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Synthesis, Characterization and Biological Activities of New 1-[(2, 5-Dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl Pyrazoline Derivatives

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A series of new 1-[(2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl) -2-(N-benzoyl)2,5-dichloroanilino)]-5-phenyl pyrazoline have been synthesized in 44-76 % yield, by the reaction of N-cinnamoyl-N-2'-cyanoethyl-2,5-dichloroaniline with ethyl-2-[(N-benzoyl) 2,5-dichloroanilido]acetohydrazide. Pyrazolines are yellow, cream and brown colour solids, having high melting points. Identity of these products has been established by elemental analysis and spectral data. Newly synthesized compounds (**6a-t**) have been tested for their antibacterial activity against gram positive bacteria *S. albus, S. aureus* and gram negative bacteria *E. coli* and *Pseudomonas poisonous*. The compounds (**6a, 6b, 6c, 6f, 6g, 6j, 6m** and **6r**) shown significant activity and the compounds (**6i, 6k, 6l, 6p, 6t**) have shown moderate activity. The same compounds were tested for their antifungal activity against *Candida albicans*, *Aspergillus niger* and *Alternaria alternata* at concentration of 30 mg/mL using sabouraud dextrose agar media. The compounds (**6c, 6j, 6m** and **6r**) shown significant activities and compounds (**6a, 6b, 6f** and **6g**) were found to be moderately active against *Candida albicans* and *Aspergillus niger*. All the other compounds did not show significant activity against the fungi at the concentration used. Some new compounds have been tested for antitubercular activity *in vitro* using *Mycobacterium tuberculosis*. The compounds were incorporated into Lowenstein Jensen egg medium having concentrations of 10 and 100 mg/mL and were inoculated with *Mycobacterium tuberculosis*, H_{27}, Rv strains, incubated at 37 ºC and observed. The compounds (**6a, 6b, 6c, 6f, 6g, 6j** and **6m**) inhibited the growth of *Mycobacterium tuberculosis* at 100 mg/mL concentration other compounds were found to be inactive.

Key Words: 5-Phenyl pyrazoline, Synthesis, Characterization and biological activities.

INTRODUCTION

Considerable attention has been focused on pyrazolines and substituted pyrazolines due to their interesting biological activities. They have found to possess antifungal[1], antidepressant[2-7]], anticonvulsant[8], antiinflammatory[9-12], antibacterial[13,14],

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anticancer[15,16], antioxidant[17,18], antipyretic[19], antineoplastic activities[20,21], antiviral[22], antiamoebic[23,24], acaricidal agro chemical fungicides or insecticides[25], anticholinergic[26,27], antidiabetic[28], anti HIV[29-32], antimalarial[33], anesthetic[34], anaxiolytic[35], antiparasitic[36], antiallergic[37], antimicrobial[38-40], antituberculosis[41-44], tyrosinase inhibitor[45], blue photo luminescence and electro luminescence[46], food and chemical toxicology[47], herbicidal[48-50], hypoglycemic[51], hypotensive[52], immuno suppressive[53], antitumor[54,55]. Moreover, many selectively chloro-substituted organic compounds show peculiar pharmacological and agrochemical properties. The work reported herein was aimed at the preparation of some new pyrazoline derivatives with anticipated biological activities.

EXPERIMENTAL

All chemicals were used of A.R. grade (either of B.D.H. or Excel-R or Extra pure E. Merck quality). The structures of the compounds were determined by elemental analysis, IR and NMR spectral data. All melting points were measured on an electro thermal melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP 3-300 or a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded in DMSO-d_6 on a Varian Mercury VX 200 NMR using TMS as the internal reference. Mass spectra were measured on a GCMS-QP 1000 EX spectrophotometer at 70 eV. Purity of the compounds is checked on TLC using Silica Gel-G. Elemental analysis is performed on Carlo-Erba1108 analyzer.

Synthesis of ethyl-2-[2,5-dichloroanilido]ethanoate [1]: A mixture of 2,5-dichloro aniline (10 mL) and diethyl malonate (20 mL) was refluxed for 45 min in a round bottomed flask fitted with an air condenser of such a length (14") that ethanol formed escaped and diethyl malonate flowed back into the flask. Contents were cooled, ethanol (30 mL) was added, when malon-2,5-dichlorodianilide separated out. It was filtered under suction. The filtrate was poured on to crushed ice (*ca.* 160 g) and stirred when ethyl-2-(2,5-dichloroanilido) ethanoate precipitated as green mass. On recrystallization from aqueous ethanol (50 %), ester was obtained as white crystals. Yield 81 %, m.p. 88 ºC, m.w. 276. Anal. calculation for $C_{11}H_{11}NO_3Cl_2$: found. (%): C 39.20, H: 03.24, O: 14.25, N: 4.14, Cl: 21.09, calcd. (%) C: 39.21, H: 03.26, O: 14.26, N: 04.15, Cl: 21.16. IR [KBr, ν_{max}, cm^{-1}]: 1665-1660 [C=O diketone], 1290 [-C-O- ester], 760-755 [2,5-disubstituted benzene], 1250 [C-Cl stretching], 1590, 1520, 1440 [C=C ring stretching], 3150 [N-H stretching], 3040 [C-H aromatic], 1330-1322 [C-H stretching]. PMR (DMSO): δ 4.42 (2H, s, CO-CH_2-CO), 4.0 (2H, s, NH_2), 7.4-8.6 (3H, m, Ar-H), 9.2 (1H, s, CO-NH D_2O exchangeable), 10.6 [1H, s, Ar-NH D_2O exchangeable].

Synthesis of ethyl-2-[(N-benzoyl) 2,5-dichloroanilido] ethanoate [2]: Benzoyl chloride (8.46 g; 0.06 mol), dioxane (6 mL), ethyl-2-(2,5-dichloroanilido) ethanoate (16.5 g; 0.06 mol) and triethylamine (6.06 g; 0.06 mol) were placed in a round

bottomed flask carrying reflux condensor having calcium chloride guard tube. The contents were heated on a boiling water bath for 2 h and kept over night when triethylamine hydrochloride separated. It was filtered under suction and the filtrate was poured on to crushed ice (*ca.* 180 g) and stirred when ethyl-2-[(N-benzoyl) 2,5-dichloroanilido]ethanoate separated or solid. It was filtered under suction, dried and purified by recrystallization from aqueous methanol (1:1) in white crystals. Yield 78.4 %, m.p. 94 °C analytical calculation for $C_{18}H_{15}NO_4Cl_2$: [FW = 380], calcd. (%): N 02.95, C 45.64, H 03.38, O 13.50, Cl 15.00, found. (%): N 02.94, C 45.62, H 03.37, O 13.52, Cl 15.02. IR [KBr, ν_{max}, cm^{-1}]: 1720 [C=O diketone], 1300 [-C-O- ester], 762[2,5-disubstituted benzene], 1090 [C-Cl stretching], 1590, 1520, 1440 [C=C ring stretching], 3160 [N-H stretching], 3040 [C-H aromatic], 1330-1322 [C-H stretching]. PMR (DMSO): δ 4.44 [2H, s, CO-CH_2-CO], 4.1 [2H, s, NH_2], 7.2-8.5 [3H, m, Ar-H], 9.4 [1H, s, CO-NH D_2O exchangeable], 10.8 [1H, s, Ar-NH D_2O exchangeable].

