Synthesis, Antimicrobial and Antitumor Activity of Some 3, 5-Diaryl and 1, 3, 5-Triaryl-2-Pyrazoline Derivatives

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Abstract: A series of 3,5-diaryl-Δ^2-pyrazolines **(2a-h)** were prepared by the reaction of 1,3-diaryl-2-propen-1-ones **(1)** with hydrazine hydrate and used as precursor for the preparation of 1-acetyl-2-pyrazolines **(3a-d)**. A series of1,3,5-triaryl-2-pyrazolines **(6a-h)** and **(7a-g)** were prepared by the reaction of 1,3-diaryl-2-propen-1-ones **(1)** with phenyl hydrazine and/or 2,4-dinitrophenylhydrazine. Similarly, 3,5-diaryl isoxazoline derivatives **(9a,b)** were prepared by the 1,3-cyclocondensation of 1,3-diaryl-2-propen-1-ones **(1)** with hydroxylamine hydro-chloride. Also 1-carbamoyl-, and 1-thiocarbamoyl-2-pyrazoline derivatives **(8a-e)** and **(8f-j)** were synthesized. The structures of the new compounds were proved by means of their IR, ^{1}H-,^{13}C-NMR, MS spectroscopic data and microanalysis. All the new compounds were examined for their *in vitro* antimicrobial activity. Some newly synthesized compounds were examined for their *in vitro* anticancer activity. In the present investigation, we discuss the structure-activity relationships and biological activities of these compounds.
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Key words: Pyrazolines, Isoxazolines, Antimicrobial activity, Antitumor activity.

Introduction

Over the years, pyrazoles and isoxazoles have emerged as an intereseting class of five membered heterocycles with an astonishingly wide range of applications in pharmaceutical chemistry.Compounds including a pyrazole neucleus are known to possess analgesic, anti-inflammatory[1,2], antipyretic , anti-depressant, tranquillizing, muscle relaxant, psycho-analeptic, anticonvulsant, hypotensive[3-10], mono amine oxidase inhibitor[11-14], anticancer[15,16] and antimicrobial activities[17]. Isoxazoline deriva- tives possess potent antithrombic effect and impro-pharmaco – kinetic proprerties[18,19]. In fact, a variety of isoxazole derivatives exhibit COX-I / COX-II inhibiting activity. It was found that Celecoxib, a pyrazole derivatives and Valdecoxib an isoxazole derivative have no effect on platelet aggregation and donot reduce increased PG levels in cerebrospinal fluid[18]. Many synthetic procedures exist for the synthesis of substituted pyrazoles and isoxazoles[19,20]. However, the development of simple an efficient methodologies to get five membered heterocycles is one of the major aspects in organic synthesis[20,21]. In fact, the Michael acceptors are valuable intermediates in a variety of synthetic trans-formations and useful as building blocks in the synthesis of biologically active heterocycles[21]. The present investigation deals with the synthesis of pyrazole, 1-acetyl pyrazole, pyrazolyl pyrazole and isoxazole derivatives from unsaturated ketones by cyclocondensation with different nucleophiles.

Chemistry

The synthetic scheme involves the cyclo-condensation of the appropriate α,β-unsaturated ketone **(1)** with hydrazine hydrate in the presence of ethanol to give **(2a-i)**. Likewise **(6a-h)** and **(7a-g)** were prepared by the reaction of **(1)** with phenyl hydrazine and/or 2,4-dinitrophenyl hydrazine in boiling ethanol. In addition the N-carbamoyl pyrazolines **(8a-e)** and the N-thiocarbamoyl pyrazo-lines **(8f-j)** were prepared by the reaction of **(1)** with semicarbazide and/or thiosemicarbazide in boiling ethanol containing anhydrous sodium acetate. Similarly, the reaction of **(1)** with hydroxylamine hydrochloride in boiling pyridine affected cyclo-condensation to the corresponding isoxazolines **(9a,b)**.. The N-acetyl pyrazoline derivatives **(3a-d)** were treated with aromatic aldehydes (under Claisen-Schmidt conditions) to give the corres-ponding N-pyrazolyl propenones **(4a,b)**. Compound **(4a)** was cyclised to the corresponding pyrazolyl pyrazoline **(5)** upon treatment with hydrazine hydrate in boiling ethanol (Scheme **1**). The IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral data (Tables **2,5**) and microanalyses (Tables **1,3,4**) were used to a certain the structure of all the compounds.

Structure and chemical data of the synthesised compounds are given in (Table **1**). The IR spectra of the compounds showed *ν*C=N band in the region 1572-1514 cm^{-1}. In (Table **2**) the ^{1}H-NMR spectra, Ha, Hb and Hx protons of the pyrazoline ring were shown as doublet of doublets around δ 2.9-3.23, 3.73-3.86 and 4.9-5.45 ppm (J_{ab}=16.6, J_{ax}=7.5 and J_{bx}=9.15 Hz). The protons belonging to the aromatic

nucleii and substitutent groups were observed within the expected chemical shift values (Tables **2, 5**).

Biological results

i) Antimicrobial activity

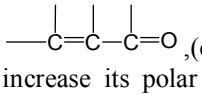
Compounds (**2**) and (**6-8**) were tested for *in vitro* antimicrobial activity against the Gram positive bacteria *Staphylococcus aureus* (RCMB000106), *Bacillis subtilis* (RCMB000107), the Gram-negative bacteria *Pseudomonas aeruginosa* (RCMB000102), *Escherichia coli* (RCMB000103) and the fungi *Aspergillus fumigatus* (RCMB002003), *Penicillium italicum* (RCMB052001), *Syncephalastrum racemosum* (RCMB005003) *Candida albicans* (RCMB-005002). The primary screen was carried out by agar-diffusion method[22] using nutrient agar medium. The minimum inhibitory concentration for the most active compounds against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method[23]. Chloramphenicol was used as standard antibacterial and Terbinafin was used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in (Tables **6-8**).

ii) Anticancer activity

Some pyrazoline derivatives **(2d,2f,3b,6b** and **6d)**were submitted and evaluated at single conc-entration of 10^{-5}M towards panel of cancer cell lines. The human tumor cell lines were derived from different cancer types such as colon and breast cancer (Table **9**). Primary anticancer assays were performed according to the Egypt. NCI protocol[24]. The compounds were added at a single concentration and the cell culture was incubated for 48h. End point determinations were made with a protein binding dye sulforhodamine B (SRB)[25]. The results for each compound were reported as the percent growth of treated cells when compared to untreated cells (Table **9**).

