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Synthesis and Biological Evaluation of new 1,2,4-triazinones functionalized with mono-substituted pyrazole heterocycles

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Abstract

A new series of functionalized 1,2,4-triazinones (**11a-g**) were synthesized *via* 1,3-dipolar cycloaddition reactions of L-α-amino esters (**1a-g**) with N-(5-methyl-1H-pyrazol-3-yl) nitrile amine, generated in *situ* from the reaction of N-(5-methyl-1H-pyrazol-3-yl)-2-oxopropanehydrazonyl chloride (**9**) and triethyl amine. The structures of the newly synthesized compounds were confirmed by spectroscopic methods (HRMS, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR). Furthermore, their antibacterial, antioxidant and antitumor activity were evaluated. The results clearly showed that compounds **11a**,**11d** and **11e** acted as highly active antioxidants; while on the other hand, all synthesized compounds exhibited a weak antitumor and antibacterial activity.

***Keywords*:** 5-methylpyrazol-3-amine, N-(5-methylpyrazol-3-yl) hydrazonoyl chloride, nitrile amines, pyrazolo[1,2-d][1,2,4]triazin-1-one, 1,2,4-triazin-6-one.

1. Introduction

Triazinones derivatives are reported in the literature as very important heterocyclic compounds due to their potential promising biological activities, such as herbicidal [1], anticancer [2], anti-HIV [3], anti-inflammatory [4], antibacterial [5], fungicidal [6], insecticidal [7], antimicrobials [8], and play a crucial role in several marketed drugs [9]. For example, the triazinones **1-3** (Fig 1) exhibit good cytotoxicity against breast cancer [10]; the imidazotriazinone **4** is used to treatment of cGMP disease [11] and the 1,2,4-triazino triazaphosphinine **5** presents good antibacterial activity [12]. Moreover, some triazinone components can also be found in natural products, therapeutics and drugs. Some examples are pymetrozine, as an insecticide against sucking insect pests [13], or 6-aza-5-methyl-20-deoxyisocytidine shown to adjust the stability of DNA [14].

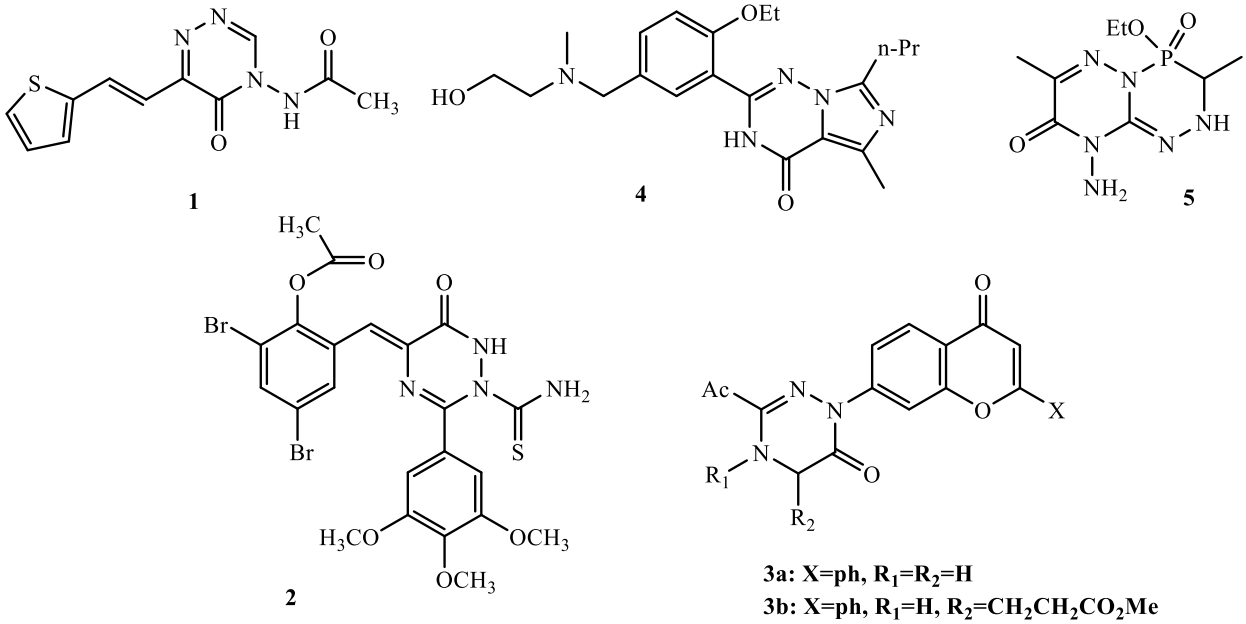


Figure 1. Examples of biological active **1,2,4**-Triazinone containing compounds.