Synthesis of ethyl-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazide [3]: Ethyl-2-[(N-benzoyl) 2,5-dichloroanilido]ethanoate (10.98 g; 0.03 mol), ethanol (8 mL) and hydrazine hydrate (15 mL; 70 %) were mixed together and stirred for 35 min. Ethyl-2-[(N-benzoyl) 2,5-dichloroanilido] acetohydrazide was filtered under suction and recrystallized from ethanol in white crystals. Yield 76 %, m.p. 172 °C, m.w. 366 analytical calculation for $C_{16}H_{13}N_3O_3Cl_2$: calcd. (%): N 09.04,C 41.32,H 03.01,O 10.33, Cl 15.28, found. (%): N 09.01, C 41.30, H 03.00, O 10.31, Cl 15.27. IR [KBr, ν_{max}, cm^{-1}]: 3160 [N-H stretching], 3048 [C-H aromatic], 1660 [C=O diketone], 1432 [C-Cl aromatic], 1595, 1520, 1445 [C=C ring stretching]. PMR (DMSO): δ 4.44 (2H, s, CO-CH_2-CO), 4.1 (2H, s, NH_2), 7.2-8.5 (3H, m, Ar-H), 9.4 (1H, s, CO-NH D_2O exchangeable), 10.9 (1H, s, Ar-NH D_2O exchangeable).

Mono cyanoethylation of 2,5-dichloroaniline [4]: A 250 mL three necked flask equipped with a stirrer, reflux condenser and thermometer was charged with 2,5-dichloro aniline (0.1 mol, 16.2 g), acrylonitrile (0.1 mol, 10.6 g) and cupric acetate monohydrate (1.02 g, 4 % by weight of the amine). The mixture was stirred and refluxed on boiling water bath for 3 h. The dark mixture was then transferred to a 250 mL distilling flask fitted with a 15.2 cm modified vigorous column and the unchanged acrylonitrile was first collect at 100 mm (water pump). The distillation was continued and the unchanged 2,5-dichloro aniline b.p. 252 ºC/0.5 mm was recovered. The N-cyanoethyl-2,5-dichloroaniline was obtained as light yellow coloured viscous liquid at 178-179 ºC/mm which solidified after keeping over-night. Yield: 15.7 g (97 %), m.p. 82 ºC.

Preparation of cinnamoyl chloride [5]: Cinnamic acid (10 g, 0.067 mol) and thionyl chloride (12 mL) were taken in a round bottomed flask fitted with a reflux condenser carrying a calcium chloride guard tube. The contents were refluxed on a water bath for 2 and 0.5 h in a fume cupboard until the evolution of HCl gas ceased from the guard tube. After cooling liquid was carefully transferred to a claisen flask and distilled under reduced pressure when unreacted thionyl chloride distilled over first. Cinnamoyl chloride was collected at 165-166 ºC/18-20 mm pressure.

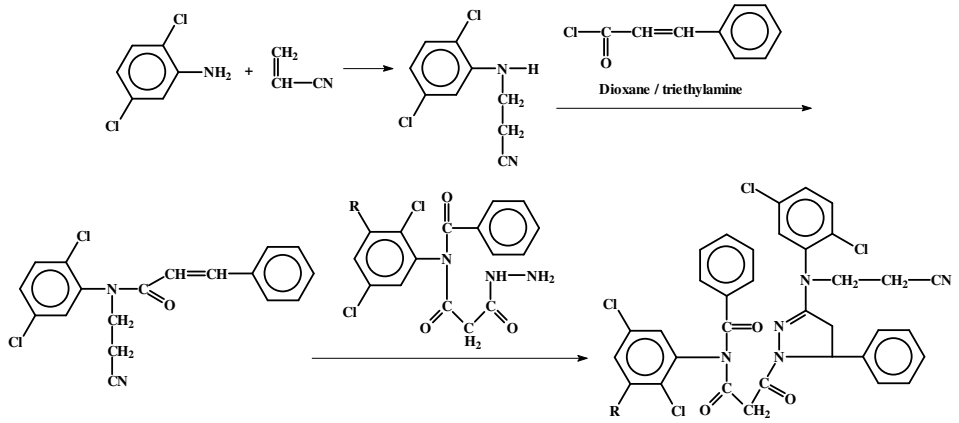
Synthesis of N-cinnamoyl -N-2'-cyanoethyl-2,5-dichloroaniline [6]: Solution of cinnamoyl chloride (3.5 g, 0.02 mol), dioxane (2 mL), N-2'-cyanoethyl-2,5-dichloro aniline (7.90 g, 0.02 mol) and triethylamine (2.1 g) were placed in a round bottomed flask having a Liebig condenser carrying calcium chloride guard tube. The contents were heated for 2 h on a boiling water bath. On keeping overnight triethylamine hydrochloride separated as solid. It was filtered and contents concentrated when crystals separated out. Two crystallization from ethanol gave shining white needles. Yield 55 %, m.p.: 156 ºC, anal. calcd. (%) for $C_{18}H_{14}N_2OCl_2$; m.w. 345; N: 4.5, Cl: 11.3; found. (%) N: 4.3, Cl: 11.2, IR [KBr, ν_{max}, cm^{-1}]: 3280-3050 (C-H stretching, aromatic), 2955 and 2890 (C-H stretching, aliphatic (asymmetric) and C-H stretching, aliphatic (symmetric), 2215 (C-N stretching), 1655 (C=C stretching, benzene ring), 1645 C=O (stretching, tertiary amide), 1615, 1575, 1455, (C=C ring stretching), 1050, 750, (2,5-disubstituted benzene).