Results and Discussion

We have synthesized a series of Δ^2-pyrazoline heterocycles, **(2a-i)**, **(5)**, **(6a-h)**, **(7a-g)**, **(8a-j)** and isoxazoline derivatives **(9a,b)** by the reaction of **(1)** with the appropriate nucleophiles as presented in (Scheme **1**).

According to the results shown in table **1**, it is clear that the introduction of an electron-withdrawing atom (4-Br) in the acetophenone aromatic moiety at the terminal position of the system  ,(compound **1h**) seemed to increase its polar character and, therefore its tendency to undergo 1,3- cyclocondensation to Δ^2-pyrazolines **(2)** in moderately good yield (84%). On the other hand, the introduction of a halo/or nitro groups into the aromatic moiety of the aldehyde (4-Cl, 4-NO_2, 4-F), influenced the yield of the Δ^2-pyrazolines but to a lesser extent (yield was 60, 70 and 67%, respectively). The same conclusion was realized in the formation of the chalcone **(4b)** (yield was Ca 85%). This was in agreement with the previous findings[26,27].

i) Biological results

The results of preliminary antibacterial testing of compounds **(2a,b,f,g)**; **(6a,b,e)**; **(7a,d,e,g)**; **(8a,b)** are shown in (Table **6**), The results revealed that compounds **(2f,7d,7e** and **7g)** displayed excellent activity against Gram-positive bacteria. Similarly, compounds **(2f,6e,7d** and **7g)** displayed excellent activity against Gram-negative bacteria and Gram-positive bacteria. Compounds **(2g** and **7a)** displayed moderate activity against Gram-positive bacteria and compounds **(2g,6a,7a** and **9b)** have shown moderate activity against Gram-negative bacteria. Compounds **(2a, 2b, 6a, 6b, 6e** and **9a)** exhibited least activity against both bacteria, while all the tested compounds have shown very slight to no activity against the Gram-negative bacteria *Pseudomonas aeruginosa* (Table **6**).

On the other hand, the results of the preliminary antifungal testing of compounds **(2a,b,f,g)**, **(6a,b,e)**, **(7a,d,e,g)** and **(9a,b)** are shown in (Table **7**), The results revealed that compounds **(2f,7d,7e** and **7g)** good activity against the tested fungi especially *A. fumigatus, P. italicum, S. racemosum*. Compounds **(2f, 6e, 7d** and **9b)** low activity against *C. albican,* and compounds **(7e)** and **(7g)** had no activity on the same fungi.

Further, compound having the thiophene moiety, **(6a)** showed comparatively good activity Gram-negative bacteria *E. coli*. Also, compound having group $N(CH3)_2$ such as **(2f)**, **(7d)** exhibited an excellent activity against the same bacteria. Similarly compound **(2f)** which had bromine and $N(CH_3)_2$ groups at the two aromatic nuclei attached to the pyrazoline nucleus had shown good antibacterial and antifungal activity. Also compound **(7d)** has shown the same result to a less extent, while compound **(7e)** which contained a $N(CH_3)_2$ group at one aromatic nucleus and no bromine at the aromatic nucleus attached to the pyrazoline moiety has shown relatively good activity toward all the tested microbes. Further compound **(7g)** which contains two nitro groups at the N-phenyl pyrazoline and two phenyl group at position 3,5- of the pyrazoline ring displayed a relatively high reactivity against the tested microbes.

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table **8**).

Table (6): Antibacterial activity of (2a,b,f,g); (6a,b,e); (7a,d,e,g) and (8a,b).

Sample No.	*Gram-positive bacteria**						*Gram-negative bacteria**					
	Staphylococcus aureus			*Bacillus subtilis*			*Escherichia coli*			*Pseudomonas aeruginosa*		
	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0
2a	0	0	+	0	0	+	0	0	+	0	0	0
2b	0	0	+	0	0	+	0	0	+	0	0	0
2f	0	+	+	+	++	++	+	++	++	0	0	+
2g	0	+	+	0	+	+	0	+	+	0	0	0
St.*	+	++	++	++	++	+++	+	++	++	+	++	++
6a	0	0	0	0	0	0	0	+	+	0	0	0
6b	0	0	+	0	0	+	0	0	+	0	0	0
6e	0	0	+	0	0	+	+	++	++	0	0	0
St.*	+	++	++	++	++	+++	+	++	++	+	++	++
7a	0	+	+	0	+	+	0	+	+	0	0	0
7d	+	+	++	+	++	++	+	+	++	0	0	+
7e	+	+	++	++	++	++	0	+	+	0	0	0
7g	+	+	++	+	++	++	+	++	++	0	0	+
St.*	+	++	++	++	++	+++	+	++	++	+	++	++
9a	0	0	+	0	0	+	0	0	+	0	0	0
9b	0	0	0	0	0	+	0	+	+	0	0	0
St.*	+	++	++	++	++	+++	+	++	++	+	++	++

* Dissolved in DMSO
* Chloramphenicol as standard antibacterial control.
Well diameter: 0.6cm ….. (100µml of each concentration was tested).
Inhibition values = 0.1 - 0.5cm beyond control = +; Inhibition values = 0.6 – 1.0cm beyond control = ++; Inhibition values = 1.1 – 1.5cm beyond control = +++ and 0 = not detected.

Table (7): Antifungal activity (2a,b,f,g); (6a,b,e); (7a,d,e,g) and (9a,b).

