 0.64); 105.00 (100). Anal. Calcd. for $C_{27}H_{19}ClFN_5O$ (483.92): C, 67.01; H, 3.96; N, 14.47. Found: C, 67.08; H, 4.01; N, 14.58.

*4.1.4.13. 4-(4-Chlorophenyl)-2-[5-(4-fluorophenyl)-3-(4-tolyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6e**):*

M.p. 269-271°C, yield: 66%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2922, 2850 (CH aliphatic), 2194 (CN), 1661 (C=O), 1597 (C=N). ^{1}H NMR: δ 2.26 (s, 3H, CH$_3$), 2.54 (s, 3H, N-CH$_3$), 2.95 (dd, 1H,

C4-H_A pyrazoline, J_{AX}= 6.3 Hz, J_{AM}= 17.8 Hz), 3.41 (dd, 1H, C4-H_M pyrazoline, J_{MX}= 11.8 Hz, J_{MA}= 17.8 Hz), 4.23 (dd, 1H, C5-H_X pyrazoline, J_{XA}= 6.3 Hz, J_{XM}= 11.8 Hz), 7.13-7.88 (m, 12H, aromatic H). Anal. Calcd. for $C_{28}H_{21}ClFN_5O$ (497.95): C, 67.54; H, 4.25; N, 14.06. Found: C, 67.57; H, 4.26; N, 14.13.

*4.1.4.14. 4-(4-Chlorophenyl)-2-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6f**):*

M.p. 245-247 ^oC, yield: 75%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2920, 2850 (CH aliphatic), 2194 (CN), 1660 (C=O), 1614 (C=N). ^1H NMR: δ 2.50 (s, 3H, N-CH_3), 2.99 (dd, 1H, C4-H_A pyrazoline, J_{AX}= 6.5 Hz, J_{AM}= 17.7 Hz), 3.38 (dd, 1H, C4-H_M pyrazoline, J_{MX}= 11.9 Hz, J_{MA}= 17.7 Hz), 4.26 (dd, 1H, C5-H_X pyrazoline, J_{XA}= 6.5 Hz, J_{XM}= 11.9 Hz), 7.23-7.95 (m, 13H, aromatic H). MS, m/z (%): 500.20 (M^+, 3.46); 138.95 (100). Anal. Calcd. for $C_{27}H_{19}Cl_2N_5O$ (500.38): C, 64.81; H, 3.83; N, 14.00. Found: C, 64.86; H, 3.88; N, 14.05.

*4.1.4.15. 4-(4-Chlorophenyl)-2-[3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6g**):*

M.p. 262-263^oC, yield: 72%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2922, 2848 (CH aliphatic), 2193 (CN), 1662 (C=O), 1595 (C=N). ^1H NMR: δ 2.51 (s, 3H, N-CH_3), 3.07 (dd, 1H, C4-H_A pyrazoline, J_{AX}= 6.3 Hz, J_{AM}= 18.2 Hz), 3.51 (dd, 1H, C4-H_M pyrazoline, J_{MX}= 11.6 Hz, J_{MA}= 18.2 Hz), 3.80 (s, 3H, OCH_3), 4.31 (dd, 1H, C5-H_X pyrazoline, J_{XA}= 6.3 Hz, J_{XM}= 11.6 Hz), 7.15-7.95 (m, 12H, aromatic H). ^{13}C NMR: δ 26.5 (N-CH_3), 41.2 (C-4 pyrazoline), 53.5 (C-5 pyrazoline), 55.9 (OCH3), 120.7-138.4 (aromatic Cs), 151.5 (C-2 pyrazoline), 159.8 (C-OCH_3), 160.5 (C-2 pyrimidine), 162.3 (C=O). Anal. Calcd. for $C_{28}H_{21}Cl_2N_5O_2$ (530.40): C, 63.40; H, 3.99; N, 13.20. Found: C, 63.44; H, 4.02; N, 13.31.

*4.1.4.16. 4-(4-Chlorophenyl)-2-[3,5-bis-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6h**):*

M.p. >300^oC, yield: 74%. IR υ_{max}/cm^{-1}: 3061 (CH aromatic), 2922, 2850 (CH aliphatic), 2193 (CN), 1654 (C=O), 1598 (C=N). ^1H NMR (C*D*Cl_3): δ 2.34 (s, 3H, N-CH_3), 3.05 (dd, 1H, C4-H_A pyrazoline, J_{AX}= 6.8 Hz, J_{AM}= 17.9 Hz), 3.85 (dd, 1H, C4-H_M pyrazoline, J_{MX}= 11.8 Hz, J_{MA}= 17.9 Hz), 3.88 (s, 6H, 2 OCH_3), 5.80 (dd, 1H, C5-H_X pyrazoline, J_{XA}= 6.7 Hz, J_{XM}= 11.8 Hz), 7.10-7.91 (m, 12H, aromatic H). Anal. Calcd. for $C_{29}H_{24}ClN_5O_3$ (525.99): C, 66.22; H, 4.60; N, 13.31. Found: C, 66.21; H, 4.67; N, 13.35.

4.2. Antiproliferative activity

4.2.1. Cell culture:

The tumor cells were obtained from ATCC. Cells were incubated under standard cell culture conditions at 37 ^oC in a humidified atmosphere with 5% CO2. Cells were grown in RPMI 1640 supplemented with 5% fetal bovine serum, 2 mM glutamine, 100 units/mL streptomycin, and 0.25 mg/mL amphotericin. Cellswere harvested at 70 and 90% confluence with trypsin/EDTA and used immediately. Cell count and viability was determined by Trypan blue staining followed by hemocytometry. Only cultures displaying >95% cell viability were used for performing the experiments [28].

4.2.2. Growth assay:

Tissue culture treated microtiter 96-well plates were seeded at a density of 5000 cells/well. The plates were incubated for 18-24 h prior to any treatment. All test compounds were solubilized in 100% DMSO and diluted with media to obtain final DMSO concentration of 0.1%. Cells were dosed. Cell viability was measured 72 h after treatment by Cell Titer Glo Assay (Promega), which is a luminescent assay that is an indicator of live cells as a function of metabolic activity and ATP content. The assay was performed according to the manufacturer's specifications. Luminescence was measured by a Perkin Elmer Victor® multi-label plate reader.

Effects of the synthesized compounds on tumor cell growth were assessed and potency was expressed in terms of the compounds IC_{50} values. After testing 8 concentrations for each compound with four replicates per concentration, dose-response curves were constructed and analyzed using PrismTM 4 (Graph-Pad software, San Diego, CA). IC_{50} values were calculated in µM from those graphs displaying the dose-survival percentage curve for each compound using a four parameter logistic equation.

References.

[1] C. Jin, Y-J. Liang, H. He, L. Fu, Eur. J. Med. Chem. 46 (2011) 429-432.

[2] P. Biginelli, Gazz. Chim. Ital. 23 (1893) 360-413.

[3] C. O. Kappe, Eur. J. Med. Chem. 35 (2000) 1043-1052.

[4] J. C. Barrow, P. G. Nantermet, H. G. Selnick, K. L. Glass, K. E. Rittle, K. F. Gilbert, R. S. L. Chang, S. S. O. 'Malley, T. V. Olah, J. D. Ellis, A. Barrish, K. Kassahun, D. Nagarathnam, C. J. Forray, J. Med.Chem. 43 (2000) 2703-2718.

[5] G. C. Rovnyak, S. D. Kimball, B. Beyer, G. Cucinotta, J. D. Di-Marco, J. Gougoutas, A. Hedberg, M. Malley, J. P. McCarthy, R. Zhang, S. Moreland, J. Med. Chem. 38 (1995) 119-129.