On the other hand; pyrazole-containing compounds remarkably show significant attention in the literature due to their potential applications in chemotherapy. Some pyrazoles act as antileukemic derivatives [15], antitumor agents [16] and anti-inflammatory drugs [17]. For example, **celecoxib 6** shows anti-inflammatory effects and inhibits COX-2, **fomepizole 7** prevents alcohol dehydrogenase; and **sildenafil 8** restrains phosphodiesterase [18] (Fig 2). Also some of the pyrazole compounds play a significant role in material chemistry [19].

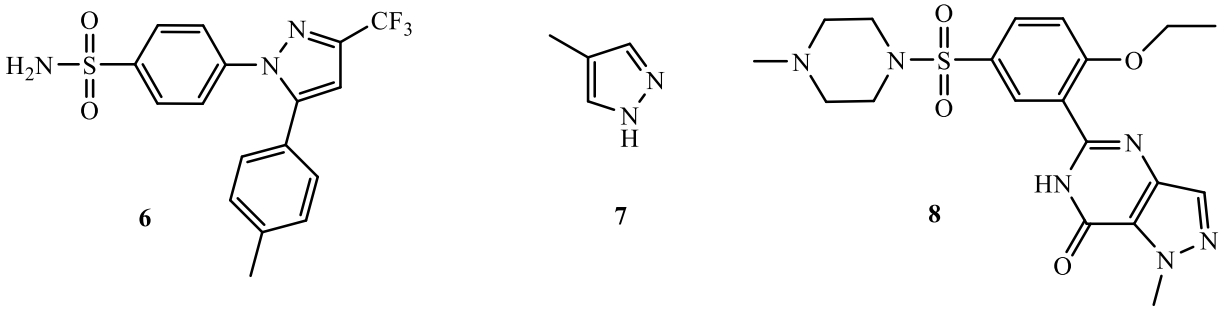


Figure 2: Examples of pyrazole containing drug molecules.

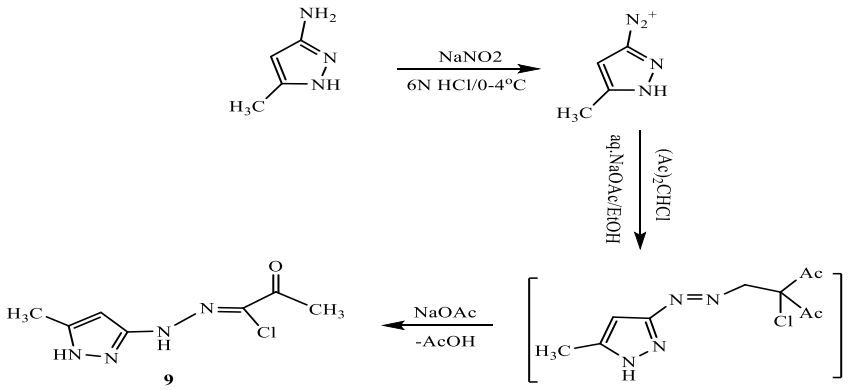
1,2,4-triazinones can be synthesized *via* different common methods, such as cyclization of α-acylamine carboxyhydrazides [20], reaction of nitrileamines with α-aminoacetonitrile [21] and cyclocondensation of nitrileamines with 2-hydrazinoacetate or α-aminoesters [22-24]. However, cyclocondensation represents the most efficient synthetic

method and therefore, is the one generally followed. Herein; we investigate the synthesis (*via* a 1,3-dipolar cyclocondensation reaction with various nitrilimines with respective α-aminoesters) and the spectroscopic data for new 1-(pyrazol-3-yl)-1,2,4-triazin-6-one (**11a-g**) compounds, together with their study for *in vitro* cytotoxicity, antibacterial and antioxidant activities.