Sample no.	*Asperigillus fumigatus**			*Penicillum italicum**			*Syncephalastrum racemosun**			*Candida albicans**		
	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0
2a	0	0	+	0	0	+	0	0	+	0	0	+
2b	0	0	+	0	0	+	0	0	+	0	0	+
2f	+	++	++	+	++	++	+	++	++	0	+	+
2g	0	0	0	0	0	0	0	0	0	0	0	0
St.*	++	+++	+++	++	+++	+++	++	++	+++	++	++	++
6a	0	0	+	0	0	+	0	0	+	0	0	+
6b	0	0	+	0	0	+	0	0	+	0	0	+
6e	0	0	0	0	+	+	0	0	0	0	+	+
St.*	++	+++	+++	++	+++	+++	++	++	+++	++	++	++
7a	+	++	++	0	0	+	0	0	+	0	0	+
7d	+	+	++	+	+	0	+	++	++	0	+	+
7e	+	++	++	0	0	+	+	+	++	0	0	0
7g	+	++	++	0	+	+	+	++	++	0	0	0
St.*	++	+++	+++	++	+++	+++	++	++	+++	++	++	++
9a	0	0	+	0	0	+	0	0	+	0	0	+
9b	0	0	0	0	0	0	0	+	+	0	+	+
St.*	++	+++	+++	++	+++	+++	++	++	+++	++	++	++

* Dissolved in DMSO
* Terbinafin as standard antifungal control.

Table(8): Minimum inhibitory concentration (MIC) Mg/ml of (2f, 7a, 7e and 7g).

Minimum inhibitory concentration, MIC (Mg/ml)								
Comp.	*S.aureus*	*B.subtilis*	*E.coli*	*P.aeruginasa*	*A.fumigatus*	*P.italicum*	*S.racemosum*	*C.albicans*
2f	2.500	0.313	0.313	5.000	0.625	0.625	0.625	2.500
7a	2.500	2.500	2.500	--	0.625	5.000	2.500	5.000
7e	0.625	0.313	2.500	--	0.313	5.000	0.625	--
7g	0.625	0.313	0.625	5.000	0.625	2.500	0.313	--

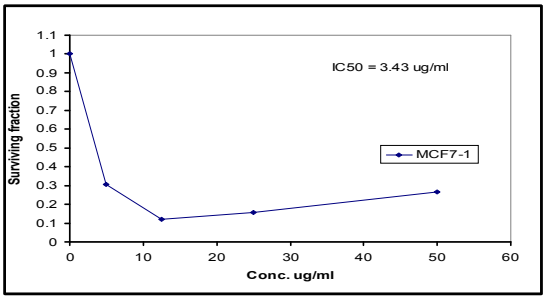
ii) Anticancer results

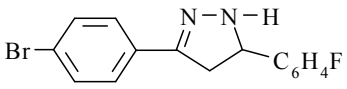
Compounds **(2d, 2f, 3b, 6b** and **6d)** were tested for cytotoxicity and antitumor effect. The mean IC_{50} value for compound **(2d)** was 3.43 µg/ml and for compound **(2f)** was 7.09 µg/ml indicating that the two compounds possess potent broad-spectrum anticancer activity. Compound **(3b)** has shown a good anticancer activity towards breast cancer with IC_{50} of 6.8 µg/ml and a moderate activity toward colon of IC_{50} of 12.5 µg/ml. The anticancer activity of **(6b)** against breast cancer was relatively good since it exhibited IC_{50} of 10.3 µg/ml and the the activity of compound **(6d)** against colon cancer was moderately promising since it exhibited IC_{50} of 16.5 µg/ml (Table **9**)

Table (9): IC_{50} values in µg/ml for compounds (2d,2f,3b,6b and 6d) in selected human cancer cell lines .

Compound	HCT 116[a]	MCF7[b]
2d	—	3.43
2f	7.09	—
3b	6.8	12.5
6b	10.3	—
6d	—	16.5

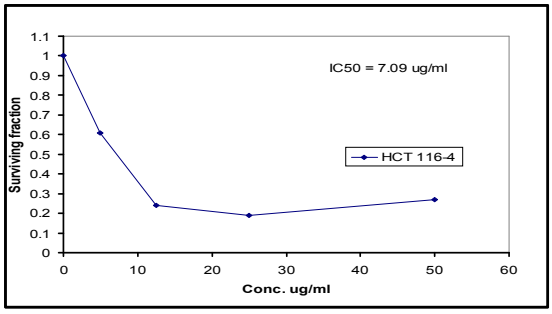
a Breast cancer ; b Colon cancer

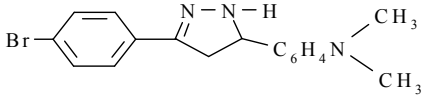




2d

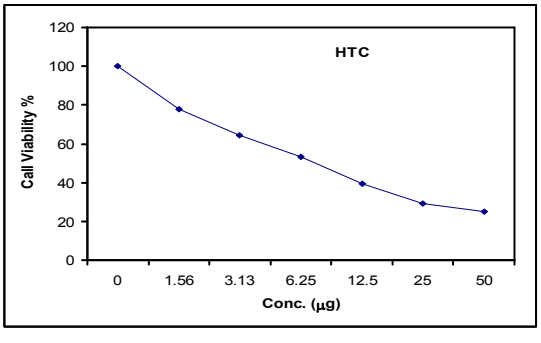
Fig. (1)

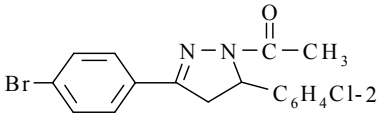




2f

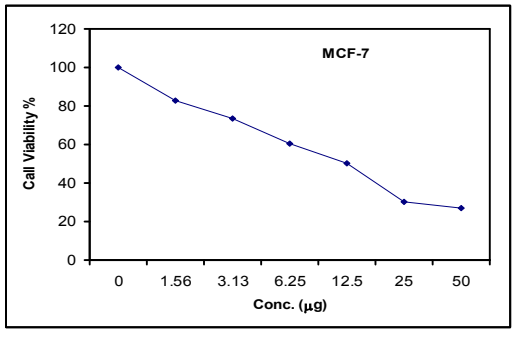
Fig. (2)