[6] D. Nagarathnam, S. W. Miao, C. M. Harrel, C. Gluchowski, J. Med.Chem. 42 (1999) 4764-4777.

[7] L. Heys, C. G. Moore, P. J. Murphy, Chem. Soc. Rev. 29 (2000) 57-67.

[8] C. O. Kappe, Acc. Chem. Res. 33 (2000) 879-888.

[9] T. M. Kapoor, T. U. Mayer, M. H. Coughlin, T. J. J. Mitchison, Cell. Biol. 150 (2000) 975-988.

[10] M. M. Edrees, T. A. Farghaly, F. A. A. El-Hag, M. M. Abdalla, Eur. J. Med. Chem. 45 (2010) 5702-5707.

[11] S. Manfredini, R. Bazzanini, P. G. Baraldi, M. Guarneri, D. Simoni, M. E. Marongiu, A. Pani, E. Tramontano, P. La Colla, J. Med. Chem. 35 (1992) 917-924.

[12] S. Manfredini, R. Bazzanini, P. G. Baraldi, M. Bonora, M. Marangoni, D. Simoni, A. Pani, F. Scintu, E. Pinna, Anti-Cancer Drug Des. 11 (1996) 193-204.

[13] H.-A. Park, K. Lee, S.-J. Park, B. Ahn, J.-C. Lee, H. Y. Cho, K.-I. Lee, Bioorg. Med. Chem. Lett. 15 (2005) 3307-3312.

[14] A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, M. Wachi, J. Yamagishi, J. Med. Chem. 47 (2004) 3693-3696.

[15] S. G. Kucukguzel, S. Rollas, H. Erdeniz, M. Kiraz, A. C. Ekinci, A. Vidin, Eur. J. Med. Chem. 35 (2000) 761-771.

[16] T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang, P. C. Isakson, J. Med. Chem. 40 (1997) 1347-1365.

[17] G. Menozzi, L. Mosti, P. Fossa, F. Mattioli, M. Ghia, J. Heterocycl. Chem. 1997, 34, 963-968.

[18] R. Sridhar, P. T. Perumal, S. Etti, G. Shanmugam, M. N. Ponnuswamy, V. R. Prabavathy, N. Mathivanan, Bioorg. Med. Chem. Lett. 14 (2004) 6035-6040.

[19] G. R. Bebernitz, G. Argentieri, B. Battle, C. Brennan, B. Balkan, B. F. Burkey, M. Eckhardt, J. Gao, P. Kapa, R. J. Strohschein, H. F. Schuster, M. Wilson, D. D. Xu, J. Med. Chem. 44 (2001) 2601-2611.

[20] J-J. Liu, H. Zhang, J. Sun, Z-C. Wang, Y. S. Yang, D-D. Li, F. Zhang, H-B. Gong, H-L. Zhu, Bioorg. Med. Chem. 20 (2012) 6089–6096.

[21] P-C. Lu, D-D. Li, Q-S. Li, X. Lu, Z-P. Xiao, H-L. Zhu, Bioorg. Med. Chem. Lett. 21 (2011) 5374–5377.

[22] S. Kambe, K. Saito, H. Kishi, A. Sakurai, H. Midorikawa, Synthesis 4 (1979) 287-289.

[23] J. Modha, N. Datta, H. Parekh, Il Farmaco 56 (2001) 641–646.

[24] F. Hayat, A. Salahuddin, S. Umar, A. Azam, Eur. J. Med. Chem. 45 (2010) 4669-4675.

[25] H. L. Yadav, P. Gupta, R. S. Pawar, P. K. Singour, U. K. Patil, Med. Chem. Res. 20 (2011) 461–465.

[26] V. Kanagarajan, J. Thanusu, M. Gopalakrishnan, Eur. J. Med. Chem. 45 (2010) 1583–1589.

[27] A. N Choughary, V. Juyal, Int. J. Pharm. Pharm. Sci., 3 (2011) 125-128.

[28] A. H. Abadi, D. A. Abouel-Ella, J. Lehmann, H. N. Tinsley, B. D. Gary, G. A. Piazza, M. A. Abdel-Fattah, Eur. J. Med. Chem. 45 (2010) 90-97.

Scheme captions:

- **Scheme 1.** Preparation of compounds **3a** and **3b**.

- **Scheme 2.** Preaparation of compounds **4a-h**.

- **Scheme 3.** Synthesis of compounds **5a-h** and **6a-h.**

Figure captions:

- **Figure 1.** Structures of some DHPM's and pyrazolines with antitumor activity.

- **Figure 2.** General structure of target compounds.

Table caption:

Table 1. IC_{50} values (μM) of the in vitro antiproliferative activity of the tested compounds against A 549 (lung), HT 29 (colon), MCF 7 and MDA-MB 231(breast) cell lines.

Highlights:

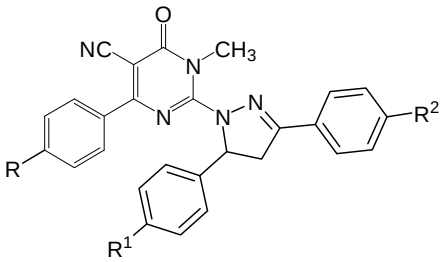
- Pyrimidine-pyrazoline hybrids were synthesized.

- Antiproliferative activity against four human cell lines was performed.

- Compounds **5e** and **5g**, showed high activity against three of the cell lines.

- IC_{50} of compound 5c against A 549 (lung) cell line was 1.76 µM.

Table 1. IC_{50} values (μM) of the in vitro antiproliferative activity of the tested compounds against A 549 (lung), HT 29 (colon), MCF 7 and MDA-MB 231(breast) cell lines.



5a-h, 6a-h

Cpd	R	R^1	R^2	A 549	HT 29	MCF 7	MDA-MB 231
5a	H	H	H	52.86	2.49	35.35	3.99
5b	H	H	Cl	3.25	19.17	3.28	28.02
5c	H	H	OCH_3	1.76	14.67	17.97	12.83
5d	H	H	F	39.17	8.15	38.14	11.82
5e	H	CH_3	F	2.00	4.62	23.11	5.10
5f	H	Cl	H	26.93	6.60	12.25	10.64
5g	H	Cl	OCH_3	6.07	9.12	4.80	11.09
5h	H	OCH_3	OCH_3	26.52	9.42	126.76	16.23
6a	Cl	H	H	28.09	19.51	34.37	29.14
6b	Cl	H	Cl	24.74	15.00	46.32	25.12
6c	Cl	H	OCH_3	23.81	10.95	52.38	19.53
6d	Cl	H	F	27.84	13.70	36.83	20.89
6e	Cl	CH_3	F	35.59	10.52	115.97	19.30
6f	Cl	Cl	H	43.86	8.72	25.28	12.42
6g	Cl	Cl	OCH_3	35.65	15.10	27.27	24.84
6h	Cl	OCH_3	OCH_3	39.69	18.21	99.81	28.29
Dox	-	-	-	3.13	2.75	1.20	0.11

