Convenient one-pot synthesis and biological evaluation of novel 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide derivatives as new anti-tumor agents against both liver carcinoma (HepG2) and lung carcinoma (A549) cell lines

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Abstract

Pyrazle constituents have garnered consideration mainly because of their presumed biological and curative properties. Therefore, the goal of the presented research is to create some novel pyrazole-1-carbothiohydrazide derivatives with predicted biological functions using an accessible one-pot synthesis employing 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**) as a facile antecedent. The component **1** and 1,3-diphenylpropane-1,3-dione (**2**) interacted resulting in pyrazole derivative **3** when ethanol or acetic acid were supplied. However, when the substance **1** interacted in ethanol with either **2** or acetyl acetone **5**, using the catalytic proportion of triethylamine offered, it yielded compounds **4** and **6** respectively. Ingredient **1** was subjected to additional reactions with ethyl cyanoacetate (**7**), stearic acid **10**, or equivalent carboxylic acids **12a-c**, resulted in the formation of **9**, **11**, and **13a-c** consequently. Additionally, when **1** was combined with α-haloketones **14** or **16**, it yielded the corresponding 1,3,4-thiadiazine derivatives, **15** and **17**. Every designed product's chemical structure was established according to spectroscopic and analytical results. The cytotoxic activities of the synthesized chemicals were assessed against two carcinoma cell lines, and compared to the standard drug Cisplatin using the colorimetric MTT assay. Furthermore, the results revealed that chemical **17** was the most active against the liver and lung carcinoma cell lines giving potent IC_{50} value of 5.35 and 8.74 μM, respectively, compared with reference drug cisplatin (3.78 and 6.39 μM). Interestingly, ingredient **17** when evaluated for their toxicity against normal lung fibroblast (MRC-5) cells exhibited low toxic effects indicating the safe use. Generally, our results exerted promising bioactive compounds.

Keywords pyrazole-1-carbothiohydrazide, ethyl cyanoacetate, 1,3,4-thiadiazine derivatives, cytotoxic activities, Cisplatin.

1- Introduction

Following cardiovascular and cerebrovascular disorders as the world's two main causes of mortality, cancer is one of the biggest threats to human health [1,2]. Chemotherapy is crucial for treating different types of cancer. However, due to the continuous development of drug resistance, especially multidrug resistance, and the limited specificity of currently available chemotherapeutics, it is often unsuccessful in treating cancer [3,4]. Thereby, research into more potent anticancer chemotherapy medications is desperately needed. Alternatively, pyrazoles have two neighbouring nitrogen atoms that can function as hydrogen donors or acceptors, possessing distinct electronegativities and responses that aid in the synthetic process and incorporation of substituents, or enhancing the molecules' polarity or ability to dissolve in water [5,6], resulting in a variety of universal biological characteristics, including bacterial lethality properties [7,8], malarial murder effects [9], anti-inflammatory features [10], viral killing qualities [11], antitubercular traits [12], and cancer-fighting abilities [13,14]. Furthermore, pyrazole derivatives, particularly pyrazole hybrids, exhibited substantial tumor fighting properties both in vivo and in vitro via a variety of approaches including inducing apoptosis, controlling autophagy, and frustrating the cell cycle [15]. As such, the current study aims to produce new heterocyclic chemicals with hypothesized biological actions. This can be performed via employing 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide as an effective precursor for creation unique pyrazole derivatives using facile one-pot synthesis and determining how effective they are as anti-tumor medications.

2- Experimental

Using a Gallenkamp apparatus, melting points in open glass capillaries were found. The Shimadzu FTIR 8101 PC infrared spectrophotometer and Pye Unicam SP 3-300 were used to capture the infrared spectra of potassium bromide discs. Chemical shifts are represented as δ values in the ^{1}H and ^{13}C NMR spectra, which were recorded on a Jeol JMS-AX 500 MHz instrument with TMS serving as an internal reference. A Shimadzu GCMQP 1000 EX device was employed to obtain mass spectra (EI) at 70 eV. The aforementioned studies were conducted by three institutions in Egypt: the National Research Centre in Giza, the Micro-analytical Centre at Cairo University in Giza, and the Regional Centre for Mycology and Biotechnology at Al-Azhar University in Cairo. 3,5-Dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**), [16] 2-bromo-1,3-diphenyl propane-1,3-dione (**16**), [17] were produced in my previous studies, 1,3-diphenylpropane-1,3-dione (**2**), [18] was constructed using techniques described in the scientific literature.

*Reaction between 3,5-dimethyl-1H-pyrazole-1-carbothiohydrazide (**1**) and 1,3-diketones.*

In a 20 ml solution of ethanol, 0.448 g (2 mmol) of 1,3-diphenylpropane-1,3-dione (**2**) and 0.340 g (2 mmol) of the pyrazole-1-carbothiohydrazide derivative **1** were combined. The mixture was then allowed to chilled to atmospheric temperature after five hours of refluxing. After that, it was immersed in a mixture of ice and water. The resulting solid was filtered and scrubbed with water. Next, it was dried and purified through recrystallization using an appropriate solvent, resulting in the formation of (10*E*)-*N*'-((Z)-3-hydroxy-1,3-diphenylallylidene)-3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**3**). Chemical **3** can also be acquired by repeating the previous process, but employing acetic acid instead of ethanol.
IR (KBr) νmax/cm^{-1}: 3189 (NH), 3064 (OH); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.85 (s, 1H, CH), 6.23 (s, 1H, CH), 7.32-8.16 (m, 10H, Ar-H), 12.72 (s, 1H, OH), 17.20 (s, 1H, NH); ^{13}C NMR (DMSO-d6): δ 12.75, 13.05, 93.20, 110.48, 125.05, 127.37, 128.46, 128.79, 132.96, 134.55, 141.49, 152.38, 185.27; MS m/z (%) calculated $C_{21}H_{20}N_4SO$: 376.48, found: 376.53 (M^+, 14.64), 317.76 (42.97), 287.09 (100.00), 262.33 (68.02), 245.36 (70.87), 93.44 (98.81).