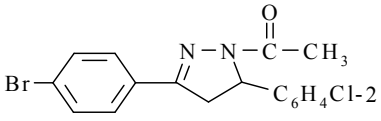




3b

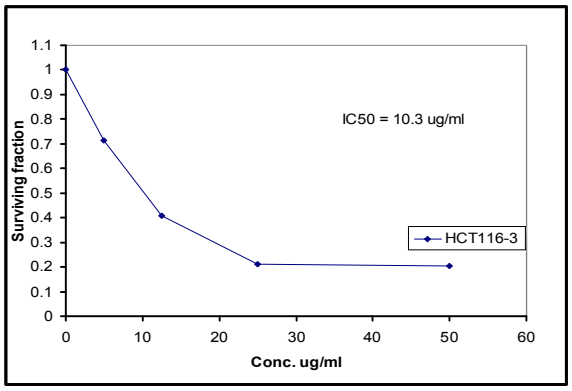
Fig. (3)

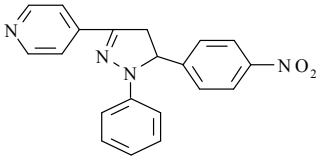




3b

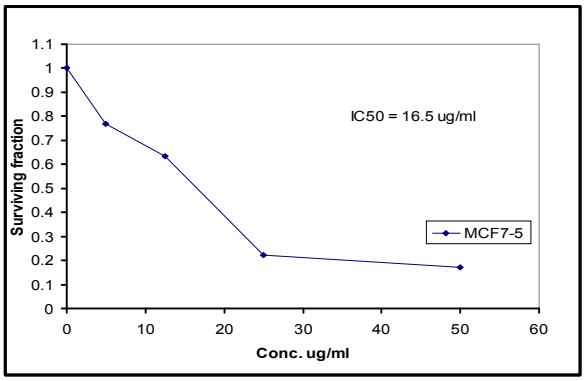
Fig. (4)

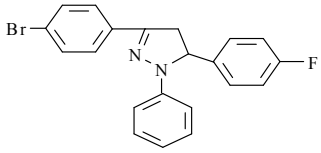




6b

Fig. (5)





6 d

Fig. (6)

Material and Methods

Melting points were measured in capillary tubes on a Gallen-Kamp melting point apparatus. The IR spectra were recorded in KBr pellets on a Pye-Unicam SP3-300 infrared spectrophotometer. The ^{1}H– and^{13}C-NMR spectra were run in DMSO-d_6 on a Varian Gemini 200 NMR spectrometer using TMS as an internal reference. Mass spectra were recorded on a GCMS-QP 1000 EX mass spectrometer at 70 eV. Microanalyses were carried out at the Micro analytical Center, Cairo University, Egypt. C, H, N, S and X analysis were carried out for all the synthesized compounds and the results were all within ± 0.045% of the theoretical values.

Experimental

The starting compounds (E)-1,3-diaryl-2- propen-1-ones **(1)** were prepared as per the literature procedure[27].

General procedure for the preparation of 3,5-diaryl-Δ^2-pyrazoline (2a-i).

A mixture of unsaturated ketones **1** (15 m mol) and hydrazine hydrate (15 m mol) in 30 ml of absolute ethanol was refluxed for 4-6 hrs. After concentration and cooling, the product obtained was recrystallised from the proper solvent to give **2a-i** (Table **1**).

General procedure for the preparation of 1-acetyl-3,5-diaryl-Δ^2-pyrazoline (3a-d).

A mixture of the pyrazolines **2a, 2e, 2h, 2i** (15 m mol) and acetic anhydride (15 ml) in 10 ml of absolute butanol was refluxed for 3 hrs. The excess solvent was removed in vacuo and the resultant solid was recrystallised from the proper solvent to give **3a-d** (Table **1**).

General procedure for the reaction of 1-(3,5-diaryl-5,5-dihydropyrazol-1-yl) ethanone (3a,b) with aromatic aldehydes. Formation of (4a,b).

To a solution of **2a** or **2c** (43 m mol) in 15 ml of ethanol was added aqueous NaOH (10 ml of 10 m mol) while stirring and cooling at 0-5°C. To this mixture was added (43 m mol) of ρ-nitro benzaldeyde or benzaldehyde (from a dropping funnel) and stirring was continued for 3h maintaining the same temperature, then left over night in the refrigerator. The resultant solid was collected by filtration and recrystallised from the proper solvent as **4a,b** (Table **1**).

Reaction of (4a) with hydrazine hydrate. Formation of 5-(4-chloro phenyl) -3-(2-thienyl) -1-[5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)]-4,5-dihydro-1H-pyrazole (5).

To a solution of **4a** (15 m mol) in 15 ml of absolute ethanol was added hydrazine hydrate (15 m mol) in 5 ml of absolute ethanol and the mixture was refluxed for 6 hrs. After concentration and cooling, the solid obtained was recrystallised from ethanol as **5** (Table **1**).

General procedure for the preparation of 1-phenyl- and 1-substituted phenyl-3,5-diaryl pyrazolines (6a-h) and (7a-g).

A mixture of the unsaturated ketones **1** (15 m mol), phenyl hydrazine and/or 2,4-dinitro phenyl hydrazine (15 m mol) in 30 ml of absolute ethanol was refluxed for 6-8 hrs. After concentration and cooling, the product obtained was recrystallised from the proper solvent to give **6a-h** and **7a-g** (Table **3**).

General procedure for the preparation of 1-carbamoyl- and 1-thiocarbamoyl-3,5-diaryl-pyrazolines (8a-j).