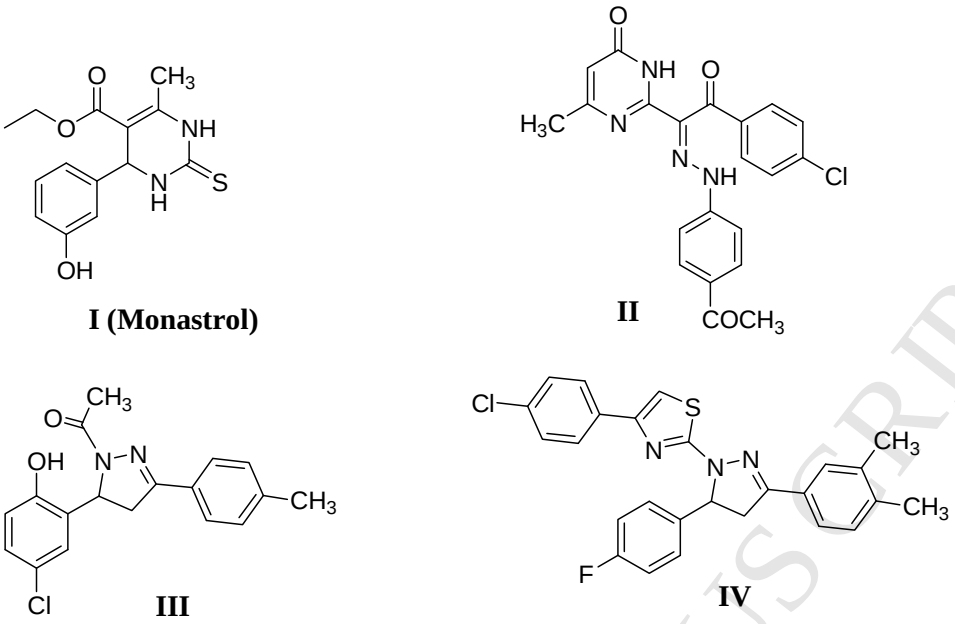


Figure 1. Structures of some DHPM's and pyrazolines with antitumor activity.

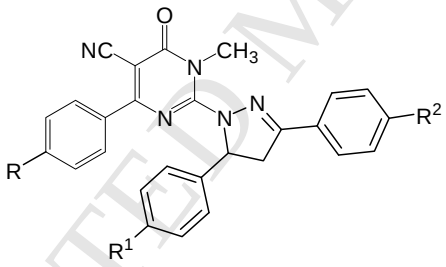
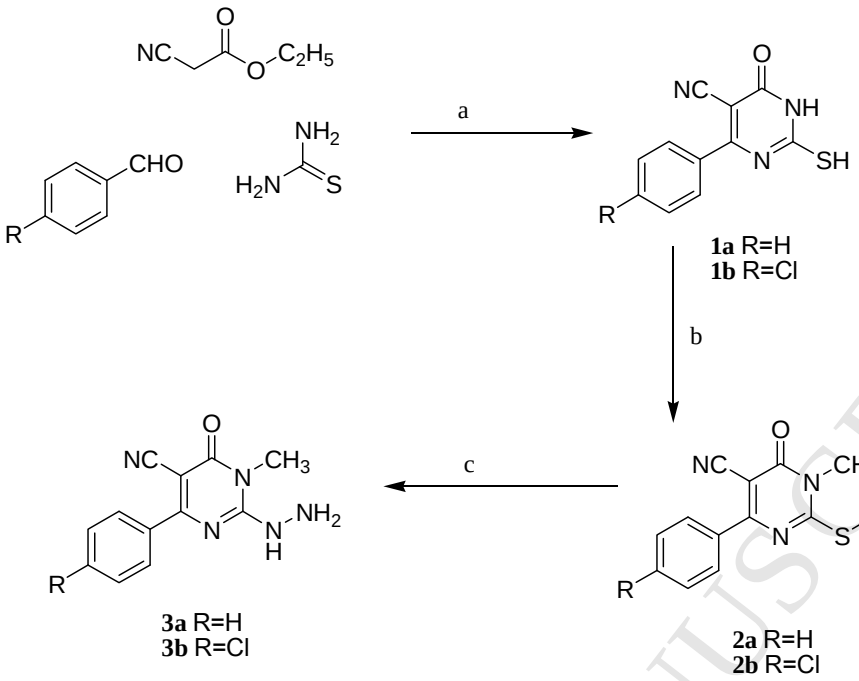
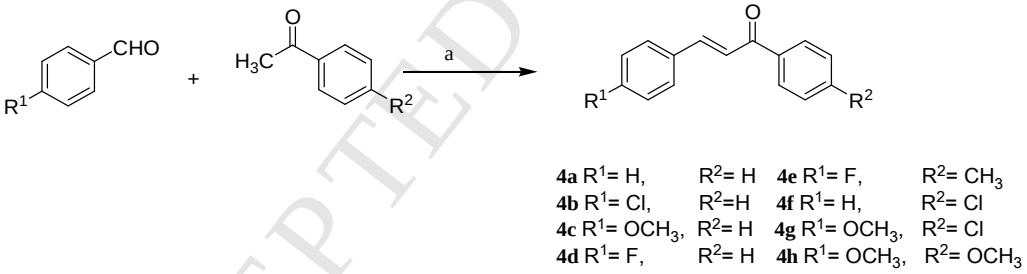


Figure 2. General structure of target compounds.



Scheme 1. Preparation of compounds **3a** and **3b**.

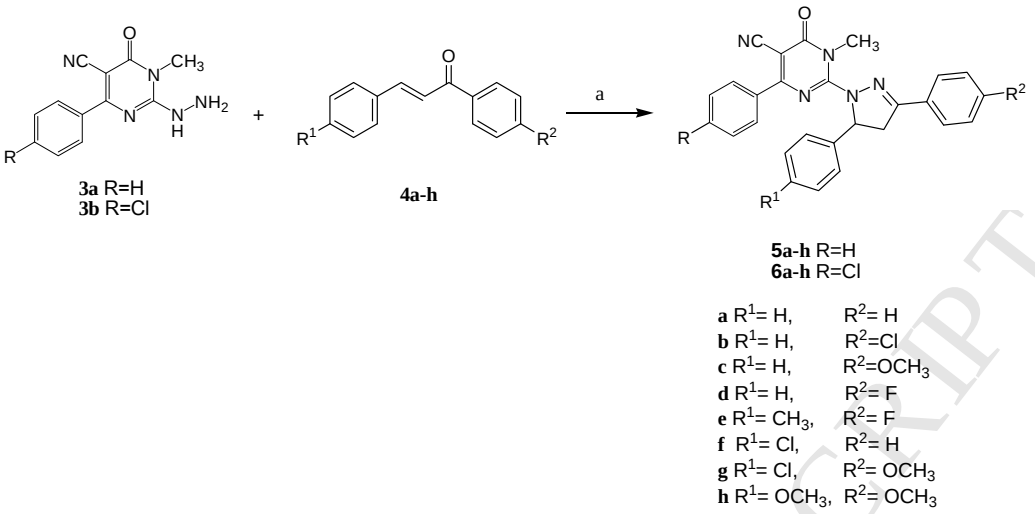
Reagents and conditions. a: potassium carbonate/ absolute ethanol, reflux, 12h; **b:** methyl iodide, potassium carbonate, dry DMF, RT., 3h; **c:** hydrazine hydrate/ absolute ethanol, reflux, 12h.