An equivalent amount of either 1,3-diphenylpropane-1,3-dione (**2**) (0.448 g, 2 mmol) or acetyl acetone **5** (0.200 g, 2 mmol) was added to a pyrazole-1-carbothiohydrazide derivative **1** (0.340 g, 2 mmol) solution in ethanol (20 ml) upon the catalytic quantity of triethylamine being supplied. The mixture was reflux heated for five hours and then allowed to cool down to the temperature of the environment. Then submerged in a combination of ice and water, and neutralized by adding diluted hydrochloric acid. The resulting solid product was then filtered and rinsed using water before being drained. To recrystallize the product, the appropriate solvent was applied. This process yielded both 3,5-dimethyl-*N*-((Z)-3-oxo-1,3-diphenylprop-1-enyl)-1*H*-pyrazole-1-carbothiohydrazide (**4**) and 1-(5-methyl-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,3,4-thiadiazin-6-yl)ethanone (**6**) respectively.

3,5-dimethyl-*N*-((*Z*)-3-oxo-1,3-diphenylprop-1-enyl)-1*H*-pyrazole-1-carbothiohydrazide (4)

IR (KBr) νmax/cm^{-1}: 3460, 3209 (NH_2), 1591 (C=O); ^{1}H NMR (DMSO-d6): δ 2.14 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.86 (s, 1H, CH), 6.21 (s, 1H, CH), 7.33-8.16 (m, 10H, Ar-H), 17.14 (s, 1H, NH_2); ^{13}C NMR (DMSO-d6): δ 13.28, 13.59, 93.81, 110.99, 127.94, 129.03, 129.35, 133.49, 134.16, 135.18, 152.89, 155.24, 185.85, 196.05; MS m/z (%) calculated $C_{21}H_{20}N_4SO$: 376.48, found: 376.00 (M^+, 8.03), 369.65 (17.84), 268.22 (34.12), 239.02 (28.11), 130.22 (28.70), 63.25 (100.00).

1-(5-methyl-2-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,3,4-thiadiazin-6-yl)ethanone (6)

IR (KBr) νmax/cm^{-1}: 3430 (NH), 1587 (C=O); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 6.21 (s, 1H, CH), 14.33 (s, 1H, NH); ^{13}C NMR (DMSO-d6): δ 13.29, 13.63, 19.01, 111.14, 136.73, 142.16, 153.07, 153.56, 164.92,

186.08; MS m/z (%) calculated $C_{11}H_{14}N_4SO$: 250.32, found: 250.54 (M^+, 17.45), 227.37 (15.42), 205.48 (45.82), 147.24 (100.00), 126.04 (40.17), 69.18 (49.97).

*Synthesis of (3-amino-5-hydroxy-1H-pyrazol-1-yl)(3,5-dimethyl-1H-pyrazol-1-yl)methane-thione (**9**)*

In a solution comprising 0.340 g (2 mmol) of pyrazole-1-carbothiohydrazide derivative **1** and 20 ml of absolute ethanol, a comparable quantity of ethyl cyanoacetate (**7**) (0.226 g, 2 mmol) was dispersed. The resulting amalgamation was heated in a reflux system for six hours and then dropped into a combination of ice and water. After filtration, the obtained solid was purified using water, evaporated, and crystallized using an adequate solvent to yield chemical **9**.
IR (KBr) νmax/cm^{-1}: 3265, 3207 (NH_2), 3071 (OH); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 5.30 (s, 2H, NH_2), 6.23 (s, 1H, CH), 7.26 (s, 1H, CH), 12.71 (s, 1H, OH); ^{13}C NMR (DMSO-d6): δ 13.28, 13.60, 111.06, 142.12, 153.02, 154.80, 155.22, 165.27, 185.96; MS m/z (%) calculated $C_9H_{11}N_5SO$: 237.28, found: 237.78 (M^+, 15.56), 207.00 (20.21), 159.95 (21.71), 119.02 (49.44), 101.37 (99.51), 75.10 (100.00).

*Reaction of 3,5-dimethyl-1H-pyrazole-1-carbothiohydrazide (**1**) with fatty and carboxylic acids*

0.340 g (2 mmol) of the pyrazole-1-carbothiohydrazide derivative **1** and stearic acid **10** (2 mmol) or equivalent carboxylic acid **12a-c**, whereby 2-mmol of 4-hydrobenzoic acid, 2-mmol of gallic acid, or 2-mmol of salicylic acid were blended with 20 ml ethanol. After refluxing for five hours, the contents were allowed to chill to the ambient temperature before being submerged in a blend of both cold water and ice. After the extraction process, the water was employed to wash the solid that had been achieved. Next, it was dried and refined through recrystallization utilizing a compatible solvent. This resulted in the production of 1,3,4-thiadiazole derivative **11** and S-imino-hydroxybenzothioate derivatives **13a-c**, consequently.

2-heptadecyl-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1,3,4-thiadiazole (11)

^{1}H NMR (DMSO-d6): δ 0.82 (t, 3H, CH_3, J=6.5 Hz), 1.20-1.43 (m, 28H, 14 CH_2), 1.46 (m, 2H, CH_2), 2.13 (t, 2H, CH_2, J=6.5 Hz), 2.16 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 6.16 (s, 1H, CH); ^{13}C NMR (DMSO-d6): δ 13.29, 13.60, 14.04, 14.41, 20.0, 22.63, 25.04, 29.12, 29.25, 29.30, 29.46, 29.58, 30.09, 30.34, 31.84, 34.20, 39.77, 40.43, 40.59, 87.21, 100.00, 110.94, 142.04, 174.91; MS m/z (%) calculated $C_{24}H_{42}N_4S$: 418.69, found: 418.82 (M^+, 67.90), 397.55 (37.04), 321.41 (59.58), 284.34 (90.97), 244.21 (100.00), 183.35 (61.77).

S-imino(3,5-dimethyl-1H-pyrazol-1-yl)methyl 4-hydroxybenzothioate derivative (13a)
IR (KBr) νmax/cm^{-1}: 3441, 3210 (NH_2), 3074 (OH), 1640 (C=O); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 5.27 (s, 1H, CH), 6.21-7.29 (m, 4H, Ar-H), 10.69 (s, 2H, NH_2), 12.72 (s, 1H, OH),; ^{13}C NMR (DMSO-d6): δ 13.29, 13.62, 111.06, 142.10, 152.99, 154.76,

155.21, 165.19, 185.97; MS m/z (%) calculated $C_{13}H_{14}N_4SO_2$: 290.34, found: 290.37 (M^+, 30.14), 238.45 (72.68), 205.96 (100.00), 154.31 (55.95), 97.94 (82.92), 77.10 (76.45).

