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# Synthesis and Studies on Some New Fluorine Containing Hydroxypyrazolines and 1H Pyrazoles-as Possible Antiproliferative Agents

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**Abstract:** A series of twenty four newly synthesized 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-fluorophenyl) pyrazolines (3) and 1H-3-aryl-5-hydroxy-5-(2,4-dichloro-5-fluorophenyl)-pyrazoles (6) were tested for cytostatic and cytotoxic effects on in a primary three cell line-one dose anticancer assay against NCI-H 460 (Lung), MCF 7(Breast) and SF 268 (CNS). Proliferation of these cancer cell lines was strongly inhibited by eleven compounds. These eleven compounds were then passed on for evaluation in the full panel of 60 cell lines derived from seven cancer types namely, Lung, Colon, Melanoma, Renal, Ovarian, CNS and Leukemia. These compounds showed antiproliferative activity on the whole cell panel. Compound 1H-pyrazole, 6d [3,4-methylenedioxy at C 3] showed highest activity with Growth Inhibition ($GI_{50}$) value <10 μM against all tested 60 cell lines except for Leukamia CCRF-CEM, HL-60TB, K-562 cell lines. Whereas hydroxypyrazolines 3i, 3k 3m, 3o, 3p and 3q showed moderate activity with $GI_{50}$ value <50 μM against all tested 60 cell lines. Compounds 3h, 3c, 6c appear to be less active with $GI_{50}$ value >100 μM for some of the tested cell lines. Compound 6a appears to be least active with $GI_{50}$ value >100 μM for almost all the tested cell lines. The Total Growth Inhibition (TGI) and Lethal Concentration ($LC_{50}$) values for the most active compound [6d] found to be >100 μM for Leukemia cell lines and for the other cell lines these values remain <20 μM and hence prove to be a cytostatic and cytotoxic for these lines. Hence these newly synthesized pyrazole and pyrazoline derivatives showed promising antiproliferative property.

**Key words:** Hydroxypyrazolines, 1H-pyrazoles, antiproliferative activity

## INTRODUCTION

Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial, antifungal, anti-inflammatory and antidepressant activities (Elgeuro, 1984). The pyrazoline function is quite stable and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activities. The presence of fluorine in the molecules at strategic positions alters the activity (Filler and Kabayashi, 1992). Chalcone dibromides are very useful synthons in the synthesis of bioactive molecules such as pyrazolines, pyrazoles, isoxazoles, flavones, flavonols, flavanones, aurones, coumarones, tetralones, aziridines etc. The anti-

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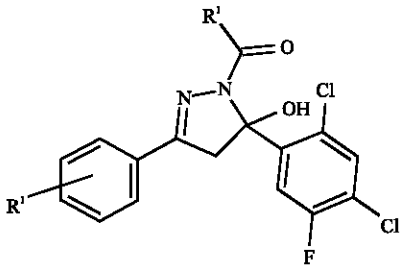



**Structure 1**

infective and anti-inflammatory properties of chalcone derivatives were reviewed by Nowakowaska (Nowakowska, 2007). The literature survey revealed that after 1989, the synthesis of hydroxy pyrazolines (Structure 1) was reported mostly from Holla's group (Holla *et al*., 1989; Holla *et al*., 2006; Holla *et al*., 2006; Holla *et al*., 2006; Karthikeyan *et al*., 2007). Bonacorso and others (Bonacorso *et al*., 2006) reported the regiospecific one step synthesis of heteroaroyl-2-pyrazolines under mild conditions. Prompted by the biological activity of the pyrazole derivatives the present work is undertaken.

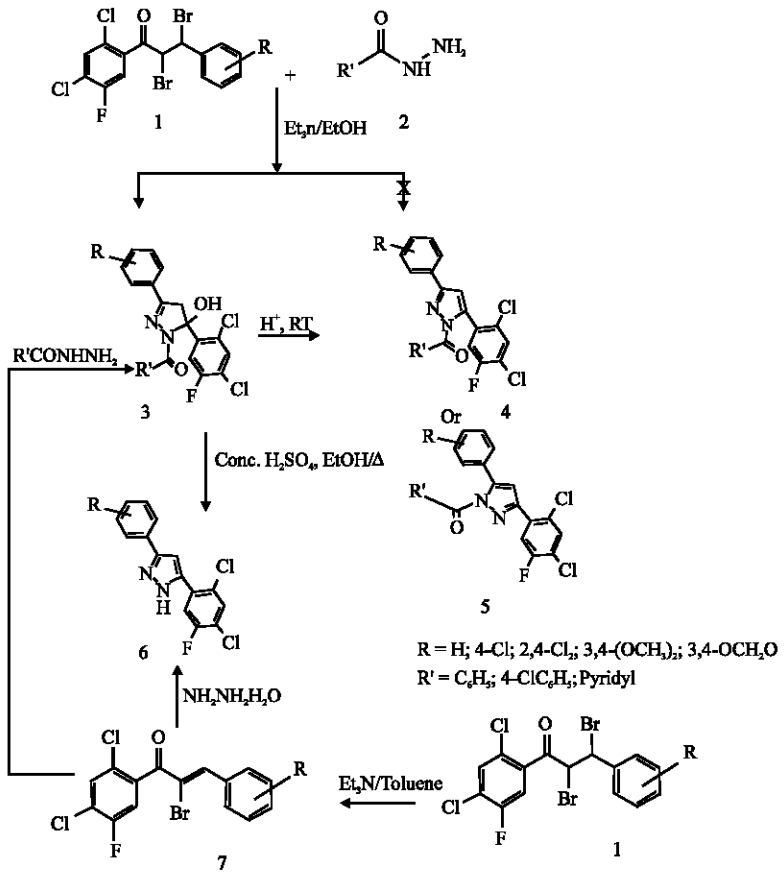
This study presents the synthesis of hydroxypyrazolines, 1H-pyrazoles and to screen the newly synthesized heterocycles for their antiproliferative activity.