4a	R^1= H,	R^2= H	**4e**	R^1= F,	R^2= CH_3
4b	R^1= Cl,	R^2=H	**4f**	R^1= H,	R^2= Cl
4c	R^1= OCH_3,	R^2= H	**4g**	R^1= OCH_3,	R^2= Cl
4d	R^1= F,	R^2= H	**4h**	R^1= OCH_3,	R^2= OCH_3

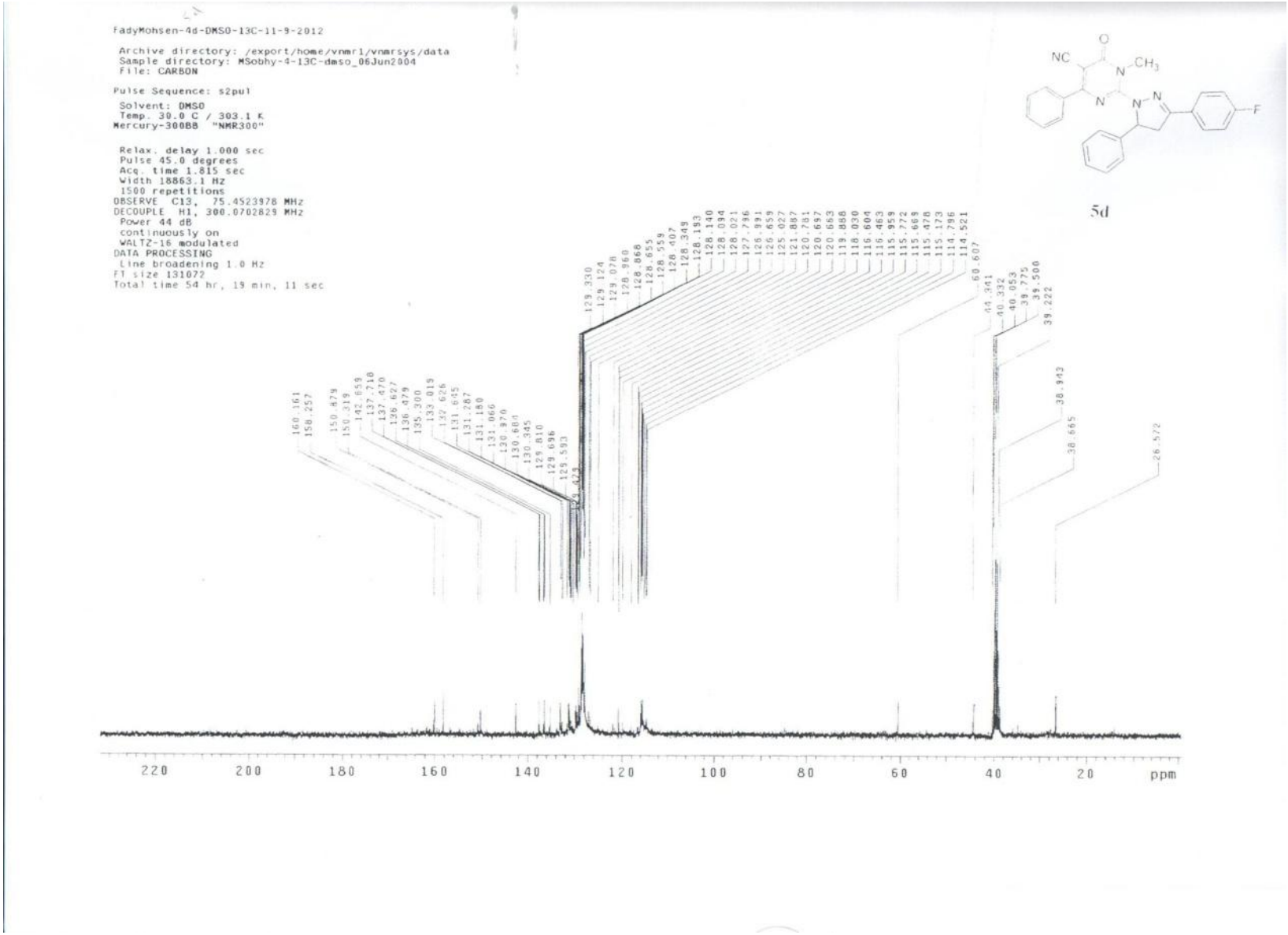
Scheme 2. Preparation of compounds **4a-h**.

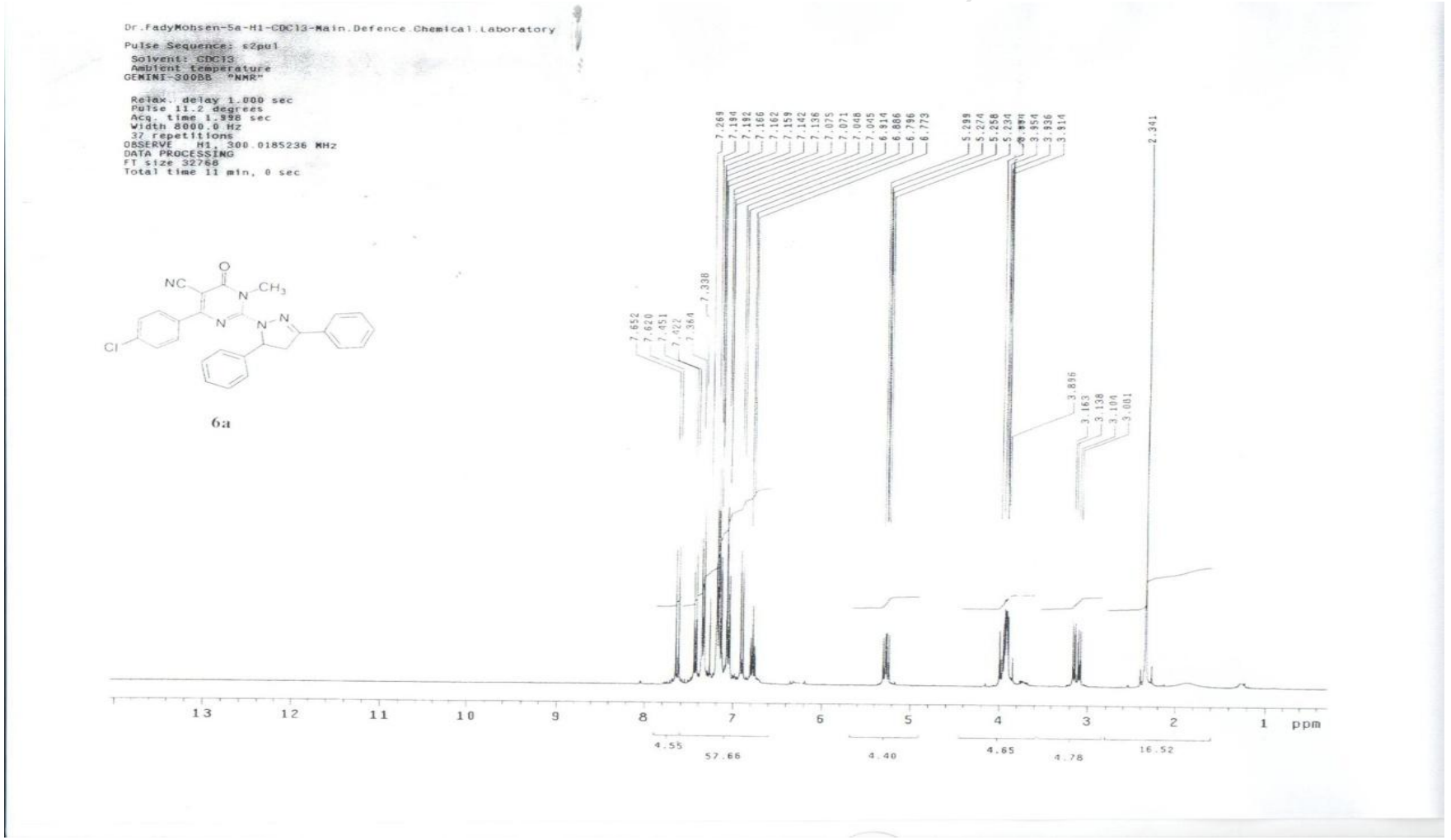
Reagents and conditions. a: sodium hydroxide/ absolute ethanol, RT., 24h.