S-imino(3,5-dimethyl-1*H*-pyrazol-1-yl)methyl 3,4,5-trihydroxybenzothioate derivative (13b)

IR (KBr) νmax/cm^{-1}: 3442, 3207 (NH_2), 3074, 2911, 2854 (3OH), 1643 (C=O); ^{1}H NMR (DMSO-d6): δ 2.16 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 5.27 (s, 1H, CH), 6.23 (s, 2H, Ar-H), 7.28 (s, 2H, NH_2), 10.52 (s, 1H, OH), 12.72 (s, 2H, 2OH); ^{13}C NMR (DMSO-d6): δ 12.81, 13.11, 109.23, 110.53, 126.41, 133.81, 141.56, 152.45, 154.69, 164.60, 185.33; MS m/z (%) calculated $C_{13}H_{14}N_4SO_4$: 322.34, found: 322.51 (M^+, 22.21), 263.08 (75.23), 221.82 (53.65), 152.01 (57.46), 103.50 (100.00), 89.88 (93.12).

S-imino(3,5-dimethyl-1*H*-pyrazol-1-yl)methyl 2-hydroxybenzothioate derivative (13c)

IR (KBr) νmax/cm^{-1}: 3442, 3208 (NH_2), 3074 (OH), 1644 (C=O); ^{1}H NMR (DMSO-d6): δ 2.18 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.28 (s, 2H, NH_2), 5.26 (s, 1H, CH), 6.22-7.25 (m, 4H, Ar-H), 12.71 (s, 1H, OH); ^{13}C NMR (DMSO-d6): δ 13.31, 13.63, 110.99, 142.08, 152.91, 155.15, 185.83; MS m/z (%) calculated $C_{13}H_{14}N_4SO_2$: 290.34, found: 290.83 (M^+, 13.15), 274.48 (43.55), 233.80 (63.50), 185.32 (49.99), 151.14 (61.93), 96.50 (100.00).

*Reaction between 3,5-dimethyl-1H-pyrazole-1-carbothiohydrazide (**1**) and α-haloketones.*

After introducing a modest portion of triethylamine to an amalgamation of pyrazole-1-carbothiohydrazide derivative **1** (0.340 g, 2 mmol) into ethanol (20 ml), a comparable quantity of either chloroacetic acid **14** (0.189 g, 2 mmol) or 2-bromo-1,3-diphenyl propane-1,3-dione (**16**) (0.606 g, 2 mmol) was included. The resulting combination was reflux simmered for five hours and then left to recede to the environmental level. Afterwards, it was immersed in a chilled water-ice bath and diluted hydrochloric acid was added for neutralization. The obtainable crystalline substance was passed through filter, dried out, and then recrystallized leveraging the required solvent to acquire the pertinent 1,3,4-thiadiazine derivatives, **15** and **17**, accordingly.

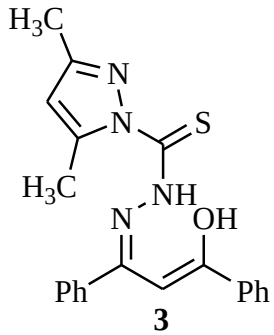
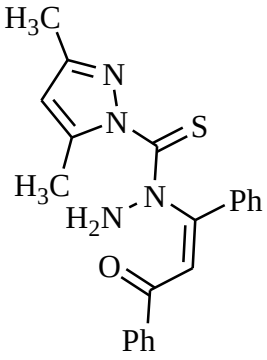
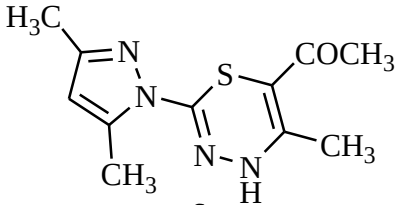
2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6*H*-1,3,4-thiadiazin-5-ol (15)

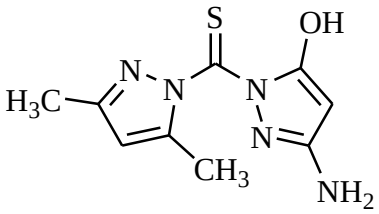
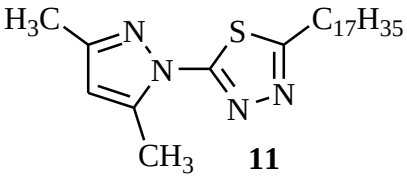
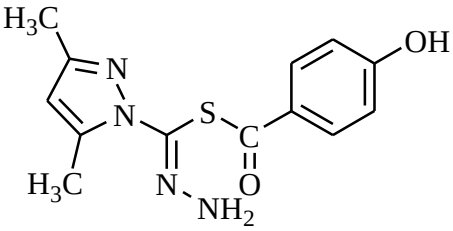
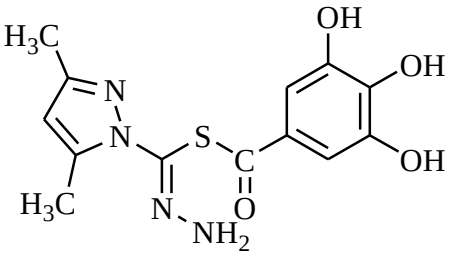
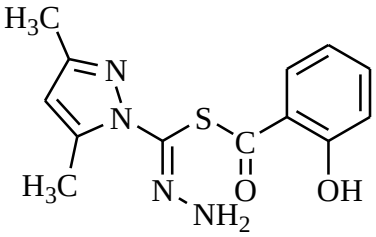
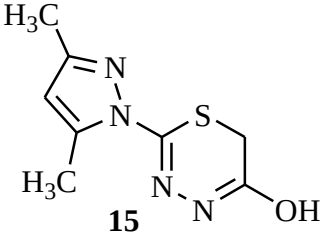
IR (KBr) νmax/cm^{-1}: 3434 (OH); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 6.21 (s, 1H, CH), 12.98 (s, 1H, OH); ^{13}C NMR (DMSO-d6): δ 13.56, 13.63, 39.87, 110.91, 142.44, 153.23, 161.67, 169.73; MS m/z (%) calculated $C_8H_{10}N_4SO$: 210.25, found: 210.15 (M^+, 19.20), 206.16 (30.89), 173.16 (50.38), 161.82 (100.00), 114.65 (68.51), 78.98 (67.59).