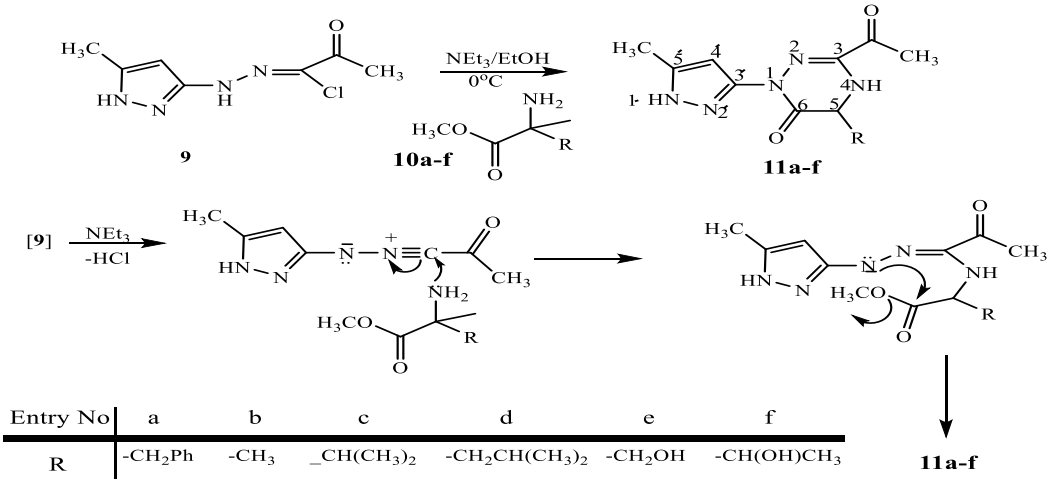
2 Results and discussion

2.1 Synthesis

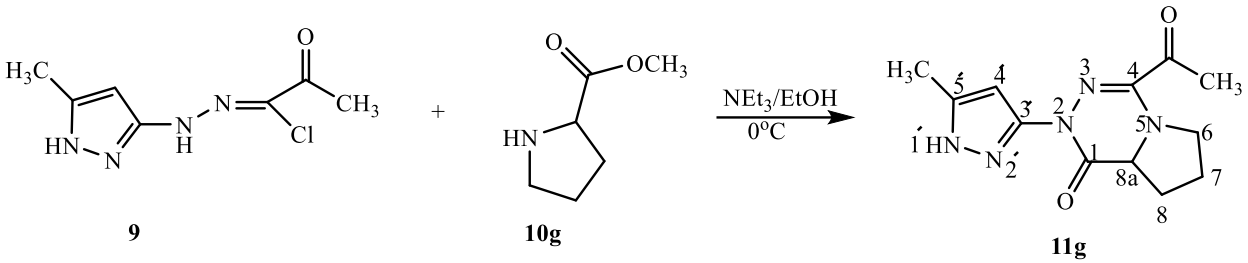
Compounds **11a–g** were prepared as described in the literature for chemically related compounds (Schemes 1-3) [25]. First, the *N*1-(5-methylpyrazol-3-yl) hydrazonoyl chloride **9** was synthesized *via* Japp-Klingemann reaction [26], by direct coupling of 5-methylpyrazol-3-diazonium chloride with 3-chloropentane-2,4-dione in ethanolic solution in the presence of sodium acetate buffer solution (Scheme 1). The intermediate 5-methylpyrazol-3-diazonium chloride solution was prepared freshly *via* diazotization of 5-methyl-3-aminopyrazole. In a second step, the corresponding hydrazonoyl chloride (**9**) yields 1,3-dipolar species (**A**) in the presence of triethyl amine as a base. These species react with the nitrogen nucleophiles L-α-Amino esters (**10a-f**), resulting in the formation of the intermediate open–chain amidrazone esters (B), which themselves undergo spontaneous intramolecular condensation (involving the amidrazone nitrogen and the ester carbonyl group) to yield the respective targeted heterocycles (**11a-f**) (Scheme 2). In a similar fashion, proline methyl ester reacted with (**9**) to give the corresponding bicyclic triazinone (**11g**) (Scheme 3). The spectroscopic and characteristic data of compounds 11a-g are given in detail in the experimental part.