Synthesis of 1-[(2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7]: A mixture of N-cinnamoyl-N-2'-cyanoethyl-2,5-dichloroaniline (0.345 g; 0.001 mol), ethyl-2-(2,5-dichloroanilido) acetohydrazide (0.262 g; 0.001 mol), dioxane (3 mL) and glacial acetic acid (2 drops) was refluxed for 5 h. The solid which separated during the course of heating was filtered under suction and purified by washing thrice with hot ethanol, when the pyrazoline was obtained as yellow needles. Yield 64 %, m.p.: 258 ºC, m.w.: 693, anal. calcd. (%) for $C_{34}H_{25}N_5O_3Cl_4$: 13.1; N: 6.5, found. (%) Cl: 13.2, N: 6.4. UV [(λ_{max}^{EtOH} nm), log ε]: 216.4 (4.94), 319.5 (4.78). IR [KBr, ν_{max}, cm^{-1}]: 3300-2870 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250 (C≡N stretching), 1665 [C=O and N-H (amide)], 1595 (C=N stretching), 1585, 1475, 1425 (C=C ring stretching, aromatic), 1045, 825, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.24-2.46 (2H, s, CH_2), 3.6-3.9 (3H, s, CH_3), 4.14-4.40 (1H, s, NH), 6.96-7.40 (13H, m, ArH). 3.18 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.66 H_Z, C_4-H_A of pyrazoline ring). 3.94 (1H, dd J_{MA} = 17.85 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.75 (1H, d, J = 16.14 Hz COCH geminal proton), 5.56 (1H, dd J_{MX} 12.85 H_Z, J_{AX} = 4.65 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 179.57 (C=O), 159.79 (C=N), 142.05, 137.66, 134.43, 131.88 (4C, ArC's), 133.48, 130.50, 128.63, 126.78, 112.23 (5C, Ar CH's), 62.69 (CH_2, ester), 61.82 (C-5, pyrazoline), 46.99 (C-4, pyrazoline), 18.88 (CH_3). -MS-FAB$^+$: m/z: 693 [M].

Synthetic sequence for new pyrazolines has been outlined in **Scheme-I**.

Some characteristics of the synthesized compounds are shown in Table-1. Analytical and spectral data (UV, IR, ^{1}H NMR, FAB$^+$-MS) confirmed the structures of the new compounds.

1-[(2,5-Dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7a]: Yield 64 %, m.p.: 258 ºC, m.w.: 693, anal. calcd. (%) for $C_{34}H_{25}N_5O_3Cl_4$: 13.1; N: 6.5, found. (%) Cl: 13.2, N: 6.4.



Scheme-I: Reaction scheme for the complete synthesis of compounds

TABLE-1
(UNSUBSTITUTED/SUBSTITUTED) 1-[(2, 5-DICHLOROANILINOMALONYL)-3-(N-2'-CYANOETHYL)-2-(N-BENZOYL)-2,5-DICHLOROANILINO)]-5-PHENYL PYRAZOLINE

Comp. No.	R	Colour	m.p. (ºC)	Yield (%)	m.w.	m.f.
7a	H	Yellow	258	64	693.0	$C_{34}H_{25}N_5O_3Cl_4$
7b	CH_3 (*o*)	Cream	270	44	707.0	$C_{35}H_{27}N_5O_3Cl_4$
7c	CH_3 (*m*)	Light yellow	266	51	707.0	$C_{35}H_{27}N_5O_3Cl_4$
7d	CH_3 (*p*)	Light yellow	248	56	707.0	$C_{35}H_{27}N_5O_3Cl_4$
7e	Cl (*o*)	White	262	50	727.5	$C_{34}H_{24}N_5O_3Cl_5$
7f	Cl (*m*)	Light yellow	260	61	727.5	$C_{34}H_{24}N_5O_3Cl_5$
7g	Cl (*p*)	Cream	265	63	727.5	$C_{34}H_{24}N_5O_3Cl_5$
7h	OCH_3 (*o*)	Yellow	248	68	723.0	$C_{35}H_{27}N_5O_4Cl_4$
7i	OCH_3 (*m*)	White	254	70	723.0	$C_{35}H_{27}N_5O_4Cl_4$
7j	OCH_3 (*p*)	Cream	259	76	723.0	$C_{35}H_{27}N_5O_4Cl_4$
7k	F (*p*)	Yellow	246	54	711.0	$C_{34}H_{24}N_5O_3F_1Cl_4$
7l	Br (*o*)	Dark brown	256	52	772.0	$C_{34}H_{24}N_5O_3BrCl_4$
7m	OC_2H_5 (*o*)	L. brown	262	62	738.0	$C_{36}H_{29}N_5O_4Cl_4$
7n	OC_2H_5 (*m*)	Brown	253	66	738.0	$C_{36}H_{29}N_5O_4Cl_4$
7o	OC_2H_5 (*p*)	Brown	246	62	738.0	$C_{36}H_{29}N_5O_4Cl_4$
7p	CO_2H (*o*)	Brown	259	67	738.0	$C_{35}H_{25}N_5O_5Cl_4$
7q	CO_2H (*m*)	Brown	252	61	738.0	$C_{35}H_{25}N_5O_5Cl_4$
7r	CO_2H (*p*)	L. brown	255	54	738.0	$C_{35}H_{25}N_5O_5Cl_4$
7s	Br (*m*)	Brown	246	58	772.0	$C_{34}H_{24}N_5O_3BrCl_4$
7t	Br (*p*)	Brown	258	52	772.0	$C_{34}H_{24}N_5O_3BrCl_4$

All compounds gave satisfactory elemental analysis.

UV [(λ_{max}^{EtOH} nm), log ε]: 216.4 (4.94), 319.5 (4.78). IR [KBr, ν_{max}, cm^{-1}]: 3300-2870 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250

(C≡N stretching), 1665 [C=O and N-H (amide)], 1595 (C=N stretching), 1585, 1475, 1425 (C=C ring stretching, aromatic), 1045, 825, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.24-2.46 (2H, s, CH_2), 3.6-3.9 (3H, s, CH_3), 4.14-4.40 (1H, s, NH), 6.96-7.40 (13H, m, ArH). 3.18 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.66 H_Z, C_4-H_A of pyrazoline ring). 3.94 (1H, dd J_{MA} = 17.85 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.75 (1H, d, J = 16.14 Hz COCH geminal proton), 5.56 (1H, dd J_{MX} 12.85 H_Z, J_{AX} = 4.65 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 179.57 (C=O), 159.79 (C=N), 142.05, 137.66, 134.43, 131.88 (4C, ArC's), 133.48, 130.50, 128.63, 126.78, 112.23 (5C, Ar CH's), 62.69 (CH_2, ester), 61.82 (C-5, pyrazoline), 46.99 (C-4, pyrazoline), 18.88 (CH_3). -MS-FAB$^+$: m/z: 693 [M].