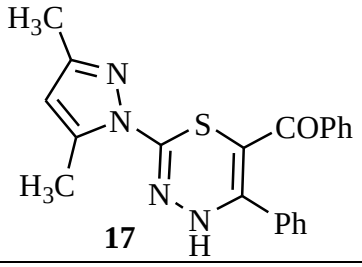
(2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-phenyl-4*H*-1,3,4-thiadiazin-6-yl)(phenyl)methanone (17)

IR (KBr) νmax/cm^{-1}: 3429 (NH), 1597 (C=O); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.55-8.17 (m, 10H, Ar-H and s, 1H, CH), 17.15 (s, 1H, NH); ^{13}C NMR (DMSO-d6): δ 93.83, 127.96, 129.03, 129.39, 133.55, 135.17, 185.88; MS m/z (%) calculated $C_{21}H_{18}N_4SO$: 374.46, found: 374.18 (M^+, 20.68), 342.73 (69.73), 263.95 (83.20), 132.99 (85.39), 112.07 (90.65), 79.62 (75.07).

Table 1: The chemically produced ingredients' physical traits

Chemical	Structure	% Yield	Physical State	Color	M.P.(ºC)
3	 **3**	**96**	**crystalline solid**	**creamy**	**100**
4	 **4**	**92**	**crystalline solid**	**creamy**	**90**
6	 **6**	**83**	**crystalline solid**	**Yellow**	**154**

9	 **9**	**87**	**crystalline solid**	**Yellow**	**158**
11	 **11**	**82**	**crystalline solid**	**White**	**98**
13a	 **13a**	**78**	**crystalline solid**	**Pale Yellow**	**168-170**
13b	 **13b**	**85**	**crystalline solid**	**Yellow**	**176**
13c	 **13c**	**81**	**crystalline solid**	**creamy**	**168**
15	 **15**	**89**	**crystalline solid**	**creamy**	**130**

17	 **17**	**93**	**crystalline solid**	**Yellow**	**98**

Cytotoxicity assay:

The tested human carcinoma cell lines; HepG2 (human lung carcinoma cell line), A549 cells (human lung carcinoma cell line), and MRC5 cells (human lung fibroblast normal cell line) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/mL gentamycin (Lonza, Belgium). The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week during the period of experiment.

For antitumor assays, the tumor cell lines were suspended in medium at cell density of $5x10^4$ cells/well in Corning® 96-well tissue culture plates and then incubated for 24 hours. The tested compounds were then added into 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 48 h, the numbers of viable cells were determined by the MTT assay [19]. Briefly, the media was removed from the 96-well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µL of the 12 mM MTT stock solution {5 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide purchased from Sigma-Aldrich (St. Louis, MO) in 1 mL of Phosphate buffered saline} to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc 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

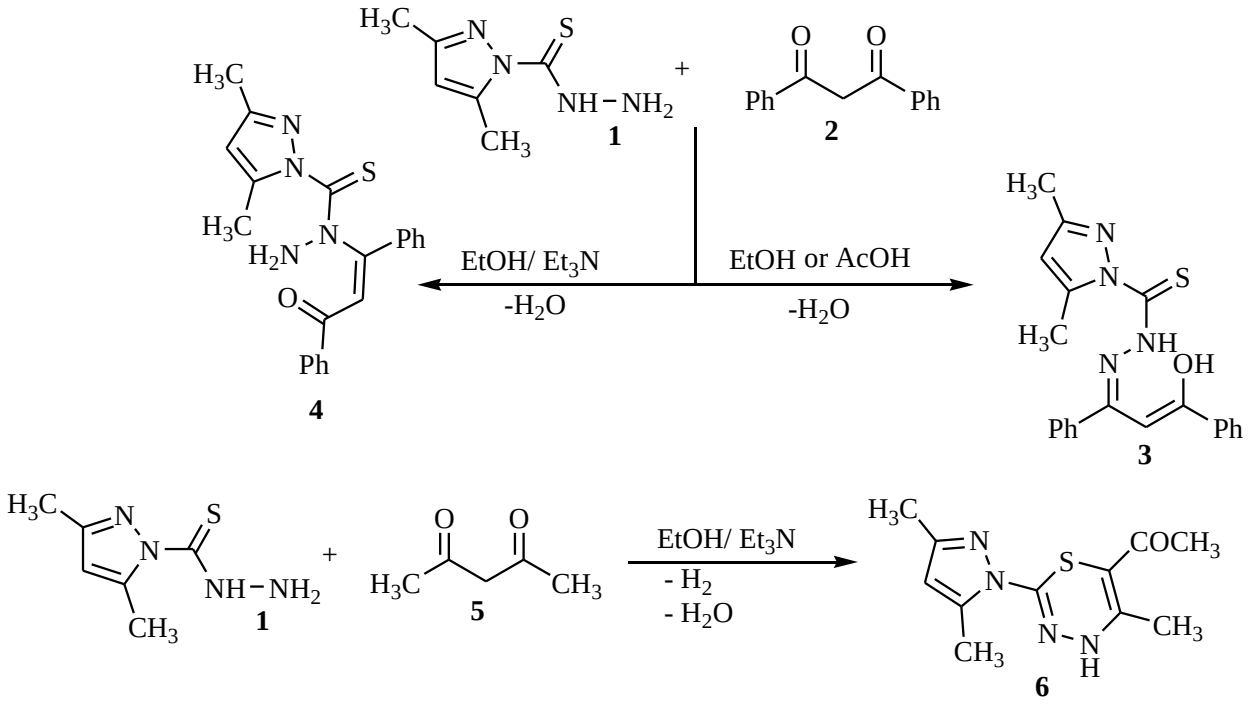
treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using GraphPad Prism software (San Diego, CA. USA)[20].

Safety and selectivity index (SI): The effects of the tested compounds and cisplatin reference drug were measured on normal human lung fibroblast (MRC-5) cell line (obtained from the American Type Culture Collection, ATCC, Rockville, MD) as mentioned previously to produce a dose–response curve and to calculate the 50% cytotoxic concentration (CC_{50}) using GraphPad Prism software. The SI was calculated by dividing the CC_{50} by the IC_{50} values [21].