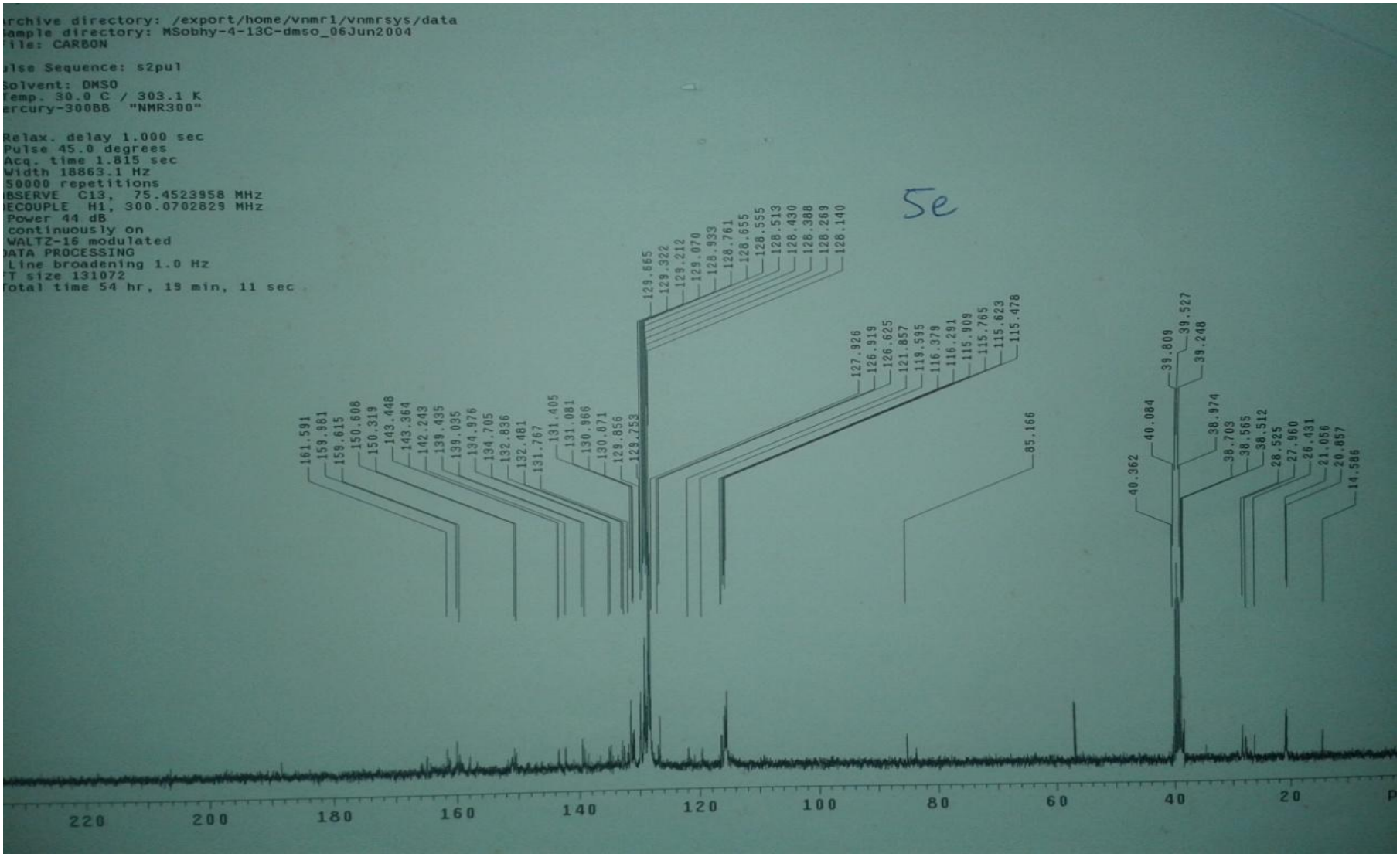
Scheme 3. Synthesis of compounds **5a-h** and **6a-h.**

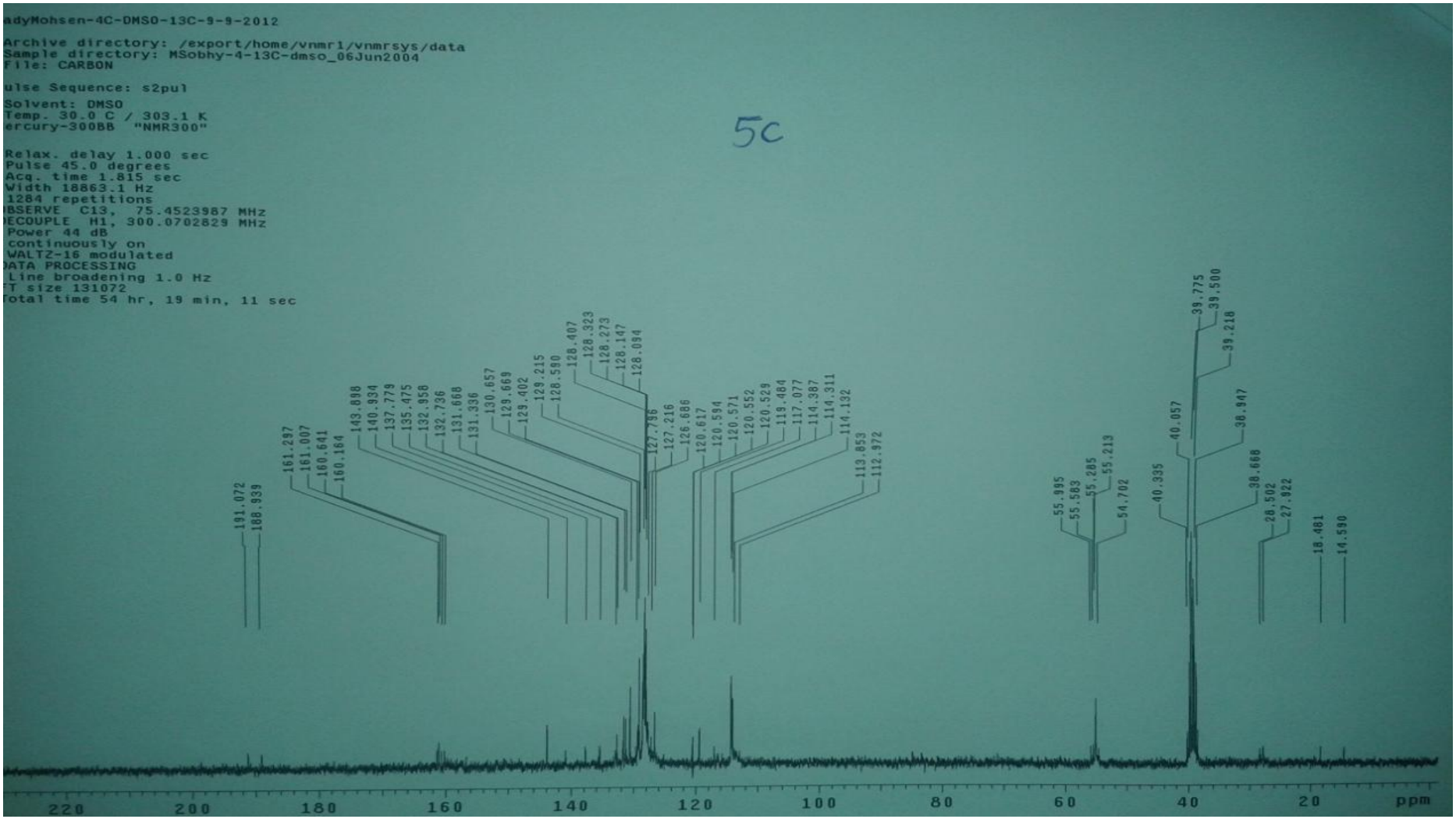
Reagents and conditions. a: sodium hydroxide/ absolute ethanol, reflux, 72h.