## MATERIALS AND METHODS

The synthesis of the target molecules 3 and 6 which is given in the Scheme 1 was carried out by B. Sooryanarayana Rao at the Department of Chemistry, Mangalore University according to the reported procedure (Holla *et al*., 1989) during his Ph.D. programme. Melting points were taken in open capillary tubes and are uncorrected. IR spectra in KBr pellets were recorded on JASCO FT-IR 5300 Infrared spectrophotometer. $^1$H NMR spectra were recorded in DMSO-$d_6$ on a Varian (300 MHZ) spectrometer using TMS as an internal standard and the mass spectra were recorded on aVG-s-70 micro mass, mass spectrometer operating at 70eV. The purity of the compounds were checked by TLC using ethylacetate:methanol [8:2] solvent system. Iodine was used as visualizing agent. The characterization data are given in the Table 1 and 2 and spectral data are given in Table 3. The anticancer studies were carried out at National Institute of Health, Bethesda, Maryland, USA under the Drug Discovery programme

### Antiproliferative Activity

The newly synthesized compounds 3 and 6 were screened for their antiproliferative activities at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI according to the procedure suggested by Boyd and Paull (1995) in a primary three cell line-one dose anticancer assay against NCI-H 460 (Lung), MCF 7(Breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on an incubated micro titer plate. The test agents were added at a single concentration and the culture was incubated for 48 h. Endpoint determinations were made with Sulforhodamine B, a protein binding dye. Results for each test agents were reported as the percent growth of the treated cells when compared with the untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) in a primary three cell line-one dose anticancer assay were considered as active and these compounds were then passed on to 60-cell line screening studies.




Scheme 1: Synthesis of 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-fluorophenyl) pyrazolines (3) and 1H-pyrazoles (6)

Table 1: Characterization data of 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazolines (3)

| Compounds | | | mp | Yield | Analysis (%) found (calculated) | | |
|---|---|---|---|---|---|---|---|
| No. | R | $R^1$ | (°C) | (%) | C | H | N |
| 3a | H | $C_6H_5$ | 221-23 | 85 | 61.31(61.54) | 3.40(3.49) | 6.43(6.52) |
| 3b | 4-$OCH_3$ | $C_6H_5$ | 238-40 | 78 | 60.21(60.13) | 3.63(3.70) | 5.98(6.10) |
| 3c | 3,4-$(OCH_3)_2$ | $C_6H_5$ | 214-16 | 78 | 58.47(58.89) | 3.80(3.88) | 5.64(6.10) |
| 3d | 3,4-$OCH_2O$) | $C_6H_5$ | 228-30 | 74 | 58.22(58.35) | 3.16(3.17) | 5.86(5.92) |
| 3e | 4-Cl | $C_6H_5$ | 232-34 | 76 | 56.52(56.96) | 3.09(3.02) | 5.98(6.04) |
| 3f | 2,4-$Cl_2$ | $C_6H_5$ | 180-82 | 73 | 53.26(53.04) | 2.64(2.61) | 5.48(5.62) |
| 3g | H | 4-$ClC_6H_5$ | 170-72 | 72 | 55.56(55.93) | 3.18(3.24) | 6.10(6.04) |
| 3h | 4-$OCH_3$ | 4-$ClC_6H_5$ | 183-85 | 76 | 54.83(55.01) | 3.46(3.44) | 5.72(5.67) |
| 3i | 3,4-$(OCH_3)_2$ | 4-$ClC_6H_5$ | 192-94 | 68 | 54.12(54.38) | 2.71(2.76) | 3.31(3.35) |
| 3j | 3,4-$OCH_2O$) | 4-$ClC_6H_5$ | 185-87 | 78 | 53.24(53.51) | 3.23(3.30) | 5.46(5.52) |
| 3k | 4-Cl | 4-$ClC_6H_5$ | 180-82 | 76 | 53.18(53.01) | 2.52(2.61) | 5.53(5.62) |
| 3l | 2,4-$Cl_2$ | 4-$ClC_6H_5$ | 162-64 | 72 | 49.16(49.58) | 2.21(2.25) | 5.53(5.62) |
| 3m | H | pyridyl | 180-82 | 79 | 58.29(58.60) | 3.28(3.21) | 9.67(9.77) |
| 3n | 4-$OCH_3$ | pyridyl | 183-85 | 70 | 57.02(57.39) | 3.39(3.47) | 9.02(9.13) |
| 3o | 3,4-$(OCH_3)_2$ | pyridyl | 193-95 | 68 | 56.08(56.33) | 3.61(3.67) | 8.48(8.57) |
| 3p | 3,4-$OCH_2O$) | pyridyl | 198-200 | 77 | 55.34(55.69) | 2.89(2.95) | 8.78(8.86) |
| 3q | 4-Cl | pyridyl | 208-10 | 71 | 54.43(54.25) | 2.73(2.79) | 8.91(9.04) |
| 3r | 2,4-$Cl_2$ | pyridyl | 194-96 | 67 | 50.27(50.50) | 2.43(2.40) | 8.37(8.42) |