1- [(*o*-Methyl)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl) 2, 5-dichloroanilino)]-5-phenyl pyrazoline [7b]: Yield 44 %, m.p.: 270 ºC, m.w. 707, anal. calcd. (%) for $C_{35}H_{27}N_5O_3Cl_4$, N: 4.4; found. (%) N: 4.5, Cl: 8.8; found. (%) Cl: 8.7. UV [(λ_{max}^{EtOH} nm), log ε]: 214.6(4.90), 319.4 (4.82). IR [KBr, ν_{max}, cm^{-1}]: 3300-2890 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond) (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2242 (C≡N stretching), 1650 [C=O and N-H (amide)], 1580 (C=N stretching), 1585, 1478, 1430 (C=C ring stretching, aromatic), 1045, 822, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.23-2.48 (2H, s, CH_2), 4.16-4.30 (1H, s, NH), 6.90-7.45 (13H, m, ArH). 3.10 (1H, dd, J_{AM} = 16 H_Z, J_{AX} = 4.60 H_Z, C_4-H_A of pyrazoline ring). 3.98 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 13.80 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d, J = 16.43 Hz COCH geminal proton), 5.70 (1H, dd J_{MX} 12.40 H_Z, J_{AX} = 4.50 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 181.58 (C=O), 158.74 (C=N), 143.07, 136.54, 133.40, 130.74 (4C, ArC's), 131.47, 130.36, 126.62, 124.70, 114.31 (5C, Ar CH's), 63.66 (CH_2, ester), 60.81 (C-5, pyrazoline), 46.91 (C-4, pyrazoline), 18.82 (CH_3). -MS-FAB$^+$: m/z: 707 [M].

1- [(*m*-Methyl)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7c]: Yield 51 %, m.p.: 266 ºC, m.w.: 707, anal. calcd. (%) for $C_{35}H_{27}N_5O_3Cl_4$, Cl: 10.2; N: 5.0, found. (%) Cl: 10.1, N: 5.1. UV [(λ_{max}^{EtOH} nm), log ε]: 212.2 (4.92), 318.6 (4.78). IR [KBr, ν_{max}, cm^{-1}]: 3300-2950 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2240 (C≡N stretching), 1670 [C=O and N-H (amide)], 1575 (C=N stretching), 1560, 1430, 1410 (C=C ring stretching, aromatic), 1050, 815, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.32-2.56 (2H, s, CH_2), 4.35-4.55 (1H, s, NH), 6.40-7.20 (13H, m, ArH). 3.10 (1H, dd, J_{AM} = 17 H_Z, J_{AX} = 4.55 H_Z, C_4-H_A of pyrazoline ring). 3.88 (1H, dd J_{MA} = 17.70 Hz, J_{MX} = 13.55 Hz, C_4-H_M of pyrazoline ring), 4.68 (1H, d, J = 16.16 Hz COCH geminal proton), 5.66 (1H, dd J_{MX} 12.60 H_Z, J_{AX} = 4.40 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 167.56 (C=O), 154.61 (C=N), 143.01, 136.62, 133.43, 130.85 (4C, ArC's),

132.48, 130.55, 128.66, 125.77, 112.28 (5C, Ar CH's), 62.67 (CH_2, ester), 61.83 (C-5, pyrazoline), 45.92 (C-4, pyrazoline), 18.84 (CH_3). -MS-FAB$^+$: m/z: 707 [M].

1- [(*p*-Methyl)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7d]: Yield 56 %, m.p.: 248 ºC, m.w.: 707, anal. calcd. for $C_{35}H_{27}Cl_4N_5O_3$, Cl: 11.2; N: 5.5, found. (%) Cl: 11.1, N: 5.6. UV [(λ_{max}^{EtOH} nm), log ε]: 227.3 (4.96), 319.6 (4.70). IR [KBr, ν_{max}, cm^{-1}]: 3300-3040 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250 (C≡N stretching), 1620 [C=O and N-H (amide)], 1570 (C=N stretching), 1550, 1460, 1430 (C=C ring stretching, aromatic), 1040, 825, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.14-2.41 (2H, s, CH_2), 4.28-4.35 (1H, s, NH), 6.80-7.60 (13H, m, ArH). 3.28 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.61 H_Z, C_4-H_A of pyrazoline ring). 3.87 (1H, dd J_{MA} = 17.79 Hz, J_{MX} = 13.58 Hz, C_4-H_M of pyrazoline ring), 4.68 (1H, d, J = 16.45 Hz COCH geminal proton), 6.11 (1H, dd J_{MX} 13.30 H_Z, J_{AX} = 4.65 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ ppm 174.55 (C=O), 157.77 (C=N), 139.15, 135.65, 133.44, 131.80 (4C, ArC's), 131.42, 129.85, 126.62, 124.64, 111.17 (5C, Ar CH's), 64.61 (CH_2, ester), 62.81 (C-5, pyrazoline), 45.92 (C-4, pyrazoline), 17.93 (CH_3). -MS-FAB$^+$: m/z: 707 [M].

1- [(*o*-Chloro)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7e]: Yield 50 %, m.p.: 262 ºC, m.w.: 727.5, anal. calcd. (%) for $C_{34}H_{24}Cl_5N_5O_3$, Cl: 12.2; N: 4.8, found. (%) Cl: 12.1, N: 4.6. UV [(λ_{max}^{EtOH} nm), log ε]: 215.5 (5.10), 319.2 (5.16). IR [KBr, ν_{max}, cm^{-1}]: 3300-3110 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2290 (C≡N stretching), 1680 [C=O and N-H (amide)], 1540 (C=N stretching), 1530, 1490, 1440 (C=C ring stretching, aromatic), 1080, 890, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 3.10-3.18 (2H, s, CH_2), 4.19-4.55 (1H, s, NH), 6.87-7.20 (13H, m, ArH). 3.10 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.62 H_Z, C_4-H_A of pyrazoline ring). 4.05 (1H, dd J_{MA} = 18.10 Hz, J_{MX} = 13.90 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d, J = 16.19 Hz COCH geminal proton), 5.45 (1H, dd J_{MX} 13.15 H_Z, J_{AX} = 5.10 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ ppm 164.79 (C=O), 154.72 (C=N), 147.22, 143.60, 138.44, 132.83 (4C, ArC's), 130.79, 128.85, 123.63, 121.72, 115.26 (5C, Ar CH's), 64.60 (CH_2, ester), 60.92 (C-5, pyrazoline), 47.15 (C-4, pyrazoline), 19.10 (CH_3). -MS-FAB$^+$: m/z: 727 [M], 728 [M + 1].