A mixture of unsaturated ketones **1** (15 m mol),

freshly fused sodium acetate (45 m mol) and semicarbazide/or thiosemicarbazide (15 m mol) (dissolved in 1 ml of water) in 30 ml of absolute ethanol was refluxed for 6-8 hrs. After concentration and cooling, the product obtained was recrystallised from the proper solvent to give **8a-j** (Table **4**).

General procedure for the preparation of 1,3-diaryl isoxazolines (9a-b).

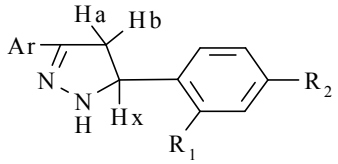
A mixture of unsaturated ketones **1** (15 m mol) and hydroxylamine hydrochloride (15m mol) in (15 ml) of dry pyridine was refluxed for 6hrs. After cooling it was poured onto a mixture of HCl –ice (50:50 by volume) and the solid that formed was collected by filtration, washed well with water, then diluted ethanol and recrysallized from the proper solvent to give **9a,b** in moderate yields (Table **4**).

Table (1) : Physical data of Δ^2-pyrazolines (2a-i), (3a-d), (4a,b) and (5).

Compd. No.	M.P.°C solvent of crystallization*	Yield %	Molecular formula (Mol.wt.)	Analysis calcd./found%						
				C	H	N	S	Br	Cl	F
2a	158-160 P.E	60	$C_{13}H_{11}N_2SCl$ (262.5)	59.42 59.4	4.19 4.2	10.66 10.7	12.19 12.2		13.52 13.5	
2b	228-230 P.E.	70	$C_{14}H_{12}N_4O_2$ (268)	62.68 62.7	4.47 4.5	20.89 20.9				
2c	110-112 P.E.	73	$C_{15}H_{15}N_3O$ (253)	71.14 71.1	5.92 5.9	16.60 16.6				
2d	198-200 P.E.	67	$C_{15}H_{14}N_2FBr$ (319)	56.42 56.4	3.76 3.8	8.77 8.8		25.07 25.1		5.95 6.0
2e	182-184 P.E.	79	$C_{15}H_{12}N_2ClBr$ (335.5)	53.65 53.7	3.57 3.6	8.34 8.3		23.84 23.8	10.58 10.6	
2f	134-136 P.E.	68	$C_{17}H_{18}N_3Br$ (344)	59.302 59.3	5.23 5.2	12.209 12.2		23.25 23.3		
2g	220-222 EtOH	73	$C_{17}H_{19}N_3$ (265)	76.98 77.0	7.16 7.2	15.84 16.0				
2h	138-140 P.E.	84	$C_{15}H_{13}N_2Br$ (301)	59.80 59.8	4.31 4.3	9.302 9.3		26.57 26.6		
2i	182-184 P.E.	73	$C_{15}H_{14}N_2$ (222)	81.44 81.4	6.306 6.3	12.61 12.6				
3a	138-140 EtOH	74	$C_{15}H_{13}N_2OSCl$ (304.5)	59.11 59.1	4.27 4.3	9.19 9.2	10.51 10.5		11.66 11.7	
3b	194-196 EtOH	78	$C_{17}H_{14}N_2OClBr$ (377.5)	54.04 54.0	3.71 3.7	7.41 7.4		21.19 21.2	9.403 9.4	
3c	110-112 EtOH	67	$C_{17}H_{15}N_3OBr$ (343)	59.47 59.5	4.37 4.4	8.16 8.2		23.32 23.3		
3d	148-150 EtOH	74	$C_{17}H_{16}N_2O$ (264)	77.27 77.3	6.06 6.1	10.61 10.6				
4a	144-146 EtOH	73	$C_{22}H_{16}N_3O_3SCl$ (437.5)	60.34 60.3	3.65 3.7	9.6 9.6	7.31 7.3		8.11 8.1	
4b	192-194 EtOH	85	$C_{24}H_{19}N_2OBr$ (431)	66.82 66.8	4.41 4.4	6.49 6.5		18.56 18.6		
5	70-72 EtOH	55	$C_{22}H_{18}N_5O_2SCl$ (451.5)	58.47 58.5	3.98 4.0	15.503 15.5	7.08 7.1		7.86 7.9	

***** Where P.E.= petroleum ether (b.p 60-80).

Table (2): IR, ^{1}H-,^{13}C-NMR and MS of (2a-i), (3a-d), (4a,b) and (5).



Comp. No.	IR(γcm^{-1})		^{1}H-NMR* , δppm	^{13}C-NMR	MS (%)
2a	3226 1590 1572	NH C=C C=N	8.4 (d, 1H, NH), 7.07-7.86 (m, 7H, Ar-H), 4.9 (dd, 1H, H_x), 3.35 (dd, 1H, Ha) (Jax = 7.5 Hz), 2.5 (dd, 1H, Hb) (Jbx = 9.15Hz),		