FadyMohsen-4d-DMSO-13C-11-9-2012
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory: MSobhy-4-13C-dmso_06Jun2004
File: CARBON
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 30.0 C / 303.1 K
Mercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18863.1 Hz
1500 repetitions
OBSERVE C13, 75.4523978 MHz
DECOUPLE H1, 300.0702829 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 54 hr, 19 min, 11 sec
NC
O
N
CH3
N
N
N
F
5d
160.161
158.257
150.479
150.319
142.659
137.710
137.470
136.627
136.479
135.300
133.019
132.626
131.645
131.287
131.180
131.066
130.970
130.680
130.345
129.810
129.696
129.593
129.330
129.124
129.070
128.960
128.868
128.655
128.559
128.407
128.349
128.193
128.140
128.094
128.021
127.796
126.991
126.659
125.027
121.807
120.781
120.697
120.663
119.888
118.030
116.604
116.463
115.959
115.772
115.669
115.476
115.173
114.796
114.521
60.607
41.341
40.332
40.053
39.775
39.500
39.222
38.943
38.665
26.572
220
200
180
160
140
120
100
80
60
40
20
ppm

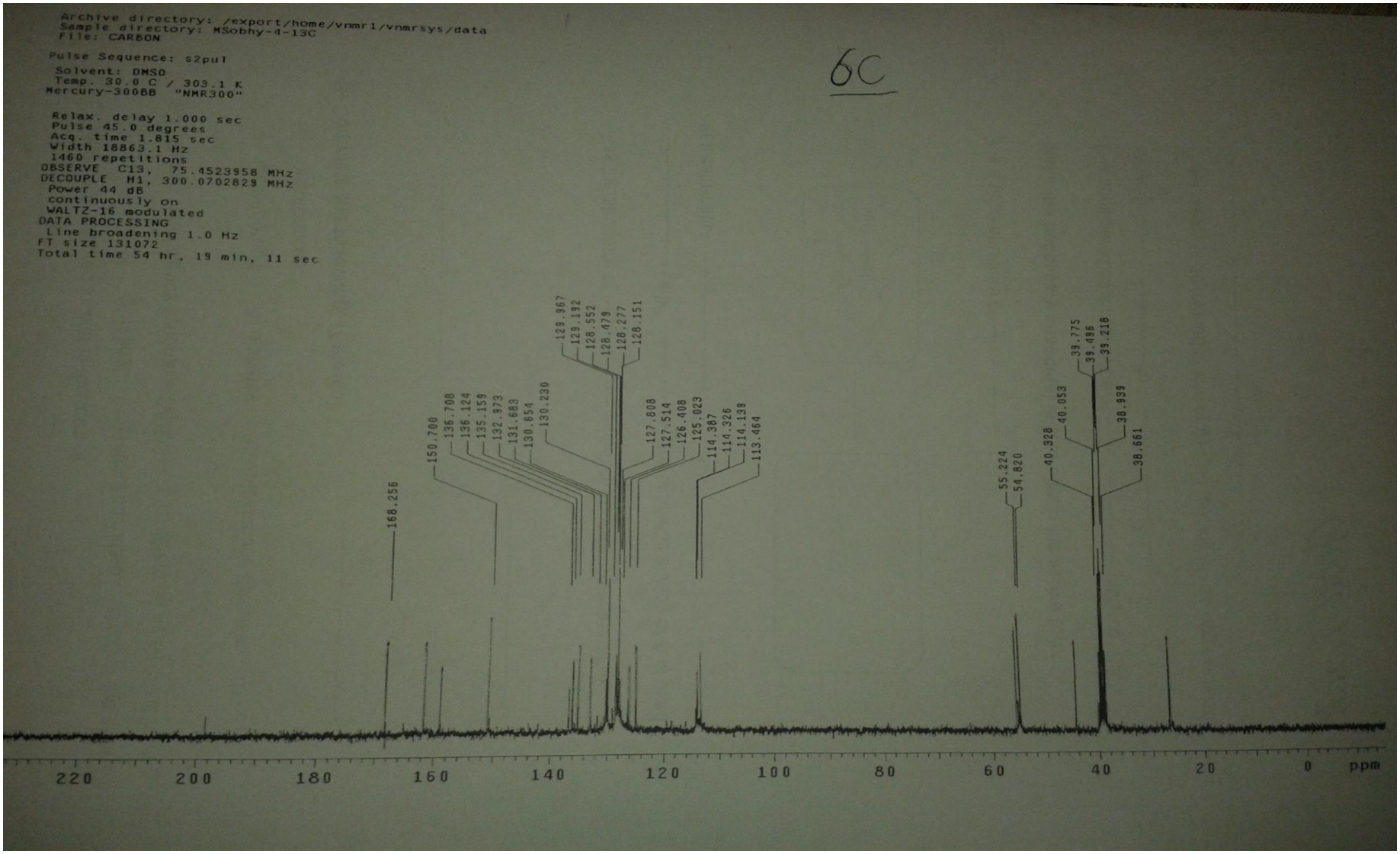

Dr.FadyMohsen-5a-H1-CDCl3-Main.Defence.Chemical.Laboratory
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300BB "NMR"
Relax. delay 1.000 sec
Pulse 11.2 degrees
Acq. time 1.998 sec
Width 8000.0 Hz
32 repetitions
OBSERVE H1, 300.0185236 MHz
DATA PROCESSING
FT size 32768
Total time 11 min, 0 sec
NC
O
N
CH3
N
N
N
Cl
7.652
7.620
7.451
7.422
7.364
7.338
7.269
7.194
7.182
7.166
7.162
7.159
7.142
7.136
7.075
7.071
7.048
7.045
6.914
6.886
6.796
6.773
5.299
5.274
5.258
5.234
3.954
3.936
3.914
3.896
3.163
3.138
3.104
3.081
2.341
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
4.55
57.66
4.40
4.65
4.78
16.52