3- Results and Discussion

(10*E*)-*N*'-((*Z*)-3-hydroxy-1,3-diphenylallylidene)-3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**3**) has been identified as the condensation product formed from the reaction of 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**) with 1,3-diphenylpropane-1,3-dione (**2**) in either 100% ethanol or glacial acetic acid under reflux, by discarding a molecule of water, as demonstrated in Scheme 1. The infrared absorption spectrum of chemical **3** established substantial bands at 3064 cm^{-1} and 3189 cm^{-1}, which correspond to the hydroxyl group and the NH group, respectively. Ingredient **3**'s ^{1}H NMR spectrum implied two singlet signals at δ 4.85 and 6.23 triggered by methine and pyrazole ring protons, respectively, an aromatic multiplets at δ 7.32–8.16 caused by phenyl hydrogens, two D_2O-exchangeable signals at δ 12.72 and 17.20 initiated by hydroxyl and amide hydrogens, respectively, as well as two singlet signals at δ 2.17 and 2.47 generated by two methyl groups. In addition, the compound's ^{13}C NMR analysis revealed, in addition to the hypothesized chemical shift, impulses generated by the sp^2 C=S carbon at δ 185.27, the sp^2 carbon of =C-OH at δ 152.38, the sp^2 methine carbon at δ 93.20, and a further two signals associated with two sp^3 methyl carbons at δ 12.75 and 13.05. Its mass assessment also pointed out the existence of a molecular ion peak at m/z 376.53 (14.64%). The process described above yields different outcomes when repeated with the addition of a catalytic proportion of triethylamine, resulting in the formation of 3,5-dimethyl-*N*-((*Z*)-3-oxo-1,3-diphenylprop-1-enyl)-1*H*-pyrazole-1-carbothiohydrazide (**4**) throughout the dissolution of a water molecule as exemplified by its spectral information, (Scheme 1). Composite **4**'s IR spectrum disclosed one absorbance band at 1591 cm^{-1}, indicating the presence of a carbonyl function, as well as two absorption bands at 3460 and 3209 cm^{-1}, characteristic of the NH_2 group. Additionally, its molar weight resulted in a segment at m/z 376.00 on its mass diagram, among others. Chemical **4**'s ^{1}H NMR spectra demonstrated a single D_2O-exchangeable signal at δ 17.14, which represented the NH_2 protons, alongside to the projected chemical shifts. According to its ^{13}C NMR studies, two resonances at δ 13.28 and 13.59 were observed from two sp^3 methyl carbons, while two signals at δ 185.85 and 196.05 were attributed to sp^2 C=S and carbonyl

carbon, respectively, Moreover, other signals were detected in the spectrum due to diverse SP^2 carbons, specifically one from the methine carbon at δ 93.81 and another from the SP^2 (CH) carbon in the pyrazole ring at 110.99, as depicted in the schematic representation of Scheme 1.

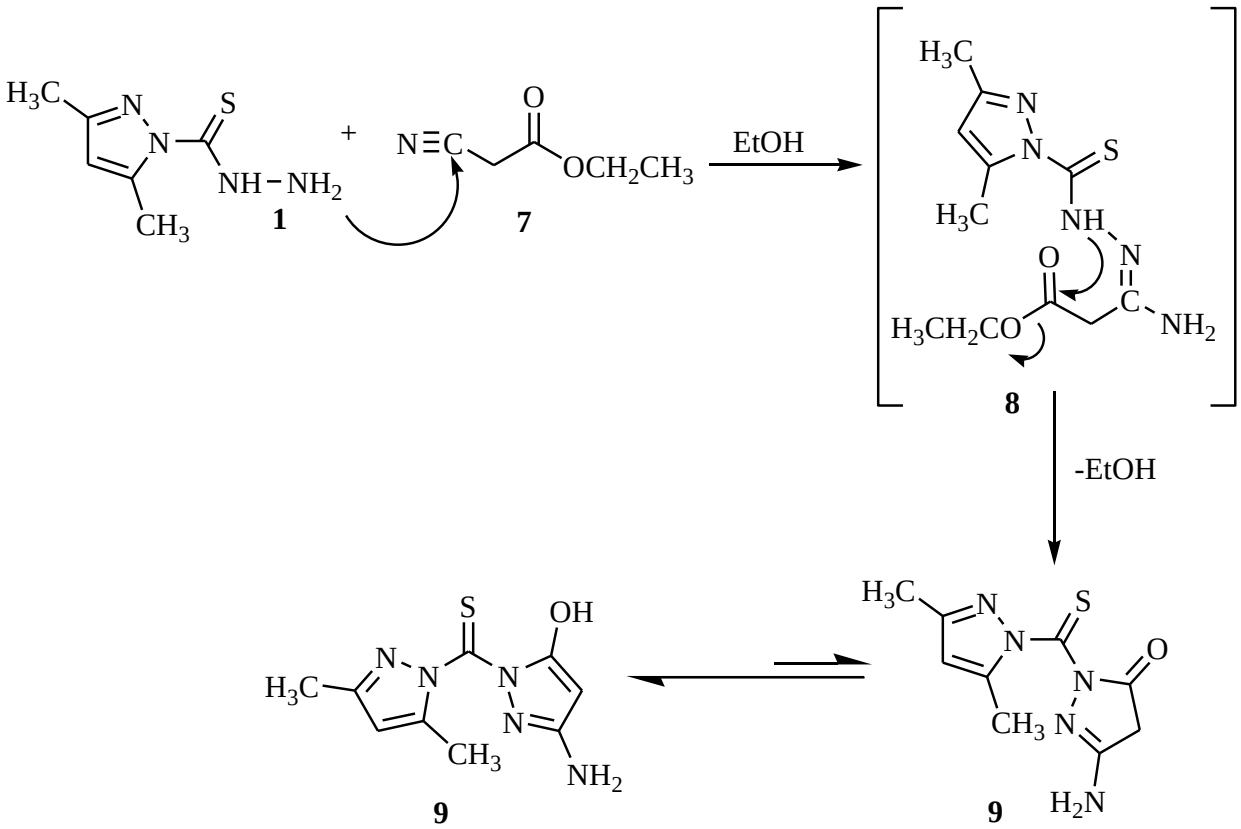


Scheme 1

When acetyl acetone **5** was incorporated into ethanol in the presence of a catalyst-containing quantity of triethylamine to treat 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**), the matching 1-(5-methyl-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,3,4-thiadiazin-6-yl)ethanone (**6**) resulted by getting rid of the hydrogen and water molecules as illustrated in Scheme 1's schematic explanation. Chemical **6**'s infrared spectrum indicated the matching notable absorbing bands at 3430 cm^{-1} and 1587 cm^{-1} because of its inclusion of amide and carbonyl groups. The proton in the pyrazole ring displayed a unique and clear signal at δ 6.21 in its ^1H NMR spectrum. Furthermore, the four methyl group protons produced four singlet signals at δ 2.17, 2.26, 2.45, and 2.59. The amide NH proton also contributed a signal at δ 14.33 that was exchangeable with D_2O. The ^{13}C NMR analysis revealed four sp^3 carbons of the methyl groups at δ 13.29, 13.63, 19.01 and five signals at δ 111.14, 136.73, 142.16, 153.07, 153.56, originating from five distinct sp^2 carbon atoms. Also, a signal at δ 186.08 was observed for a sp^2 carbonyl carbon, and another signal at δ 164.92 for a sp^2 C=N carbon in the thiadiazin ring. The mass analysis confirmed a peak at m/z 250.54 (17.45%), which coincided with its molecular ion.