Scheme 1. Preparation of [1-(5-methyl)-1H-pyrazol-3-yl) hydrazono]-1-chloropropane-2-one (**9**)



Scheme 2. Synthesis of 1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6-one (**11a-f**)



9 **10g** **11g**

Scheme 3. Synthesis of 4-acetyl-2-(5-methyl-1H-pyrazol-3-yl)-6,7,8,8a-tetrahydropyrrolo[1,2-d][1,2,4]triazin-1(2H)-one (**11g**).

2.2 Spectral data analysis for compounds 11a–g

All new compounds (**11a-g)** were characterized and confirmed based on the spectroscopic data detailed in the experimental part. A satisfactory analysis for the proposed structural formula of the target compounds was achieved by combination of the information obtained from the different used techniques. High resolution mass spectroscopy (HRMS) of 1,2,4-triazin-6(1H)-one (**11a-g**) displayed the exact mass of the suggested structures. The ^{1}H-NMR spectra for the synthesized compounds (**11a-g**) display a singlet signal assigned to the pyrazole ring (H-4') in the range 6.01-6.26 ppm. Also, a singlet signal characteristic of the NH-4 of the triazinone ring was observed in the range 5.82-5.94 ppm for compounds **11a-d** compounds, while 7.32 and 7.20 ppm were found in the case of **11e** and **11f**, respectively, due the presence of the hydrogen bond of the OH group attached to the substituent at position 5. 2D-NMR (COSY, HMQC, HMBC) and DEPT –NMR experiments display some correlations between ^{1}H and ^{13}C that can have helped to assign the different types of carbon atoms attached or neighbouring hydrogens. For example, the COSY spectra for compound **11c**, as a representative of the new compounds **11a-g**, showed a weak correlation between NH-4 (5.92 ppm) and CH-5 (4.06 ppm). Also, according to the HMBC spectra for **11c**, many correlations between the methine proton at C-5 and each of C-3, C-6 and *i*-propyl carbon atoms were detected (Table 1).

Table 1. *NMR spectroscopic data (500 MHz, $CDCl_3$) for compound* ***11c***

3-acetyl-5-isopropyl-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4 -triazin-6(1H)-one(**ST-V**)(**11c**)

Position	**δ_c ppm, type**	**δ_H ppm, type**	**HMBC**
1	-	-	-
2	-	-	-
3	142.0	-	-
4	-	5.92	C-5, C-6
5	58.8	4.06	*i*-propyl carbons, C-3, C-6
6	161.0	-	-
1’	-	-	-
2’	-	-	-
3’	145.8	-	-
4’	97.4	6.26	C- 3′, C- 5′
5’	143.6	-	-

2.3 Antitumor activity:

The antitumor activity of compounds **11a-g** was evaluated by measuring their effect on the proliferation of monolayer (A549 and MCF-7) and suspension (K562) cell lines. All tested compounds were not cytotoxic; they caused ≤ 20% maximum inhibition in cell line proliferation at 50 µg/mL (Table 2).

Table 2. *Percentage of cell survival of MCF-7, A549 and K562 following 72 h exposure to 50 µg/mL of* ***11a-g****.*

Compound	**A549**	**MCF-7**	**K562**
	% Survival ± SD		
11a	89.30% ± 0.75	80.79% ± 5.36	88.55% ± 3.95
11b	93.21% ± 2.82	86.26% ± 0.75	88.56% ± 2.80
11c	93.68% ± 3.49	91.31% ± 3.39	88.79% ± 2.39
11d	98.43% ± 0.01	94.18% ± 3.74	97.07% ± 3.20
11e	88.36% ± 0.31	96.00% ± 3.02	82.98% ± 1.68
11f	88.95% ± 3.65	95.32% ± 1.70	83.11% ± 5.48
11g	95.78% ± 3.07	85.66% ± 3.68	88.76% ± 0.99
5-Fluorouracil	3.35% ± 0.55	2.48% ± 0.34	0.69% ± 0.16

2.4 Antioxidant activity

Table 3. *Antioxidant activity of the 5-methyl-(1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazinone (****11a-g****) compounds in DPPH and ABTS assays.*