1- [(*m*-Chloro)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7f]: Yield 61 %, m.p.: 260 ºC, m.w.: 727.5, anal. calcd. (%) for $C_{34}H_{24}Cl_5N_5O_3$, Cl: 14.9; N: 5.9, found. (%) Cl: 14.7, N: 5.6. UV [(λ_{max}^{EtOH} nm), log ε]: 214.6 (4.97), 322.4 (4.81). IR [KBr, ν_{max}, cm^{-1}]: 3300-3120 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2240

(C≡N stretching), 1658 [C=O and N-H (amide)], 1605 (C=N stretching), 1570, 1460, 1430 (C=C ring stretching, aromatic), 1070, 830, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.58-2.87 (2H, s, CH_2), 4.35-4.62 (1H, s, NH), 7.10-7.55 (13H, m, ArH). 3.34 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.70 H_Z, C_4-H_A of pyrazoline ring). 4.15 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 13.20 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d, J = 16.44 Hz COCH geminal proton), 5.55 (1H, dd J_{MX} 13.30 H_Z, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 178.57 (C=O), 155.65 (C=N), 144.11, 138.64, 135.44, 132.82 (4C, ArC's), 131.88, 130.15, 126.60, 123.80, 116.26 (5C, Ar CH's), 61.66 (CH_2, ester), 59.95 (C-5, pyrazoline), 47.93 (C-4, pyrazoline), 18.95 (CH_3). -MS-FAB$^+$: m/z: 727 [M], 728 [M + 1].

1-[(*p*-Chloro)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7g]: Yield (%): 63, m.p.: 265 ºC, m.w.: 727.5, anal. calcd. (%) for $C_{34}H_{24}Cl_5N_5O_3$, Cl: 15.4; N: 6.1, found. (%) Cl: 15.4, N: 6.0. UV [(λ_{max}^{EtOH} nm), log ε]: 216.3 (5.20), 340.6 (4.88). IR [KBr, ν_{max}, cm^{-1}]: 3300-2960 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2290 (C≡N stretching), 1680 [C=O and N-H (amide)], 1620 (C=N stretching), 1575, 1465, 1415 (C=C ring stretching, aromatic), 1035, 825, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.86-3.10 (2H, s, CH_2), 4.19-4.45 (1H, s, NH), 6.90-7.42 (13H, m, ArH). 3.28 (1H, dd, J_{AM} = 17 H_Z, J_{AX} = 4.68 H_Z, C_4-H_A of pyrazoline ring). 3.70 (1H, dd J_{MA} = 17.81 Hz, J_{MX} = 13.30 Hz, C_4-H_M of pyrazoline ring), 4.20 (1H, d, J = 16.48 Hz COCH geminal proton), 5.22 (1H, dd J_{MX} 12.89 H_Z, J_{AX} = 4.57 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 169.52 (C=O), 157.78 (C=N), 152.20, 148.65, 142.44, 138.85 (4C, ArC's), 134.48, 132.53, 129.68, 123.77, 126.27 (5C, Ar CH's), 64.67 (CH_2, ester), 62.60 (C-5, pyrazoline), 47.25 (C-4, pyrazoline), 18.35 (CH_3). -MS-FAB$^+$: m/z: 727 [M], 728 [M + 1].

1-[(*o*-Methoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7h]: Yield 68 %, m.p.: 248 ºC, m.w.: 723, anal. calcd. for $C_{35}H_{27}Cl_4N_5O_4$, Cl: 13.4; N: 6.6, found. (%) Cl: 13.2, N: 6.5. UV [(λ_{max}^{EtOH} nm), log ε]: 215.3 (5.04), 318.4 (4.79). IR [KBr, ν_{max}, cm^{-1}]: 3300-2880 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2270 (C≡N stretching), 1640 [C=O and N-H (amide)], 1575 (C=N stretching), 1570, 1455, 1440 (C=C ring stretching, aromatic), 1050, 810, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.38-2.51 (2H, s, CH_2), 4.29-4.50 (1H, s, NH), 6.90-7.20 (13H, m, ArH). 3.27 (1H, dd, J_{AM} = 17 H_Z, J_{AX} = 4.55 H_Z, C_4-H_A of pyrazoline ring). 3.98 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 13.80 Hz, C_4-H_M of pyrazoline ring), 4.82 (1H, d, J = 16.23 Hz COCH geminal proton), 5.51 (1H, dd J_{MX} 11.90 H_Z, J_{AX} = 4.40 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 173.52 (C=O), 158.70 (C=N), 144.10, 138.62, 135.65, 130.85 (4C, ArC's), 133.38,

131.40, 129.46, 123.80, 116.18 (5C, Ar CH's), 63.66 (CH_2, ester), 63.68 (C-5, pyrazoline), 45.92 (C-4, pyrazoline), 19.15 (CH_3). -MS-FAB$^+$: m/z: 723 [M].

1-[(*m*-Methoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5- phenyl pyrazoline [7i]: Yield 70 %, m.p. 254 ºC, m.w.: 723, anal. calcd. (%) for $C_{35}H_{27}Cl_4N_5O_4$, Cl: 13.7; N: 6.8, found. (%) Cl: 13.6, N: 6.7. UV [(λ_{max}^{EtOH} nm), log ε]: 218.1 (4.95), 317.9 (4.68). IR [KBr, ν_{max}, cm^{-1}]: 3300-2910 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2240 (C≡N stretching), 1660 [C=O and N-H (amide)], 1590 (C=N stretching), 1585, 1480, 1410 (C=C ring stretching, aromatic), 1060, 825, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.12-2.49 (2H, s, CH_2), 4.14-4.45 (1H, s, NH), 7.10-7.40 (13H, m, ArH). 3.22 (1H, dd, J_{AM} = 19 H_Z, J_{AX} = 4.59 H_Z, C_4-H_A of pyrazoline ring). 4.10 (1H, dd J_{MA} = 17.80 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.74 (1H, d, J = 16.10 Hz COCH geminal proton), 5.70 (1H, dd J_{MX} 12.40 H_Z, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 178.56 (C=O), 153.77 (C=N), 142.05, 139.40, 132.45, 130.80 (4C, ArC's), 131.45, 129.80, 127.84, 125.70, 113.18 (5C, Ar CH's), 61.67 (CH_2, ester), 62.82 (C-5, pyrazoline), 46.65 (C-4, pyrazoline), 18.42 (CH_3). -MS-FAB$^+$: m/z: 723 [M].