When composite **1** and ethyl cyanoacetate **7** are combined and heated under reflux with ethanol, they undergo a reaction to produce (3-amino-5-hydroxy-1*H*-pyrazol-1-yl)(3,5-dimethyl-1*H*-

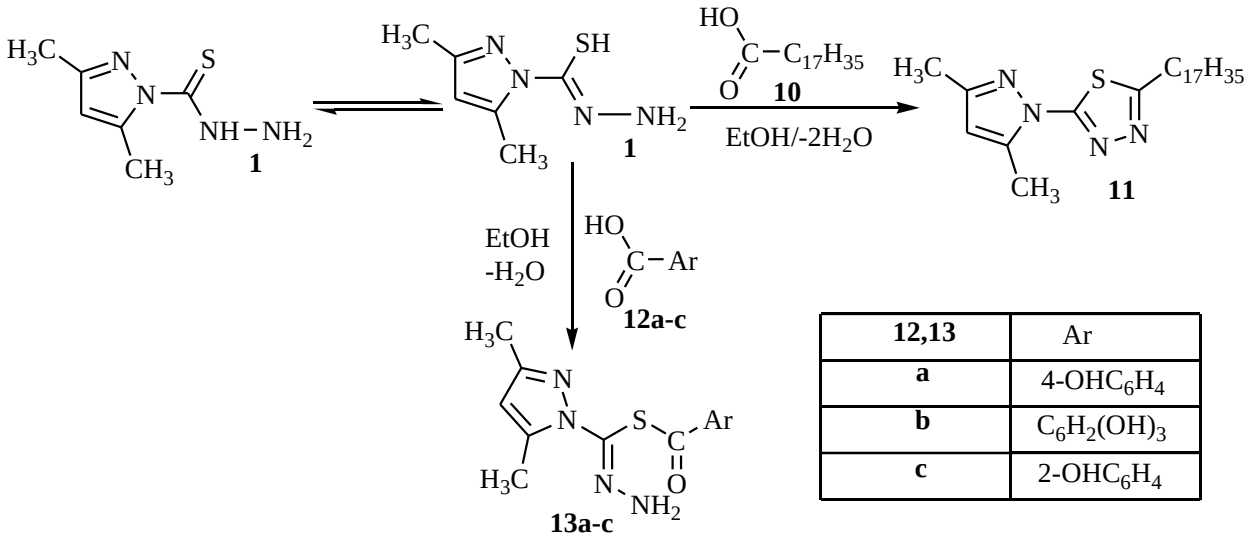
pyrazol-1-yl)methanethione (**9**) (Scheme 2). This reaction involves the addition of the NH_2 group of ingredient **1** to the nitrile group of chemical **7**, resulting in the creation of intermediate **8**. The removal of the ethanol molecule from **8** leads to the final product, composite **9**. The infrared spectra of **9** articulated an intriguing absorbance band at 3071 cm^{-1}, which is characteristic of a hydroxyl group, as well as two absorption bands at 3265 and 3207 cm^{-1}, indicating the existence of an amino function. Its mass plot showed a peak at m/z (%) 237.78 (15.56), which attributed to the molar mass of the substance. Two singlet signals were observed at δ 6.23 and 7.26, diagnostic for two C-H protons. Furthermore, two D_2O-exchangeable signals were visible at δ 5.30 and 12.71, indicating the presence of amino and hydroxyl groups in the ^1H NMR spectrum. Likewise, two singlet impulses at δ 2.17 and 2.59 were attributed to two methyl protons. The ^{13}C NMR analysis revealed two sp^3 carbons of the methyl groups at δ 13.28 and 13.60. Six signals were detected at δ 111.06, 142.12, 153.02, 154.80, 155.22, and 165.27, corresponding to six distinct sp^2 carbon atoms. Also, a signal for a sp^2 carbon of thioketone group was emerged at δ 185.96.



Scheme 2

During the reaction between 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**) and stearic acid (**10**), two water molecules were omitted, resulting in the establishing of 2-heptadecyl-5-(3,5-

dimethyl-1*H*-pyrazol-1-yl)-1,3,4-thiadiazole (**11**). However, when chemical **1** was treated with analogous carboxylic acids **12a–c**, such as 4-hydroxybenzoic acid, gallic acid, or salicylic acid, S-imino-hydroxybenzothioate derivatives **13a–c** were subsequently generated by cutting out just one water molecule, as Scheme 3 depicts. The products' infrared spectra in each scenario suggested the emergence of one band for the carbonyl function at about 1643 cm^{-1} and two separate NH_2 absorption zones between 3441 and 3207 cm^{-1}. Beyond that, the mass spectra of all the products laid out a peak that coincided to the molecular ion. Compound **13a** was selected as a representative sample from the series. Its ^1H NMR spectra revealed two peaks that were exchangeable with D_2O at δ 10.69 and 12.72, indicating the presence of NH_2 and OH protons, respectively, aside to the envisioned chemical shifts. Furthermore, the ^{13}C NMR plot of **13a** exhibited a signal at δ 185.97, which was identified as the SP^2 carbonyl carbon.