Sample NO.		**% inhibition ± SD µg/ml**			**IC_{50} ± SD µg/ml**
In DPPH		**50**	**100**	**300**	
	11a	43.65 ± 0.49 [a]	58.33 ± 1.03	72.84 ± 0.49	67.73 ± 0.68
	11b	31.40 ± 0.92	48.15 ± 1.01	66.44 ± 1.03	125.12 ± 1.11
	11c	17.79 ± 1.03	33.11 ± 0.68	58.78 ± 0.68	206.88 ± 5.18
	11d	33.11 ± 0.68	53.06 ± 0.41	81.53 ± 2.67	89.83 ± 0.87
	11e	45.54 ± 0.72	71.76 ± 0.49	84.91 ± 1.03	50.37 ± 0.22
	11f	29.50 ± 1.40	45.09 ± 0.74	54.73 ± 2.44	188.32 ± 8.14
	11g	27.70 ± 0.68	44.10 ± 0.43	69.14 ± 1.03	130.63 ± 2.56
	Trolox®				5.60 ± 0.30
In ABTS					
	11a	47.97 ± 0.46	60.68 ± 1.06	76.46 ± 0.67	54.40 ± 0.64
	11b	36.37 ± 0.32	54.81 ± 1.04	74.35 ± 0.32	89.30 ± 0.91
	11c	23.16 ± 0.77	38.19 ± 0.19	63.21 ± 0.48	170.51 ± 2.06
	11d	41.86 ± 1.14	59.49 ± 1.27	72.53 ± 0.46	79.99 ± 1.37
	11e	49.03 ± 0.81	71.60 ± 0.65	86.16 ± 1.14	46.31 ± 0.59
	11f	33.33 ± 1.93	41.10 ± 1.90	56.79 ± 0.70	183.69 ± 6.30
	11g	25.36 ± 0.19	42.32 ± 0.65	74.98 ± 0.32	125.11 ± 1.06
	Trolox®				3.90 ± 0.15

[a] Data represent the means ± standard deviation (SD)

The newly synthesized compounds (**11a-g**) were evaluated for their ability to scavenge ABTS and DPPH radicals as indications of their antioxidant potential. Their activity was categorized into weak antioxidants (**11c**, **11f**; IC_{50} value >150 µg/mL), moderate antioxidants (**11b**, **11g**; IC_{50} value 100-150 µg/mL) and highly antioxidant (**11a**,**11d** and **11e**; IC_{50} value 50-100 µg/mL) (Table **3**). The apparent non-significant difference between IC_{50} values of each compound in ABTS and DPPH assays might be due to the fact that both tests detect antioxidant activities of hydrophobic compounds.

2.5 Antibacterial Activity

The evaluation of antibacterial activity of the tested compounds revealed that none of them had caused inhibition in the growth (formation of inhibition zones) of the bacterial test strains up to the highest applied concentration (500 µg/disc).

3. Experimental

3.1 Chemicals and Apparatus

Stuart scientific melting point apparatus was used to determine the melting point. Bruker Avance-III spectrometer was used to analyze the ^{1}H- and ^{13}C-NMR spectra with $CDCl_3$ or DMSO-d_6 as solvents; the results are given in ppm (δ)-values relative to *TMS* as an internal standard; coupling constant (*J*) values are given in Hertz. The electrospray ionization technique was used in the observation of High-resolution mass spectra (HRMS) by Bruker APEX–IV. The prepared material was liquified in acetonitrile, in the spray solution containing (methanol/water 1:1 v/v + 0.1٪ formic acid) and permeated at the flow rate of 2 μL /min by a syringe pump. The standardization was carried out by Arginine cluster ranging in the mass of *m/z* 175-871. The colorless spots were observed by Ultra Violet Fluorescence Analysis Cabinet. The chemicals used for the experiment process were obtained from commercial sources without any refinement. (L)-Aminomethyl esters hydrochloride of (alanine, leucine, proline, Threonine, serine and valine (phenylalanine from Fluka)), 3-amino-5-methylpyrazole and 3-chloropentane-2,4-dione were acquired from Acros Organics (USA). Dry Trimethylamine and Ethanol were purchased from GCC (UK). Silica gel for column chromatography (70-230 mesh ASTM) was bought from Scharlau (Spain).