1-[(*p*-Methoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7j]: Yield 76 %, m.p.: 259 ºC, m.w.: 723, anal. calcd. (%) for $C_{35}H_{27}Cl_4N_5O_4$, Cl: 14.9; N: 7.4, found. (%) Cl: 14.6, 6.9. UV [(λ_{max}^{EtOH} nm), log ε]: 216.4 (4.93), 318.7 (4.76). IR [KBr, ν_{max}, cm^{-1}]: 3300-2890 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2230 (C≡N stretching), 1680 [C=O and N-H (amide)], 1610 (C=N stretching), 1590, 1520, 1460 (C=C ring stretching, aromatic), 1030, 840, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.20-2.56 (2H, s, CH_2), 4.10-4.80 (1H, s, NH), 6.85-7.10 (13H, m, ArH). 3.18 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.62 H_Z, C_4-H_A of pyrazoline ring). 3.97 (1H, dd J_{MA} = 18.20 Hz, J_{MX} = 13.50 Hz, C_4-H_M of pyrazoline ring), 4.80 (1H, d, J = 16.18 Hz COCH geminal proton), 5.60 (1H, dd J_{MX} 12.70 H_Z, J_{AX} = 4.65 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 174.55 (C=O), 158.71 (C=N), 143.10, 138.60, 137.45, 133.85 (4C, ArC's), 132.48, 130.55, 128.66, 125.75, 114.68 (5C, Ar CH's), 62.80 (CH_2, ester), 63.20 (C-5, pyrazoline), 46.80 (C-4, pyrazoline), 18.86 (CH_3). -MS-FAB$^+$: m/z: 723 [M].

1-[(*p*-Floro)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7k]: Yield 54 %, m.p.: 246 ºC, m.w.: 711, anal. calcd. (%) for $C_{34}H_{24}Cl_4FN_5O_3$, Cl: 10.8; N: 5.3, found. (%) Cl: 10.6, N: 4.9. UV [(λ_{max}^{EtOH} nm), log ε]: 222.5 (4.98), 317.9 (4.73). IR [KBr, ν_{max}, cm^{-1}]: 3300-2860 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250 (C N stretching), 1660 [C=O and N-H (amide)], 1575 (C=N stretching), 1570, 1460,

1430 (C=C ring stretching, aromatic), 1070, 860, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.18-2.34 (2H, s, CH_2), 4.16-4.70 (1H, s, NH), 6.70-7.10 (13H, m, ArH). 3.16 (1H, dd, J_{AM} = 17 H_Z, J_{AX} = 4.60 H_Z, C_4-H_A of pyrazoline ring). 3.93 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 13.70 Hz, C_4-H_M of pyrazoline ring), 4.90 (1H, d, J = 16.40 Hz COCH geminal proton), 5.55 (1H, dd J_{MX} 12.90 H_Z, J_{AX} = 4.55 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 176.47 (C=O), 156.78 (C=N), 142.05, 137.62, 135.45, 132.84 (4C, ArC's), 130.28, 129.50, 126.60, 122.70, 111.88 (5C, Ar CH's), 63.10 (CH_2, ester), 62.40 (C-5, pyrazoline), 47.10 (C-4, pyrazoline), 18.95 (CH_3). -MS-FAB$^+$: m/z: 711 [M].

1-[(*o*-Bromo)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7l]: Yield 52 %, m.p.: 256 ºC, m.w.: 772, anal. calcd. (%) for $C_{34}H_{24}Cl_4N_5O_3Br$, Cl: 10.4; N: 4.7, found. (%) Cl: 10.3, N: 4.5. UV [(λ_{max}^{EtOH} nm), log ε]: 210.2 (4.93), 318.7 (4.85). IR [KBr, ν_{max}, cm^{-1}]: 3300-2880 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2230 (C≡N stretching), 1620 [C=O and N-H (amide)], 1555 (C=N stretching), 1605, 1510, 1490 (C=C ring stretching, aromatic), 1060, 840, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.20-2.54 (2H, s, CH_2), 4.25-4.45 (1H, s, NH), 6.80-7.30 (13H, m, ArH). 3.25 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.55 H_Z, C_4-H_A of pyrazoline ring). 4.04 (1H, dd J_{MA} = 17.70 Hz, J_{MX} = 13.50 Hz, C_4-H_M of pyrazoline ring), 4.80 (1H, d, J = 16.66 Hz COCH geminal proton), 5.68 (1H, dd J_{MX} 13.10 H_Z, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 178.70 (C=O), 158.72 (C=N), 141.10, 138.40, 136.49, 130.85 (4C, ArC's), 131.48, 130.32, 127.66, 124.77, 113.38 (5C, Ar CH's), 62.60 (CH_2, ester), 61.84 (C-5, pyrazoline), 45.92 (C-4, pyrazoline), 19.06 (CH_3). -MS-FAB$^+$: m/z: 772 [M].

1- [(*o*-Ethoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7m]: Yield 62 %, m.p.: 262 ºC, m.w.: 738, anal. calcd. (%) for $C_{36}H_{29}Cl_4N_5O_4$, Cl: 11.9; N: 5.9, found. (%) Cl: 11.7, N: 5.8. UV [(λ_{max}^{EtOH} nm), log ε]: 212.5 (4.98), 318.4 (4.88). IR [KBr, ν_{max}, cm^{-1}]: 3300-2920 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2260 (C≡N stretching), 1640 [C=O and N-H (amide)], 1580 (C=N stretching), 1590, 1480, 1460 (C=C ring stretching, aromatic), 1050, 860, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.30-2.44 (2H, s, CH_2), 4.14-4.40 (1H, s, NH), 6.80-7.20 (13H, m, ArH). 3.17 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.60 H_Z, C_4-H_A of pyrazoline ring). 3.95 (1H, dd J_{MA} = 17.80 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.55 (1H, d, J = 16.35 Hz COCH geminal proton), 5.50 (1H, dd J_{MX} 12.90 H_Z, J_{AX} = 4.65 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 176.58 (C=O), 156.74 (C=N), 140.05, 136.65, 135.45, 132.90 (4C, ArC's), 131.46, 130.52, 129.66, 126.72, 112.44 (5C, Ar CH's), 62.90 (CH_2, ester), 61.88 (C-5, pyrazoline), 46.35 (C-4, pyrazoline), 18.80 (CH_3). -MS-FAB$^+$: m/z: 738 [M].