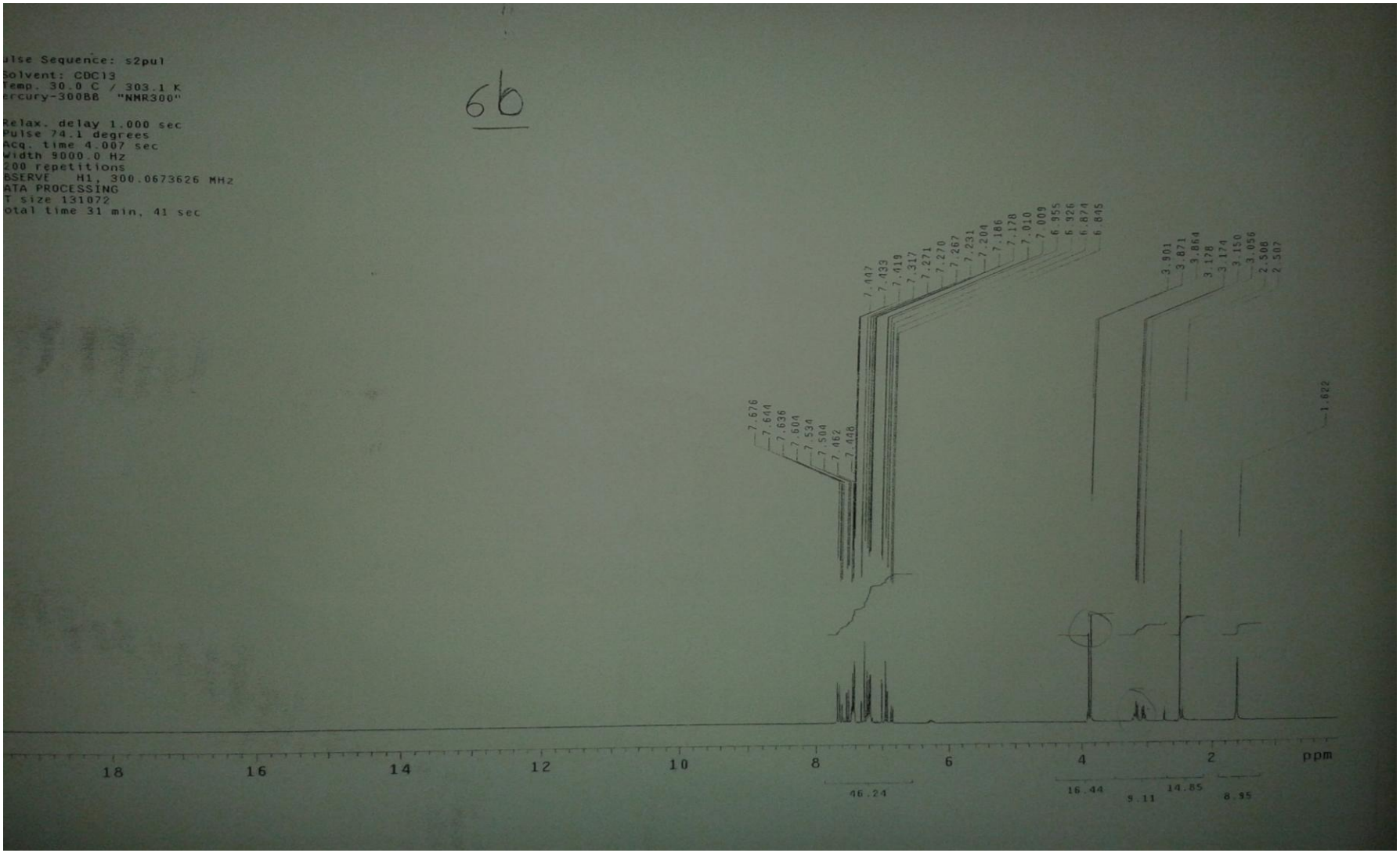
6a

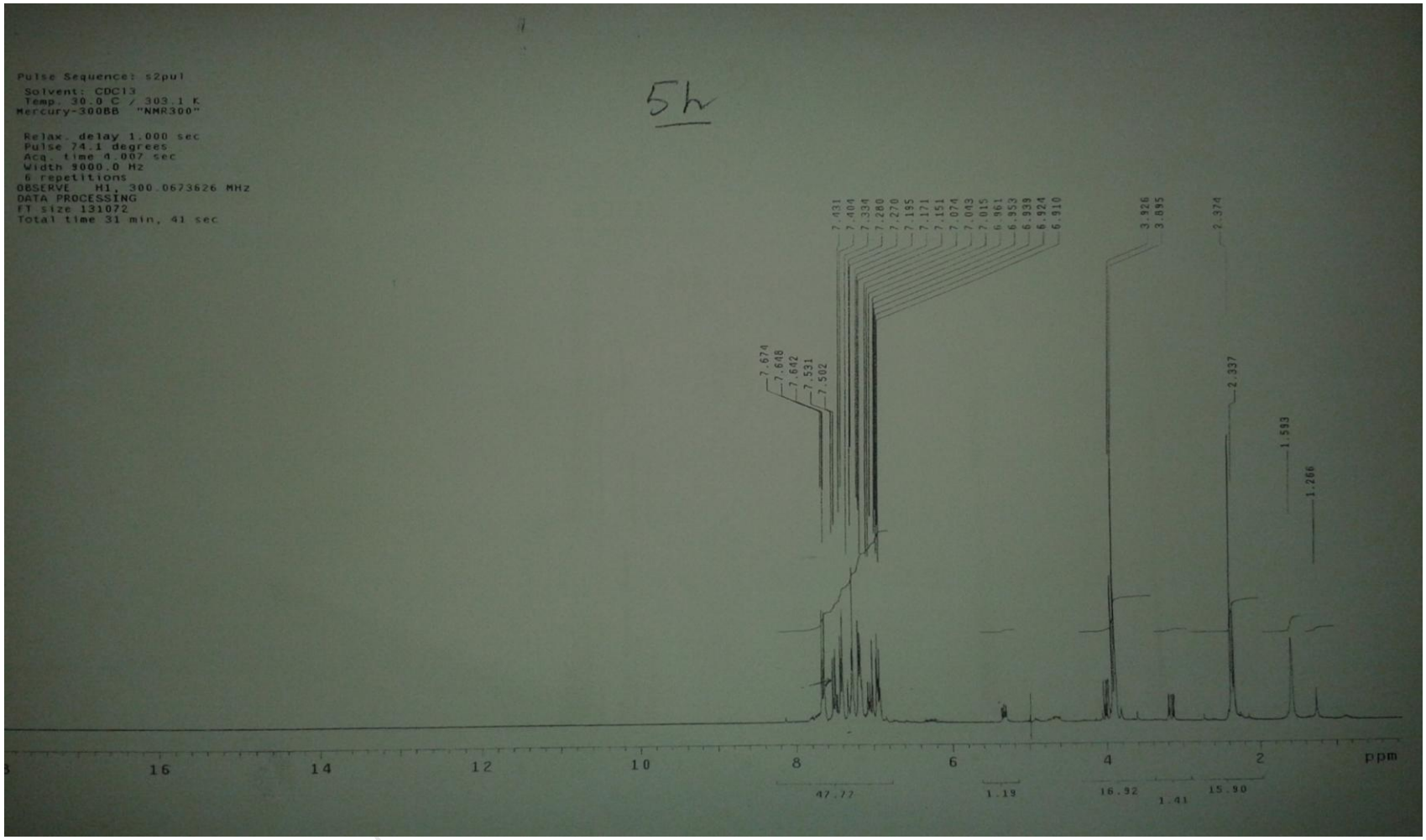

rchive directory: /export/home/vnmr1/vnmrsys/data
ample directory: MSobhy-4-13C-dmso_06Jun2004
ile: CARBON
lse Sequence: s2pul
olvent: DMSO
emp. 30.0 C / 303.1 K
ercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18863.1 Hz
50000 repetitions
BSERVE C13, 75.4523958 MHz
ECOUPLE H1, 300.0702829 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
ATA PROCESSING
Line broadening 1.0 Hz
T size 131072
otal time 54 hr, 19 min, 11 sec
Se
129.665
129.322
129.212
129.070
128.933
128.761
128.655
128.555
128.513
128.430
128.388
128.269
128.140
127.926
126.919
126.625
121.857
119.595
116.379
116.291
115.909
115.765
115.623
115.478
85.166
161.591
159.981
159.615
150.608
150.319
143.448
143.364
142.243
139.435
139.035
134.976
134.705
132.836
132.481
131.767
131.405
131.081
130.966
130.871
129.856
129.753
40.362
40.084
39.809
39.527
39.248
38.974
38.703
38.565
38.512
28.525
27.960
26.431
21.056
20.857
14.586
220
200
180
160
140
120
100
80
60
40
20


adyMohsen-4C-DMSO-13C-9-9-2012
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory: MSobhy-4-13C-dmso_06Jun2004
File: CARBON
ulse Sequence: s2pul
Solvent: DMSO
Temp. 30.0 C / 303.1 K
ercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18863.1 Hz
1284 repetitions
BSERVE C13, 75.4523987 MHz
ECOUPLE H1, 300.0702829 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
ATA PROCESSING
Line broadening 1.0 Hz
T size 131072
otal time 54 hr, 19 min, 11 sec
5c
191.072
188.939
161.287
161.007
160.641
160.164
143.898
140.934
137.779
135.475
132.958
132.736
131.668
131.336
130.657
129.669
129.402
129.215
128.590
128.407
128.323
128.273
128.147
128.094
127.736
127.216
126.686
120.617
120.594
120.571
120.552
120.529
119.484
117.077
114.387
114.311
114.132
113.853
112.372
55.995
55.583
55.285
55.213
54.702
40.335
40.057
39.775
39.500
39.218
38.947
38.668
28.502
27.922
18.481
14.590
220
200
180
160
140
120
100
80
60
40
20
ppm