12,13	Ar
a	4-OHC_6H_4
b	$C_6H_2(OH)_3$
c	2-OHC_6H_4

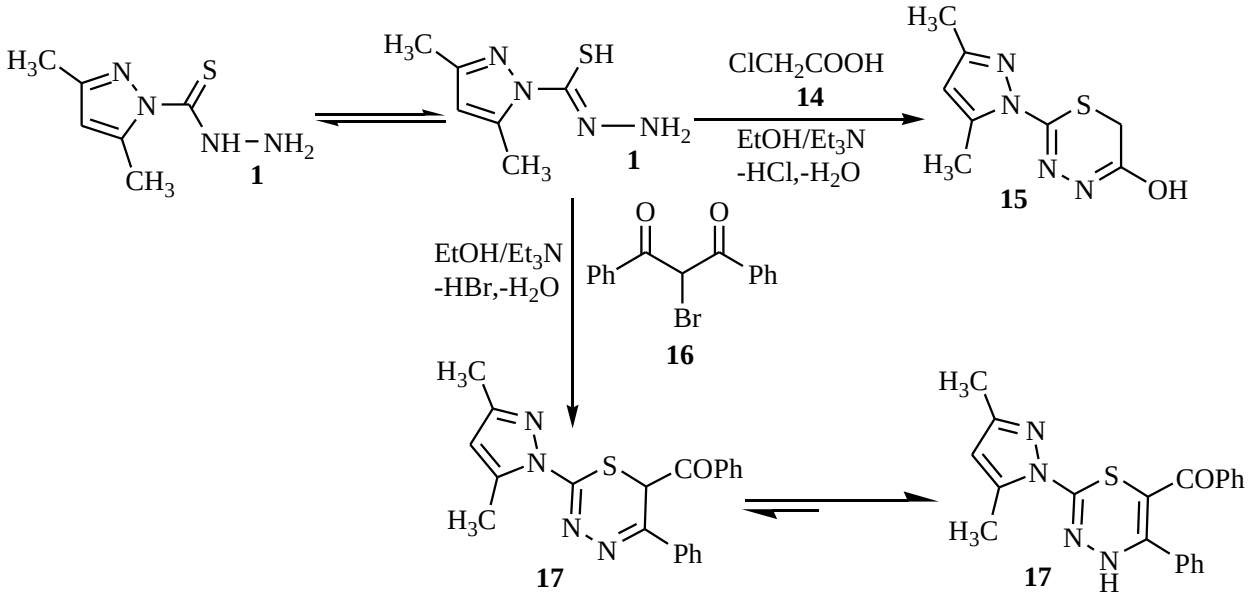
Scheme 3

Meanwhile, triethylamine has been introduced, allowing for the reaction between 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**) and α-haloketones such as, chloroacetic acid **14** or 2-bromo-1,3-diphenyl propane-1,3-dione (**16**). This leads to the creation of the relevant 1,3,4-thiadiazine derivatives, **15** and **17**, consequently , by discarding of the molecules of acid and water in each case.

Compound **15**'s infrared wavelength displayed a single absorbance band at 3434 cm^{-1}, indicating the presence of a hydroxyl group. While, the infrared wavelength of sample **17** revealed a carbonyl absorption band at 1597 cm^{-1} and another at 3429 cm^{-1}, which can be ascribed to the amide-NH group. In the ^1H NMR spectrum of composite **15**, a singlet impulse at δ 4.14 was

observed, diagnostic for the methylene hydrogens of the thiadiazin ring. Additionally, a D_2O-exchangeable signal at δ 12.98 was notable, associated with the OH hydrogen.

Whereas, compound **17** exhibits multiple oscillations at δ 7.55-8.17 caused by the aromatic hydrogens, as well as a D_2O-exchangeable endorse at δ 17.15 for the NH proton. Likewise, the ^1H NMR diagram for the two chemicals will indicate two singlet peaks at approximately δ 2.17 and 2.50, indicating the two methyl groups, and one singlet peak was detected at about δ 6.21, attributed to the C-H proton in the pyrazole ring. The ^{13}C-NMR chart for ingredient **15** revealed several signals. One signal was observed at δ 39.87, which can be diagnostic for a sp^3 methylene carbon. Additionally, two signals were observed at 161.67 and 169.73, which can be resulted from two sp^2 C=N carbons in the thiadiazin ring. Two more oscillations were observed at δ 13.56 and 13.63, which can be characteristic for two sp^3 methyl carbons. In addition to these, three responses were observed at δ 110.91, 142.44, and 153.23, that can be associated with three sp^2 carbons in the pyrazole ring. Despite this, the sp^2 C-H carbon of the pyrazole ring was responsible for a signal at δ 93.83 in the ^{13}C NMR image of sample **17**. Further, an emission at δ 185.88 was identified as the sp^2 carbonyl carbon. The aromatic sp^2 carbons were also visible at δ 127.96, 129.03, 129.39, 133.33, and 135.17. The explanation for the absence of some carbon emissions in ^{13}C NMR is that chemical **17** is not fully dissolved in DMSO-d6. Moreover, in both cases, each molecular ion exhibited a distinct emission peak on the mass chart.



Scheme 4

Biological activities of the prepared compounds:

Cytotoxic Activity

The *in vitro* growth inhibitory activity of the synthesized compounds was investigated in comparison with the well-known anticancer standard drug (cisplatin) under the same conditions using colorimetric MTT assay. Data generated were used to plot a dose response curve (Figure 1). Generally, the results revealed that all the tested compounds showed variation in inhibitory activity to the liver and lung carcinoma cell line in a concentration dependent manner. Also, the concentration of test compounds required to kill 50% of cell population was determined as the mean IC_{50} of three independent experiments (Table 2). Interestingly, the results presented in Table 2 and Figure 1A showed that compound **17** was the most active against the liver and lung carcinoma cell lines giving potent IC_{50} value of 5.35 and 8.74 μM, respectively, compared with reference drug cisplatin (3.78 and 6.39 μM). The order of activity against the hepatocellular (HepG2) carcinoma cell line was as follows: **17, 15, 13c, 3, 9, 4, 13a, 13b, 6**, and **11**. Moreover, **17** showed the highest effect on lung (A549) carcinoma cells followed by **15, 3, 9, 13c, 13a, 4, 13b, 11,** and **6**, respectively. The effects of the tested compounds and cisplatin reference drug were also measured on normal human lung fibroblast (MRC-5) cell line to calculate the 50% cytotoxic concentration (CC_{50}) as indicated in Table 2. The selectivity index (SI) was calculated by dividing the CC_{50} by the IC_{50} values. Our results indicated that compound **17** exhibited good selectivity index values.