Comp. No.	IR(γcm^{-1})		^{1}H-NMR* , δppm	^{13}C-NMR	MS (%)
			(Jab=15.6 Hz)		
	3204	NH	11.2 (d, 1H, NH), 6.63-8.50 (m, 8H,		268(12.1),269(11.4),
2b	1598	C=C	Ar-H), 5.45 (dd, 1H, H_x), 2.98 (dd, 1H,		239(13.3),225(13.6),
	1516	C=N	Ha), 3.45 (dd, 1H, Hb)		207(14.2),181(17.5),
					137(16.1),83(59.01),
					71(61.99),58(100)
	3220	NH	10.1 (d, 1H, NH), 7.04-8.56 (m, 8H,		
2c	1590	C=C	Ar-H), 5.07(dd, 1H, Hx), 3.94 (dd, 1H,		
	1570	C=N	Ha), 3.73 (dd, 1H, Hb), 3.34 (s, 3H, Ar-OCH_3)		
	3200	NH	13.49 (d, 1H, NH), 7.2-8.26 (m, 8H,		
2d	1590	C=C	Ar-H), 4.90 (dd, 1H, Hx), 3.35(dd, 1H,		
	1515	C=N	Ha), 3.05 (dd, 1H,Hb)		
	3216	NH	11.61 (d, 1H, NH), 7.19-8.18 (m, 8H,		
2e	1590	C=C	Ar-H), 5.07 (dd, 1H, Hx), 3.44 (dd, 1H,		
	1544	C=N	Ha), 3.73 (dd, 1H, Hb)		
	3336	NH	10.01 (d, 1H, NH), 6.54-7.91 (m, 8H,		
2f	1603	C=C	Ar-H), 5.34 (dd, 1H, Hx), 3.21(dd, 1H,		
	1518	C=N	Ha), 3.80 (dd, 1H,Hb) ,2.94, 2.95 (2xs, 2x3H,2xCH_3)		
	3326	NH	9.3 (d, 1H, NH), 6.69-7.87 (m, 9H,		
2g	1598	C=C	Ar–H), 5.22 (dd, 1H, Hx), 3.1 (dd, 1H,		
	1514	C=N	Hb), 3.75 (dd, 1H, Ha), 2.85,2.91 (2xs, 2x 3H, 2xCH_3)		
	3240	NH	9.59 (d, 1H, NH), 6.95-7.81(m, 9H,		
2h	1585	C=C	Ar-H), 5.41 (dd, 1H, H_x), 3.34 (dd, 1H,		
	1545	C=N	Ha), 3.86(dd, 1H, Hb)		
	3338	NH	9.81 (d, 1H, NH), 6.69-7.87(m, 10H,		
2i	1596	C=C	Ar-H), 5.23 (dd, H_x, 3.11 (dd, 1H, Ha),		
	1550	C=N	3.76(dd, 1H, Hb)		
	1648	C=O	7.29-8.34 (m, 7H, Ar-H), 4.8 (t,		
3a	1620	C=C	1H,CH-CH_2), 3.34 (d, 2H, CH-CH_2), 2.5		
	1586	C=N	(s, 3H, $COCH_3$)		
	1670	C=O		20.6 (CH_3), 39.9 (CH_2), 60.1 (CH), 146.4 (C), 120.6, 122.4, 128.0, 129.6, 130,8, 131.3, 133.0, 133.4, 133.8, 136.3 (aromatic nucleus), 146.4 (C-Br), 173.2 (C=O, acetyl), 195(C-Cl)	
3b	1620	C=C			
	1590	C=N			
	1668	C=O	7.01-8.16 (m, 9H, Ar-H), 3.29, 3.34,		
3c	1620	C=C	3.39(t, 1H,CH-CH_2), 2.2, 2.5(d, 2H,		
	1524	C=N	CH-CH_2), 2.07 (s, 3H, $COCH_3$)		
	1660	C=O			
3d	1620	C=C			
	1560	C=N			
	1664	C=O			
4a	1590	C=C			
	1504	C=N			
	1669	C=O			
4b	1569	C=C			
	1544	C=N			
	1540	C=C			
5	1588	C=N			

Table (3): Physical data of 1-phenyl- and 1-substituted phenyl-3,5-diaryl- pyrazolines (6a-h) and (7a-g).

Compd. No.	M.P.°C solvent of crystallization	Yield %	Molecular formula (Mol.wt.)	Analysis calcd./found%						
				C	H	N	S	Br	Cl	F
6a	142-144 EtOH	88	$C_{19}H_{15}N_2SCl$ (338.5)	67.35 67.4	4.43 4.4	8.27 8.3	9.45 9.5		10.48 10.5	
6b	278-280 P.E.	69	$C_{20}H_{16}N_4O_2$ (344)	69.76 69.8	4.56 4.6	16.27 16.3				

6c	108-110 P.E.	58	$C_{21}H_{19}N_3O$ (329)	67.59 76.6	5.77 5.8	12.76 12.8				
6d	134-136 P.E.	66	$C_{21}H_{16}N_2FBr$ (395)	63.79 63.8	4.05 4.1	7.08 7.1		20.25 20.3		4.81 4.8
6e	150-152 P.E.	73	$C_{21}H_{16}N_2ClBr$ (411.5)	61.23 61.2	3.88 3.9	6.804 6.8		19.44 19.4	8.62 8.6	
6f	88-90 P.E.	81	$C_{23}H_{23}N_3$ (341)	80.93 80.9	6.74 6.7	12.31 12.3				
6g	166-168 P.E.	68	$C_{21}H_{17}N_2Br$ (377)	66.84 66.8	4.509 4.5	7.42 7.4		21.22 21.2		
6h	124-126 P.E.	84	$C_{21}H_{18}N_2$ (298)	84.56 84.6	6.04 6.0	9.39 9.4				
7a	98-100 P.E.	89	$C_{21}H_{17}N_5O_5$ (419)	60.14 60.1	4.05 4.1	16.706 16.7				
7b	140-142 P.E.	68	$C_{21}H_{14}N_4O_4FBr$ (485)	51.95 52.0	2.88 2.9	11.54 11.5		16.49 16.5		3.91 3.9
7c	170-172 P.E.	78	$C_{21}H_{14}N_4O_4ClBr$ (501.5)	50.24 50.2	2.79 2.8	11.16 11.2		15.92 15.9	7.07 7.1	
7d	122-124 P.E.	64	$C_{23}H_{20}N_5O_4Br$ (510)	54.11 54.1	3.92 3.9	13.72 13.7		15.68 15.7		
7e	110-112 P.E.	79	$C_{23}H_{21}N_5O_4$ (431)	64.03 64	4.87 4.9	16.24 16.2				
7f	178-180 EtOH	67	$C_{21}H_{15}N_4O_4Br$ (467)	53.96 53.9	3.21 3.2	11.99 12.0		17.13 17.1		
7g	232-234 EtOH	83	$C_{21}H_{16}N_4O_4$ (388)	46.94 46.9	4.12 4.1	14.43 14.4				

* Where P.E.= petroleum ether (b.p 60-80°C).