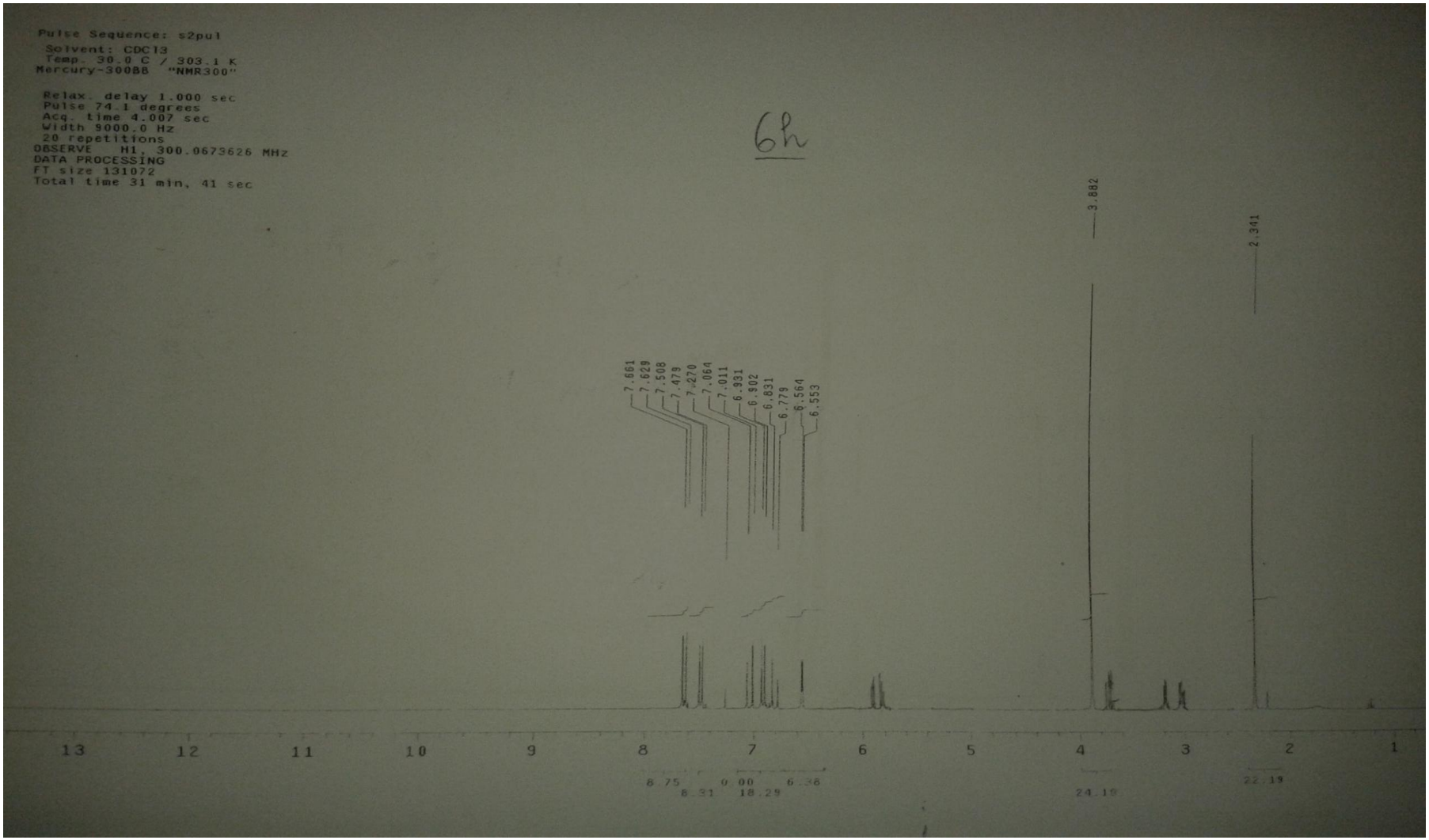

ulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
ercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 74.1 degrees
Acq. time 4.007 sec
Width 9000.0 Hz
23 repetitions
BSERVE H1, 300.0673626 MHz
ATA PROCESSING
T size 131072
otal time 31 min, 41 sec
6C
7.583
7.551
7.447
7.419
7.272
7.268
7.262
7.240
7.233
7.215
7.207
7.199
7.188
7.179
6.836
6.808
4.802
3.919
3.896
3.867
3.828
2.405
14
12
10
8
6
4
2
ppm
42.04
3.75
3.44
18.19
10.61
18.83


Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory: MSobhy-4-13C
File: CARBON
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 30.0 C / 303.1 K
Mercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18863.1 Hz
1460 repetitions
OBSERVE C13, 75.4523958 MHz
DECOUPLE H1, 300.0702829 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 54 hr, 19 min, 11 sec
6C
168.256
150.700
136.708
136.124
135.159
132.973
131.683
130.654
130.230
129.967
129.132
128.552
128.479
128.277
128.151
127.808
127.514
126.408
125.023
114.387
114.326
114.139
113.464
55.224
54.820
40.328
40.053
39.775
39.496
39.218
38.939
38.661
220
200
180
160
140
120
100
80
60
40
20
0
ppm


ulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
ercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 74.1 degrees
Acq. time 4.007 sec
Width 9000.0 Hz
200 repetitions
BSERVE H1, 300.0673626 MHz
ATA PROCESSING
T size 131072
otal time 31 min, 41 sec
6b
7.676
7.644
7.636
7.604
7.534
7.504
7.462
7.448
7.447
7.433
7.419
7.317
7.271
7.270
7.267
7.231
7.204
7.186
7.178
7.010
7.009
6.955
6.926
6.874
6.845
3.901
3.871
3.864
3.128
3.124
3.150
3.056
2.508
2.507
1.622
18
16
14
12
10
8
6
4
2
ppm
46.24
16.44
9.11
14.85
8.95


Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Mercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 74.1 degrees
Acq. time 4.007 sec
Width 9000.0 Hz
6 repetitions
OBSERVE H1, 300.0673826 MHz
DATA PROCESSING
FT size 131072
Total time 31 min, 41 sec
5h
7.674
7.648
7.642
7.531
7.502
7.431
7.404
7.334
7.280
7.270
7.195
7.171
7.151
7.074
7.043
7.015
6.961
6.953
6.939
6.924
6.910
3.926
3.895
2.374
2.337
1.593
1.266
16
14
12
10
8
6
4
2
ppm
47.77
1.19
16.92
1.41
15.90


Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Mercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 74.1 degrees
Acq. time 4.007 sec
Width 9000.0 Hz
52 repetitions
OBSERVE H1, 300.0673626 MHz
DATA PROCESSING
FT size 131072
Total time 31 min, 41 sec
5g
7.592
7.560
7.512
7.511
7.490
7.483
7.459
7.431
7.398
7.368
7.271
7.265
7.063
7.056
6.984
6.977
6.846
3.901
3.873
3.842
3.768
2.306
2.285
12
11
10
9
8
7
6
5
4
3
2
1
ppm


Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Mercury-300BB "NMR300"
Relax. delay 1.000 sec
Pulse 74.1 degrees
Acq. time 4.007 sec
Width 9000.0 Hz
20 repetitions
OBSERVE H1, 300.0673626 MHz
DATA PROCESSING
FT size 131072
Total time 31 min, 41 sec
6h
7.661
7.629
7.508
7.479
7.270
7.064
7.011
6.931
6.902
6.831
6.779
6.564
6.553
3.882
2.341
13
12
11
10
9
8
7
6
5
4
3
2
1
8.75
8.31
0.00
18.29
6.36
24.19
22.19