1- [(*m*-Ethoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7n]: Yield 66 %, m.p.: 253 ºC (d), m.w.: 738, anal. calcd. (%) for $C_{36}H_{29}Cl_4N_5O_4Cl$: 12.7; N: 6.3, found. (%) Cl: 12.4, N: 6.0. UV [(λ_{max}^{EtOH} nm), log ε]: 210.2 (4.89), 318.5 (4.72). IR [KBr, n_{max}, cm^{-1}]: 3300-2890 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2240 (C≡N stretching), 1670 [C=O and N-H (amide)], 1570 (C=N stretching), 1580, 1460, 1430 (C=C ring stretching, aromatic), 1055, 830, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.14-2.26 (2H, s, CH_2), 4.18-4.30 (1H, s, NH), 7.0-7.30 (13H, m, ArH). 3.15 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.60 H_Z, C_4-H_A of pyrazoline ring). 3.90 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 13.55 Hz, C_4-H_M of pyrazoline ring), 4.75(1H, d, J = 16.12 Hz COCH geminal proton), 5.55 (1H, dd J_{MX} 12.70 H_Z, J_{AX} = 4.50 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 174.54 (C=O), 153.78 (C=N), 143.10, 140.64, 137.45, 136.85 (4C, ArC's), 133.48, 131.55, 127.66, 124.57, 112.28 (5C, Ar CH's), 64.65 (CH_2, ester), 62.85 (C-5, pyrazoline), 46.45 (C-4, pyrazoline), 18.95 (CH_3). -MS-FAB$^+$: m/z: 738 [M].

1- [(*p*-Ethoxy)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7o]: Yield 62 %, m.p.: 246 ºC, m.w.: 738, anal. calcd. (%) for $C_{36}H_{29}Cl_4N_5O_4Cl$: 11.9; N: 5.9, found. (%) Cl: 11.6, N: 5.8. UV [(λ_{max}^{EtOH} nm), log ε]: 218.2 (4.88), 318.6 (4.72). IR [KBr, n_{max}, cm^{-1}]: 3300-2930 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250 (C≡N stretching), 1640 [C=O and N-H (amide)], 1555 (C=N stretching), 1590, 1450, 1430 (C=C ring stretching, aromatic), 1045, 840, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.20-2.46 (2H, s, CH_2), 4.10-4.45 (1H, s, NH), 6.90-7.30 (13H, m, ArH). 3.20 (1H, dd, J_{AM} = 19 H_Z, J_{AX} = 4.80 H_Z, C_4-H_A of pyrazoline ring). 3.90 (1H, dd J_{MA} = 17.60 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.70 (1H, d, J = 16.20 Hz COCH geminal proton), 5.65 (1H, dd J_{MX} 12.60 H_Z, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 181.52 (C=O), 162.78 (C=N), 142.20, 138.65, 137.42, 133.84 (4C, ArC's), 129.88, 128.50, 127.60, 126.75, 110.38 (5C, Ar CH's), 63.67 (CH_2, ester), 61.83 (C-5, pyrazoline), 46.65 (C-4, pyrazoline), 18.99 (CH_3). -MS-FAB$^+$: m/z: 738 [M].

1-[(*m*-Bromo)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7s]: Yield 58 %, m.p.: 246 ºC, m.w.: 772, anal. calcd. (%) for $C_{34}H_{24}Cl_4N_5O_3BrCl$: 10.7; N: 5.3, found. (%) Cl: 10.5, N: 4.9. UV [(λ_{max}^{EtOH} nm), log ε]: 214.3 (4.90), 318.4 (4.70). IR [KBr, n_{max}, cm^{-1}]: 3300-2890 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2240 (C≡N stretching), 1660 [C=O and N-H (amide)], 1570 (C=N stretching), 1570, 1490, 1470 (C=C ring stretching, aromatic), 1050, 830, (C-Cl stretching, 2,5-disubs-

tituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.28-2.52 (2H, s, CH_2), 4.13-4.30 (1H, s, NH), 6.90-7.55 (13H, m, ArH). 3.15 (1H, dd, J_{AM} = 18 H_Z, J_{AX} = 4.70 H_Z, C_4-H_A of pyrazoline ring). 3.95 (1H, dd J_{MA} = 17.70 Hz, J_{MX} = 13.50 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d, J = 16.10 Hz COCH geminal proton), 5.80 (1H, dd J_{MX} 12.90 H_Z, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 178.57 (C=O), 157.77 (C=N), 140.15, 136.64, 134.40, 130.80 (4C, ArC's), 130.18, 128.75, 127.66, 125.78, 113.19 (5C, Ar CH's), 61.62 (CH_2, ester), 61.70 (C-5, pyrazoline), 46.90 (C-4, pyrazoline), 18.75 (CH_3). -MS-FAB$^+$: m/z: 772 [M].

1-[(*p*-Bromo)-2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline [7t]: Yield 52 %, m.p.: 258 ºC, m.w.: 772, anal. calcd. (%) for $C_{34}H_{24}Cl_4N_5O_3Br$, Cl: 9.6; N: 4.7, found Cl: 9.4, N: 4.5. UV [(λ_{max}^{EtOH} nm), log ε]: 210.2 (4.94), 318.7 (4.76). IR [KBr, ν_{max}, cm^{-1}]: 3300-2850 [broad band due to (I) N-H stretching, secondary amide (intra molecular hydrogen bond), (II) C-H stretching, aromatic, (iii) C-H stretching, aliphatic], 2250 (C≡N stretching), 1650 [C=O and N-H (amide)], 1580 (C=N stretching), 1560, 1480, 1440 (C=C ring stretching, aromatic), 1040, 840, (C-Cl stretching, 2,5-disubstituted aromatic ring). ^{1}H NMR (250 MHz, δ ppm, DMSO-d_6): 2.20-2.44 (2H, s, CH_2), 4.15-4.45 (1H, s, NH), 6.90-7.45 (13H, m, ArH). 3.20 (1H, dd, J_{AM} = 17 H_Z, J_{AX} = 4.60 H_Z, C_4-H_A of pyrazoline ring). 3.90 (1H, dd J_{MA} = 17.85 Hz, J_{MX} = 13.65 Hz, C_4-H_M of pyrazoline ring), 4.75 (1H, d, J = 16.15 Hz COCH geminal proton), 5.55 (1H, dd J_{MX} 12.85 H_Z, J_{AX} = 4.64 Hz, C_5-H_X of pyrazoline ring). ^{13}C NMR: δ/ppm 180.55 (C=O), 161.78 (C=N), 142.15, 138.65, 136.45, 133.80 (4C, ArC's), 131.46, 128.50, 127.65, 125.70, 114.27 (5C, Ar CH's), 62.68 (CH_2, ester), 60.88 (C-5, pyrazoline), 47.20 (C-4, pyrazoline), 18.95 (CH_3). -MS-FAB$^+$: m/z: 772 [M]. Most of the pyrazolines are high melting point and light yellow or cream coloured solids. The data of new products are furnished in Table-1.