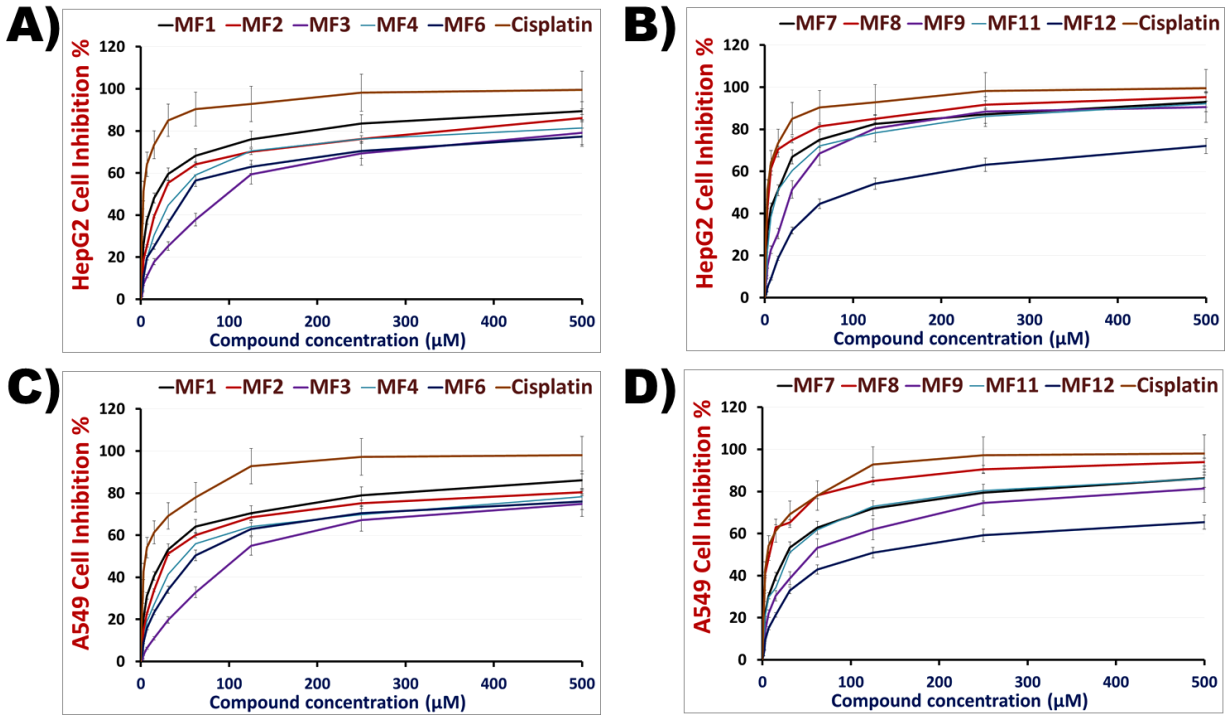


Figure 1. *In vitro* inhibitory activity of the synthesized compounds against the liver and lung carcinoma cells after 48 h treatment evaluated using MTT assay. **A** & **B**) presented in dose response curve against liver carcinoma (HepG2) cell line compared with cisplatin reference drug; **C** & **D**) presented in dose response curve against lung carcinoma (A549) cell line compared with cisplatin reference drug.
MF1 = compound **3**, **MF2** = compound **9**, **MF3** = Compound **11**, **MF4** = Compound **13a**, **MF6** = compound **13b**, **MF7** = Compound **15**, **MF8** = Compound **17**, **MF9** = Compound **4**, **MF11** = Compound **13c**, **MF12** = Compound **6**.

Table 2. The inhibitory activities of the tested compounds against liver and lung carcinoma cell lines (expressed as IC_{50} values) showing their efficiency and selectivity indices:

Compound	HepG2 (Liver Carcinoma)	A549 (Lung Carcinoma)	MRC5 (Normal Lung Fibroblast)	SI values (CC_{50}/ IC_{50})	
	IC_{50} values (µM)*	IC_{50} values (µM)*	CC_{50} values (µM)*	HepG2	A549
3	18.18±1.68	27.78± 2.04	41.62±3.14	2.29	1.49

9	25.97±1.51	30.05±2.31	36.96±2.42	1.42	1.23
11	97.79±4.03	111.09±4.17	124.28±6.71	1.27	1.12
13a	42.77±2.41	49.64±3.02	80.49±3.27	1.88	1.62
13b	52.65±3.09	61.58±3.46	92.31±4.18	1.75	1.50
15	14.67±1.21	27.35±1.97	32.03±3.19	2.18	1.17
17	5.35±0.71	8.74±0.82	21.34±0.98	3.99	2.44
4	30.18±1.96	55.67±2.75	62.74±3.62	2.08	1.13
13c	14.86±0.82	30.25±2.01	38.97±3.21	2.62	1.29
6	97.64±3.92	117.62±4.16	132.91±5.77	1.36	1.13
Cisplatin	3.78± 0.34	6.39 ± 0.73	35.58 ± 3.06	9.41	5.57

*The data are expressed in the form of mean ± standard error

4- Conclusions

The outcomes of the current investigation recommend the following inference:
Utilizing 3,5-dimethyl-1*H*-pyrazole-1-carbothiohydrazide (**1**) as a predecessor, new heterocyclic ingredients incorporating a pyrazole moiety were synthesized. These include pyrazole derivatives **3** and **4**, as well as (4*H*-1,3,4-thiadiazin-6-yl)ethanone derivative (**6**), 3,5-dimethyl-1*H*-pyrazol-1-yl)methanethione (**9**), 1,3,4-thiadiazole derivative **11**, and S-imino-hydroxybenzothioate derivatives **13a-c**. Furthermore, chemical **1** have provided access to corresponding 1,3,4-thiadiazine derivatives, **15** and **17**, through the combination of α-haloketones such as chloroacetic acid **14** or 2-bromo-1,3-diphenylpropane-1,3-dione (**16**). The chemical structures of these innovative substances have been thoroughly characterized using contemporary spectroscopic techniques. Selected synthesized ingredients were tested for their cytotoxic effectiveness against two carcinoma cell lines, HepG2 and A549. The results showed that compound **17** was the most active against both liver and lung carcinoma cell lines, with potent IC_{50} values of 5.35 and 8.74 μM, respectively. This was compared to the reference drug cisplatin, which had IC_{50} values of 3.78 and 6.39 μM for the same cell lines. Interestingly, when tested for toxicity against normal lung fibroblast (MRC-5) cells, compound **17** exhibited good

selectivity, indicating its potential for safe use. However, additional studies may be necessary to demonstrate the impact of synthesized chemicals as anti-cancer medications on other body organs.

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