3.2 General Procedure for the Syntheses of 1,2,4-triazinone Compounds (11a-g)

The compound was stirred and suspended at 0 °C (**9)** (0.24 g, 1.2 mmol) **in** 20 mL of ethanol, the appropriate L-(α)-amino acid methyl ester hydrochloride (1.4 mmol) and triethylamine (2-3 mL) in 10 mL of ethanol were added to it. The reaction was kept at 0 °C for 5-6 h. Then, the solution was poured onto water (30 mL). The resulting mixture was removed with ethyl acetate (30 mL x2), parched over anhydrous $MgSO_4$ and evaporated in the vacuum for obtaining a brown deposit. The purification process was done using column chromatography in silica gel with chloroform/ethyl acetate (1:3, v/v). This methodology was followed for all compounds except for **11e** and **11f**, as they produced a white precipitate after stirring is completed (5 hours). In these cases, the precipitate obtained was strained, washed in cold water and then dried.

3-acetyl-5-benzyl-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one (11a)

Light yellow solid, yield = 0.19 g (51.4 %), mp = 159-161°C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.30 (s, 3H, C*H*$_3$(C-5')), 2.37 (s, 3H, C*H*$_3$(CO)), 2.95 and 3.19 (m, 2H, -C*H*$_2$(benzylic)), 4.37 (m, 1H, H-5), 5.82 (s,1H, N*H*-4), 6.23 (s, 1H, H-4'), 7.11 (d, *J* = 6.9 Hz, 2H, H-*ortho*), 7.28 (m, 3H, H-*meta* +*para*). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.2 (*C*H$_3$ (C-5')), 23.8 (*C*H$_3$(CO)), 39.9 (*C*H$_2$ (CH-5)), 54.9 (C-5), 97.6 (C-4'), 127.5 (C-*para*), 128.8 (C-5'), 129.0 (C-*ortho*), 129.2 (C-

3'), 129.5 (C-*meta*), 135.0 (C-*benzyl attached*), 141.7 (C-3), 160.9 (C-6), 192.6 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{16}H_{18}N_5O_2$ [M + H] $^+$ 312.14550; found 312.14705, Calcd for $C_{16}H_{17}NaN_5O_2$ [M + Na]$^+$ 334.12745; found 334.12881.

3-acetyl-5-methyl-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one (11b)

Light brown solid, yield = 0.21 g (75 %), mp = 206-208ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.48 (d, *J* = 6.3 Hz, 3H, C*H*$_3$(CH-5)), 2.29 (s, 3H, C*H*$_3$(C-5')), 2.49 (s, 3H, C*H*$_3$(CO)), 4.24 (q, *J* = 6.3 Hz, 1H, H-5), 5.86 (s,1H, N*H*-4), 6.26 (s, 1H, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.3 (*C*H$_3$ (C-5')), 19.6 (*C*H$_3$ (CH-5)), 23.9 (*C*H$_3$(CO)), 49.2 (C-5), 97.5 (C-4'), 142.1 (C-3), 143.5 (C-5'), 146.0 (C-3'), 162.1 (C-6), 192.9 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{10}H_{13}N_5NaO_2$ [M + Na]$^+$ 258.09615; found 258.09565.

3-acetyl-5-isopropyl-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one (11c)

Light brown solid, yield = 0.27 g (86 %), mp = 145-147ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.87 and 0.99 (d, d, *J* = 7.1 and 6.8 Hz, 6H, (C*H*$_3$)$_2$CH), 2.29 (s, 3H, C*H*$_3$(C-5')), 2.32 (m, 1H, C*H*(CH-5)), 2.49 (s, 3H, C*H*$_3$(CO)), 4.06 (d, *J* = 1.9 Hz, 1H, H-5), 5.92 (s,1H, N*H*-4), 6.26 (s, 1H, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.4 (*C*H$_3$ (C-5')), 18.3 and 16.6 ((C*H*$_3$)$_2$CH), 23.9 (*C*H$_3$(CO)), 32.6 (*C*H(CH-5)), 58.8 (C-5), 97.4 (C-4'), 142.0 (C-3), 143.6 (C-5'), 145.8 (C-3'), 161.0 (C-6), 192.8 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{12}H_{18}N_5O_2$ [M + H]$^+$ 264.14550; found 264.14463.