Table (4): Physical data of 1-carbamoyl- (8a-e) and 1-thiocarbamoyl-pyrazolines (8f-j) and the isoxazolines (9a,b).

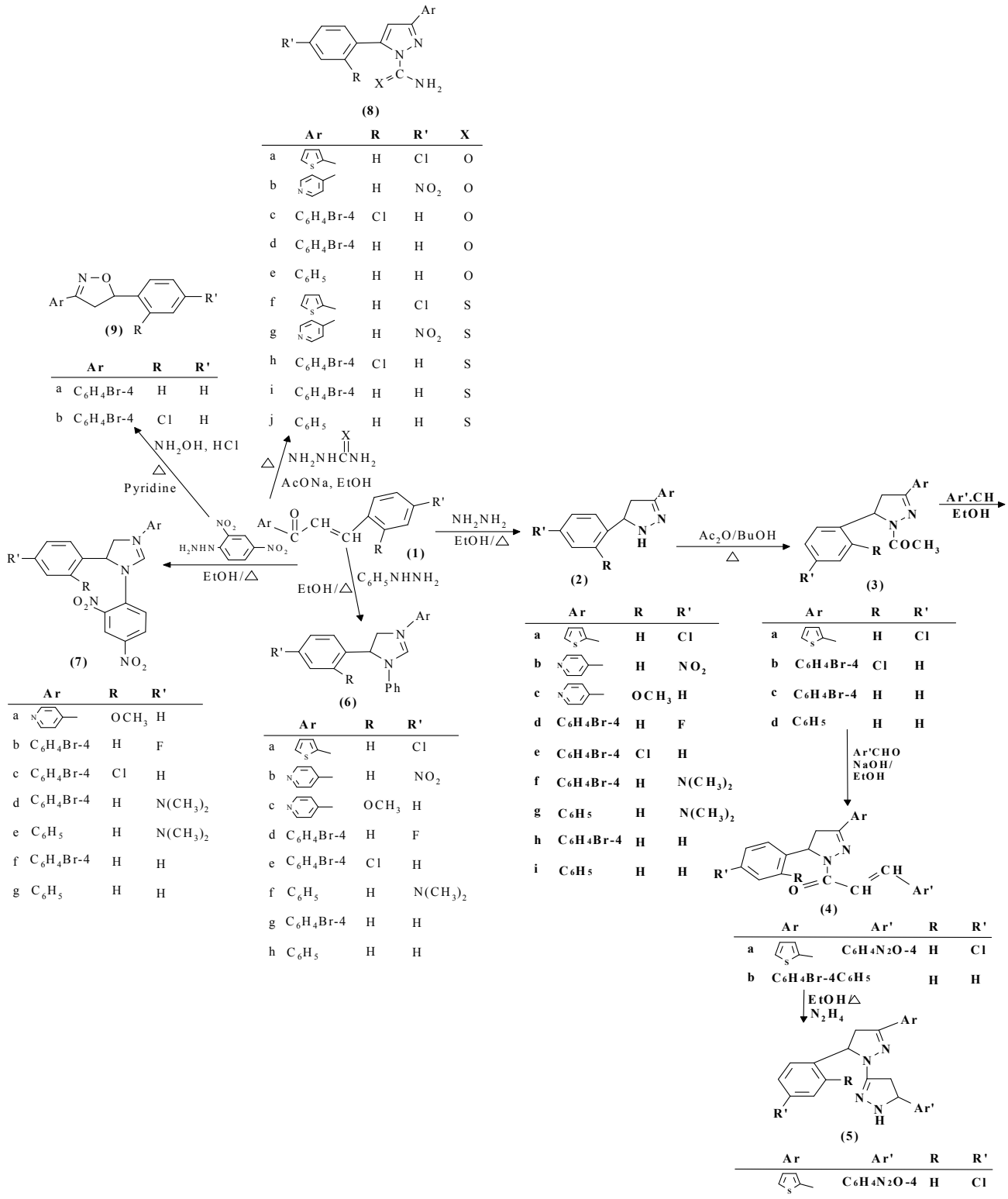
Compd. No.	M.P.°C solvent of crystallization	Yield %	Molecular formula (Mol.wt.)	Analysis calcd./found%						
				C	H	N	S	Br	Cl	F
8a	110-112 EtOH	77	$C_{14}H_{12}N_3OSCl$ (305.5)	54.99 55.0	3.92 3.9	13.74 13.7	10.47 10.5		11.62 11.6	
8b	162-164 P.E.	79	$C_{15}H_{13}N_5O_5$ (343)	57.87 579	4.18 4.2	22.508 22.5				
8c	104-106 P.E.	51	$C_{16}H_{13}N_3OClBr$ (348.5)	50.72 50.7	3.43 3.4	11.09 11.1		21.13 21.1	9.37 9.4	
8d	90-92 P.E.	75	$C_{16}H_{14}N_3OBr$ (344)	55.81 55.8	4.06 4.1	12.209 12.2		23.25 23.3		
8e	88-90 P.E.	62	$C_{16}H_{15}N_3O$ (265)	72.45 72.5	5.66 5.7	15.84 15.8				
8f	116-118 EtOH	63	$C_{14}H_{12}N_3OS_2Cl$ (337.5)	49.77 49.8	3.55 3.6	12.44 12.4	10.51 10.5	18.96 19.0		
8g	122-124 P.E.	84	$C_{15}H_{13}N_5O_2S$ (327)	55.04 55.0	3.97 4.0	21.406 21.4	9.78 9.8			
8h	96-98 P.E.	59	$C_{16}H_{13}N_3OSClBr$ (394.5)	48.66 48.7	3.29 3.3	10.46 10.5	8.11 8.1	20.28 20.3	8.99 9.0	
8i	236-238 P.E.	64	$C_{16}H_{14}N_3SBr$ (360)	53.33 53.3	3.88 3.9	11.66 11.7	8.88 8.9	22.22 22.2		

8j	94-96 P.E.	72	$C_{16}H_{15}N_3S$ (281)	68.32 68.3	5.33 5.3	14.94 14.9	11.38 11.4		
9a	140-142 EtOH	83	$C_{15}H_{11}ONClBr$ (336.5)	53.49 53.5	3.26 3.3	4.16 4.2		23.77 23.8	10.54 10.5
9b	134-136 P.E.	62	$C_{23}H_{12}NOBr$ (302)	59.605 54.6	3.97 4.0	4.63 4.6		26.49 26.5	

* Where P.E.= petroleum ether (b.p 60-80°C).