Biological evaluation

Antibacterial activity: Newly synthesized compounds (**6a-t**) have been tested for their antibacterial activity against gram positive bacteria *S. albus*, *S. aureus* and gram negative bacteria *E. coli* and *Pseudomonas poisonous* by agar plate disc diffusion method at 30 µg/mL concentration. Ampicillin and tetracycline used as a reference compound. The compound (**6a, 6b, 6c, 6f, 6g, 6j, 6m** and **6r**) shown significant activity and the compound (**6i, 6k, 6l, 6p, 6t**) have shown moderate activity.

Antifungal activity: The same compounds were tested for their antifungal activity against *Candida albicans*, *Aspergillus niger* and *Alternaria alternata* at concentration of 30 mg/mL using sabouraud dextrose agar media. The compound (**6c, 6j, 6m** and **6r**) shown significant activities and compound (**6a, 6b, 6f** and **6g**) were found to be moderately active against *Candida albicans* and *Aspergillus niger*. All the other compounds did not show significant activity against the fungi at the concentration used.

Tuberculostatic activity: Some new compounds have been tested for antitubercular activity *in vitro* using *Mycobacterium tuberculosis*. The compounds were incorporated into Lowenstein Jensen egg medium having concentrations of 10 and 100 mg/mL and were inoculated with *Mycobacterium tuberculosis*, H_{27}, R_V strains, incubated at 37 ºC and observed, weekly for the growth of organism for 8 weeks. The compound (**6a, 6b, 6c, 6f, 6g, 6j** and **6m**) inhibited the growth of *Mycobacterium tuberculosis* at 100 mg/mL concentration other compounds were found to be inactive. Results are assembled in Table-2.

TABLE-2
TUBERCULOSTATIC ACTIVITY OF NEW PYRAZOLINES

Compound No.	Growth at conc. [mg/mL]	
	10	100
7a	+	0
7b	+	0
7c	+	0
7d	+	+
7e	+	+
7f	+	0
7g	+	0
7h	+	+
7i	+	0
7j	+	+
7k	+	+
7l	+	+
7m	+	0
7n	+	+
7o	+	+
7s	+	+
7t	+	+

'+' and '0' indicate presence and inhibition of growth, respectively.

RESULTS AND DISCUSSION

Newly synthesized 1-[(2,5-dichloroanilinomalonyl)-3-(N-2'-cyanoethyl)-2-(N-benzoyl)-2,5-dichloroanilino)]-5-phenyl pyrazoline have been synthesized by the reaction of N-cinnamoyl-N-2'-cyanoethyl-2,5-dichloroaniline with ethyl-2-[(N-benzoyl)-2,5-dichloroanilido] acetohydrazide. Pyrazolines are yellow, cream and brown colour solids, having high melting points. Identity of these products has been established by elemental analysis and spectral data. Newly synthesized compounds (**6a-t**) have been tested for their antibacterial activity against gram positive bacteria *S. albus*, *S. aureus* and gram negative bacteria *E. coli* and *Pseudomonas poisonous*. The compound (**6a, 6b, 6c, 6f, 6g, 6j, 6m** and **6r**) shown significant activity and the compound (**6i, 6k, 6l, 6p, 6t**) have shown moderate activity. The

same compounds were tested for their antifungal activity against *Candida albicans*, *Aspergillus niger* and *Alternaria alternata* at concentration of 30 mg/mL using sabouraud dextrose agar media. The compound (**6c, 6j, 6m** and **6r**) shown significant activities and compound (**6a, 6b, 6f** and **6g**) were found to be moderately active against *Candida albicans* and *Aspergillus niger*. All the other compounds did not show significant activity against the fungi at the concentration used. Some new compounds have been tested for antitubercular activity *in vitro* using *Mycobacterium tuberculosis*. The compounds were incorporated into Lowenstein Jensen egg medium having concentrations of 10 and 100 mg/mL and were inoculated with *Mycobacterium tuberculosis*, H_{27}, R_V strains, incubated at 37 ºC and observed, the compound (**6a, 6b, 6c, 6f, 6g, 6j** and **6m**) inhibited the growth of *Mycobacterium tuberculosis* at 100 mg/mL concentration other compounds were found to be inactive.

Conclusion

Newly synthesized compounds (**6a-t**) have been tested for their antibacterial activity against gram positive bacteria *S. albus*, *S. aureus* and gram negative bacteria *E. coli* and *Pseudomonas poisonous*. The compound (**6a, 6b, 6c, 6f, 6g, 6j, 6m** and **6r**) shown significant activity and the compound (**6i, 6k, 6l, 6p, 6t**) have shown moderate activity. The same compounds were tested for their antifungal activity against *Candida albicans*, *Aspergillus niger* and *Alternaria alternata* at concentration of 30 mg/mL using sabouraud dextrose agar media. The compound (**6c, 6j, 6m** and **6r**) shown significant activities and compound (**6a, 6b, 6f** and **6g**) were found to be moderately active against *Candida albicans* and *Aspergillus niger*. All the other compounds did not show significant activity against the fungi at the concentration used. Some new compounds have been tested for antitubercular activity *in vitro* using *Mycobacterium tuberculosis*. The compounds were incorporated into Lowenstein Jensen egg medium having concentrations of 10 and 100 mg/mL and were inoculated with *Mycobacterium tuberculosis*, H_{27}, R_V strains, incubated at 37 ºC and observed, the compound (**6a, 6b, 6c, 6f, 6g, 6j** and **6m**) inhibited the growth of *Mycobacterium tuberculosis* at 100 mg/mL concentration other compounds were found to be inactive.

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