3-acetyl-5-isobutyl-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one(11d)

Yellow solid, yield = 0.20 g (61%), mp = 135-137ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.92 and 0.94 (d, d, *J* = 6.0 and 6.0 Hz, 6H, (C*H*$_3$)$_2$CH), 1.59 (m, 1H, C*H*(CH$_3$)$_2$), 1.72 (m, 2H, C*H*$_2$(CH-5)), 2.28 (s, 3H, C*H*$_3$(C-5')), 2.48 (s, 3H, C*H*$_3$(CO)), 4.16 (dd, *J* = 8.6 Hz, 1H, H-5), 5.94 (s,1H, N*H*-4), 6.24 (s, 1H, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.3 (*C*H$_3$ (C-5')), 21.6 and 23.0 ((C*H*$_3$)$_2$CH), 23.9 (*C*H$_3$(CO)), 24.0 (CH(CH$_3$)$_2$), 42.0 (*C*H$_2$(CH-5)), 51.8 (C-5), 97.4 (C-4'), 141.9 (C-3), 143.5 (C-5'), 145.9 (C-3'), 161.8 (C-6), 192.9 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{13}H_{20}N_5O_2$ [M + H]$^+$ 278.16115; found 278.16147, Calcd for $C_{13}H_{19}NaN_5O_2$ [M + Na]$^+$ 300.14310; found 300.14342 and Calcd for $C_{26}H_{38}NaN_{10}O_4$ [2M + Na]$^+$ 577.29751; found 577.29267.

3-acetyl-5-(hydroxymethyl)-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one (11e)

White solid, yield = 0.21 g (75 %), mp = 243-245ºC. ^{1}H-NMR (500 MHz, DMSO-d$_6$): 2.18 (s, 3H, C*H*$_3$(C-5')), 2.46 (s, 3H, C*H*$_3$(CO)), 3.51-3.71 (m, 2H, C*H*$_2$-5), 4.03 (s, 1H, H-5), 5.03 (s, 1H, OH), 7.32 (br.s, 1H, N*H*-4), 6.03 (s, 1H, H-4'). ^{13}C-NMR (125 MHz, DMSO-d$_6$): δ 11.3 (*C*H$_3$ (C-5')), 24.5 (*C*H$_3$(CO)), 56.3 (C-5), 63.4 (*C*H$_2$(OH)), 100.2 (C-4'), 139.2 (C-5'), 142.7 (C-3), 148.0 (C-3'), 161.3 (C-6), 193.3 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{10}H_{14}N_5O_2$ [M + H]$^+$ 252.10912; found 252.10907, Calcd for $C_{10}H_{13}NaN_5O_3$ [M + Na]$^+$ 274.09106; found 274.09109

3-acetyl-5-(1-hydroxyethyl)-1-(5-methyl-1H-pyrazol-3-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-one (11f)

White solid, yield = 0.15 g (48 %), mp = 245-247ºC. ^{1}H-NMR (500 MHz, DMSO-d$_6$): 1.04 (d, *J* = 6.5 Hz, 3H, C*H*$_3$-CH), 2.22 (s, 3H, C*H*$_3$(C-5')), 2.29 (s, 3H, C*H*$_3$(CO)), 3.79 (br s, 1H, H-5), 3.90 (m, 1H, C*H*-5), 4.84 (d, *J* = 6.0 Hz, 1H, OH), 6.01 (s, 1H, H-4'), 7.20 (br. s,1H, N*H*-4). ^{13}C-NMR (125 MHz, DMSO-d$_6$): δ 11.1 (*C*H$_3$ (C-5')), 19.7 (HC(OH)*C*H$_3$), 24.5 (*C*H$_3$(CO)), 59.2 (C-5), 68.2 (*C*H(OH)), 100.2 (C-4'), 139.1 (C-5'), 142.7 (C-3), 148.9 (C-3'), 161.7 (C-6), 193.3 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{11}H_{16}N_5O_3$ [M + H]$^+$ 266.12477; found 266.12641, Calcd for $C_{11}H_{15}N_5NaO_3$ [M + Na]$^+$ 288.10671; found 288.10778.

4-acetyl-2-(5-methyl-1H-pyrazol-3-yl)-6,7,8,8a-tetrahydropyrrolo[1,2-d][1,2,4]triazin-1(2H)-one (11g)

Light yellow solid, yield = 0.19 g (61%), mp = 202-204ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.92 (m, 2H, H-7), 2.27 (m, 2H, H-8), 2.29 (s, 3H, C*H*$_3$(C-5')), 2.49 (s, 3H, C*H*$_3$(CO)), 3.77 and 3.93 (m, 2H, H-6), 3.97 (t, *J* = 7.6 Hz, 1H, H-8a), 6.23 (s, 1H, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.5 (*C*H$_3$ (C-5')), 24.2 (C-7), 26.3 (*C*H$_3$(CO)), 27.5 (C-8), 50.1 (C-6), 57.6 (C-8a), 97.0 (C-4'), 143.9 (C-4), 144.0 (C-5'), 145.5 (C-3'), 161.2 (C-1), 194.4 (*C*OMe). HRMS (ESI) *m/z*: Calcd for $C_{12}H_{16}N_5O_2$ $[M + H]^+$ 262.12985; appeared 262.13105, Calcd for $C_{12}H_{15}NaN_5O_2$ $[M + Na]^+$ 284.11180; appeared 284.11282.