Table (5): IR, ^{1}H-,^{13}C-NMR and MS of 1-carbamido-pyrazolines (8a-e), 1-thiocabamido-pyrazolines (8f-j) and isoxazolines (9a,b).

Comp. No.	IR(γcm^{-1})		^{1}H-NMR* , δppm	^{13}C-NMR	MS (%)
8a	3400,3320 1646 1592	NH_2 C=O C=C	7.13-8.36 (m, 7H, Ar-H), 4.85 (s, 2H, NH_2), 3.37, 3.77 (dd, 1H, H_x), 2.55, 2.51 (dd, 1H, Hb), 2.5, 2.49 (dd, 1H, Ha)		
8b	3392,3316 1686 1586	NH_2 C=O C=C	6.18-8.30 (m, 8H, Ar-H), 5.75 (s, 2H, NH_2), 5.33, 5.39 (dd, 1H, H_x), 3.77, 3.99 (dd, 1H, Hb), 3.34, 3.59 (dd, 1H, Ha)		
8c	3400,3230 1684 1592	NH_2 C=O C=C			
8d	3456,3300 1648 1566	NH_2 C=O C=C			344(4.8), 326(9.9), 300(100), 313(5.2), 286(28.2), 271(2.2), 253(3.2), 223(13.5), 212(17.0),189(15.2), 191(12.3),183(26.1), 164(11.5),131(11.6), 102(43.6),110(6.3), 94(11.5), 75(51.5), 50(24.0)
8f	3420,3200 1278 1588	NH_2 C=S C=C	7.13-8.35 (m, 7H, Ar-H), 5.90 (s, 2H, NH_2), 4.01, 4.15 (dd, 1H, H_x), 3.81, 3.89 (dd, 1H, Hb), 3.28, 3.19 (dd, 1H, Ha)		
8g	3422,3342 1281 1591	NH_2 C=S C=C	7.45-8.72 (m, 8H, Ar-H), 5.59 (s, 2H, NH_2), 3.39, 3.36 (dd, 1H, H_x), 2.69, 2.50 (dd, 1H, Hb), 2.45, 2.38 (dd, 1H, Ha)		
8i	3375,3271 1282 1599	NH_2 C=S C=C			360(2.1), 300(2.3), 286(23.2),256(2.8), 207(8.3),185(8.2), 178(20.7),157(10.8), 155(9.1),128(10.2), 102(25.5), 75(51.7), 59(66.3), 45(32.5), 91(100)
9b	1620 550	C=C C=Br			302(97.3),300(67.8), 286(60.4),284(58.1), 277(18.4),252(17.6), 232(18.1), 224(5.5), 220(8.3), 204(58.4), 191(16.4),189(29.5), 183(18.0),176(22.2), 164(25.1),155(12.8), 151(18.6),138(13.0), 128(34.2), 105(21), 103(19), 102(74.0), 94(37.5), 76(100)



	Ar	R	R'	X
a		H	Cl	O
b		H	NO_2	O
c	C_6H_4Br-4	Cl	H	O
d	C_6H_4Br-4	H	H	O
e	C_6H_5	H	H	O
f		H	Cl	S
g		H	NO_2	S
h	C_6H_4Br-4	Cl	H	S
i	C_6H_4Br-4	H	H	S
j	C_6H_5	H	H	S

	Ar	R	R'
a	C_6H_4Br-4	H	H
b	C_6H_4Br-4	Cl	H

	Ar	R	R'
a		OCH_3	H
b	C_6H_4Br-4	H	F
c	C_6H_4Br-4	Cl	H
d	C_6H_4Br-4	H	$N(CH_3)_2$
e	C_6H_5	H	$N(CH_3)_2$
f	C_6H_4Br-4	H	H
g	C_6H_5	H	H

	Ar	R	R'
a		H	Cl
b		H	NO_2
c		OCH_3	H
d	C_6H_4Br-4	H	F
e	C_6H_4Br-4	Cl	H
f	C_6H_5	H	$N(CH_3)_2$
g	C_6H_4Br-4	H	H
h	C_6H_5	H	H

	Ar	R	R'
a		H	Cl
b		H	NO_2
c		OCH_3	H
d	C_6H_4Br-4	H	F
e	C_6H_4Br-4	Cl	H
f	C_6H_4Br-4	H	$N(CH_3)_2$
g	C_6H_5	H	$N(CH_3)_2$
h	C_6H_4Br-4	H	H
i	C_6H_5	H	H

	Ar	R	R'
a		H	Cl
b	C_6H_4Br-4	Cl	H
c	C_6H_4Br-4	H	H
d	C_6H_5	H	H

	Ar	Ar'	R	R'
a		$C_6H_4N_2O$-4	H	Cl
b	C_6H_4Br-4C_6H_5		H	H

	Ar	Ar'	R	R'
		$C_6H_4N_2O$-4	H	Cl

Scheme 1

Conclusion

It is clear from the IC_{50} values that compounds **(2d, 2f** and **3b)** showed significant high cytotoxic activity especially against MCF-7, HCT 116 cell lines. It is also evident from the data that changes in substituents influences the relative toxicity (IC_{50} values of compounds **(2d)** against **(2f)** as well as **3b**, **6b**, and **6d**). This can be attributed to their differences in either polarity which changes their lipophilicity or the conformation which alters the target protein binding properties present within the cell or on the cell membrane.

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