3.3 Antitumor activity:

The ability of the newly synthesized compounds to inhibit proliferation of the monolayer lung carcinoma A549 and the breast adencarcinoma MCF-7 cell lines, as well as the suspension myeloid leukemia K562 cell line, was evaluated as an indication of their antitumor activity. Cells were cultivated in RPMI 1640 media comprising 10% fetal bovine serum and 100 µg/mL penicillin-streptomycin solution with incubation in a humidified atmosphere at 37ºC and 5% CO_2. Tested cell lines were placed in 96 well plates with a cell density of 10^3 cells/well in a medium containing 20 µM of each tested compound. They were monitored every 24 h microscopically for any morphological changes till the end of culturing time (4 days). The cells' viability was measured using the Sulforhodamine B (SRB) method following a protocol described elsewhere with minor modifications [27]. Briefly, cells were fixed by adding 10% (w/v) final concentration of ice-cold Trichloroacetic acid solution (TCA) for 60 min, washed in the water 5 times, and were air-dried. Sulforhodamine B (SRB) solution 0.04% w/v in 1% acetic acid for 30 min was the process used for purifying the cells. The dye was extracted with 100 µL/well of 10 mM Tris and the absorbance of the extract was read using a multi-well plate spectrophotometer at 570 nm. Untreated cells were used as control. The results are presented as mean % survival compared to control ± SD for 3 independent experiments.

3.4 Antioxidant assay

Antioxidant analysis of the synthesized **11a-g** compounds was evaluated using ABTA (2,20-azinobis [3-ethyl-benzothiazoline-6-sulfonic acid]) and DPPH (1,1-Diphenyl-2-picrylhydrazyl) assays as mentioned elsewhere [28]. Briefly, different concentrations of the tested compounds were added to 1 mL of ABTS solution (7 mM ABTS in 2.45 mM potassium persulfate) at an initial absorbance A_{734nm}=0.700 ± 0.005, at ambient temperature for 6 min, and a decrease in the preparation absorbance was measured at 734 nm to calculate the % in ABTS radicals inhibition. Whilst, in the DPPH assay, different concentrations of the tested compounds were added to 1 mL of methanolic DPPH solution (0.1 mM). The mixture was vortexed, left at ambient temperature for 30 min, and the decrease in absorbance nm was analyzed at 517nm by UV/Vis spectrophotometry. The antioxidant activity of the tested samples was measured by using the formula

$$Scavenging\ effect\ (\%) = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

The scavenging activity of Trolox® standard solutions (0 to 10 µg/mL in ethanol) was assayed under similar conditions applied for tested samples. In each assay, the same volume of solvents was run as blanks.

3.5 In vitro antibacterial activity measurement

Agar diffusion tests were used in the determination of the antibacterial activities for the compound against the Gram-positive bacteria [*Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (ATCC 10145)] and the Gram-negative bacteria [*Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 13048)] as described previously [28] following the standard methods adopted by the Clinical and Laboratory Standards Institute (CLSI, 2012). The

compounds used for testing were placed on a 6-mm sterile blank disk (300 and 500 µg/disc) that were inserted on the top of Muller Hinton Agar plates (Oxoid, UK) containing 10^6 cell/mL of test bacterial strains. The measurement was performed in triplicate to assure the reliability of the results.

4.Conclusion:

New series of 1,2,4-triazinones functionalized with mono-substituted pyrazole heterocycles **11a-g** were synthesized. The antibacterial, antioxidant and antitumor activity was evaluated, compounds **11a**,**11d** and **11e** act as highly active antioxidants, while on the other hand, all synthesized compounds exhibit a weak antitumor and antibacterial activity, which confirm that the biological activity is related strongly with the substituents (R groups) on triazinone moiety .

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