Table 2: Characterization data of 3-aryl-5-(2,4-dichloro-5-flurophenyl)-1(H)-pyrazoles (6)

| Compounds No. | R | mp (°C) | Yield (%) | Analysis (%) found (calculated) C | H | N |
|---|---|---|---|---|---|---|
| 6a | H | 200-02 | 76 | 58.41(58.63) | 2.88(2.93) | 9.07(9.12) |
| 6b | 4-$OCH_3$ | 212-14 | 73 | 56.48(56.97) | 3.21(3.26) | 8.36(8.30) |
| 6c | 3,4-$(OCH_3)_2$ | 186-88 | 68 | 55.36(55.58) | 3.49(3.54) | 7.54(7.63) |
| 6d | 3,4-$(OCH_2O)$ | 204-06 | 65 | 54.37(54.70) | 2.52(2.56) | 7.89(7.98) |
| 6e | 4-Cl | 214-16 | 71 | 52.34(52.70) | 2.23(2.34) | 8.12(8.19) |
| 6f | 2,4-$Cl_2$ | 173-75 | 70 | 47.59(47.87) | 1.79(1.86) | 7.40(7.45) |

Table 3: Spectral data of some of the newly synthesized compounds

| Compound No. | Spectral data |
|---|---|
| 3a | 3a: IR (KBr disc) $\gamma_{max}$ ($cm^{-1}$): 3242(O-H), 3099 (C-H), 1644(C=O),1572 (C=C), 1081 (C-F), 728 (C-Cl).3e: IR (KBr disc)$\gamma_{max}$ ($cm^{-1}$): 3252(O-H), 3014 (C-H), 1646(C=O) 1597 (C-N), 1514 (C=C),1086 (C-F), 731 (C-Cl).3k: IR (KBr disc) $\gamma_{max}$ ($cm^{-1}$): 3945 (C-H), 1644(C=O), 1605 (C-N), 1580 (C=C), 1068 (C-F), 746 (C-Cl).3a: $^1$H NMR 300 MHZ ($CDCl_3$+DMSO-$d_6$): δ 7.86 (d, 1H, Ar-H, $J_{H\text{-}F\ ortho}$ = 7.8 Hz), 8.01(d, 1H, Ar-H J=10.6 Hz), 8.06 (d, 1H, Ar-H, $J_{H\text{-}F\ meta}$ = 6.3 Hz), 7.43-7.72 (m, 9H, Ar-H). 5.43 (s, 1H,-OH), 3.62(dd, 2H,$CH_2$ J=18.3Hz). |
| 3e | $^1$H NMR 300 MHZ ($CDCl_3$): δ 7.86 (d, 1H, Ar-H, $J_{H\text{-}F\ ortho}$ = 7.8 Hz), 8.01(d, 1H, Ar-H J=10.6 Hz), 8.06 (d, 1H, Ar-H, $J_{H\text{-}F\ meta}$ = 6.3 Hz), 7.43-7.96 (m, 9H, Ar-H). 5.39 (s, 1H,-OH), 3.62(dd, 2H,$CH_2$ J = 18.2Hz). |
| 3f | $^1$H NMR 300 MHZ ($CDCl_3$): δ 7.39 (d, 1H, Ar-H, $J_{H\text{-}F\ ortho}$ = 7.8 Hz), 7.71(d, 1H, Ar-H J = 10.6 Hz),7.94(d, 1H, Ar-H, $J_{H\text{-}F\ meta}$ = 6.3 Hz), 7.39-7.43 (m, 7H, Ar-H). 5.41 (s, 1H,-OH), 3.78(dd, 2H, $CH_2$ J = 18.6Hz). 3i: $^1$H NMR 400 MHZ ($CDCl_3$): δ 6.85(d, 1H, Ar-H, J = 8.1Hz), 7.08(d, 1H, Ar-H J = 10.6 Hz), 7.1(d, 1H, Ar-H, J= 1.8 Hz), 7.28(d, 1H, Ar-H, J= 6.9Hz), 7.93(d, 1H, Ar-H, J = 6.9Hz) 7.43 (m, 3H, Ar-H)., 6.03 (s, 2H,-$OCH_2O$-), 5.39 (s, 1H,-OH), 3.59 (dd, 2H,$CH_2$ J = 18.6Hz).3k: $^1$H NMR 400 MHZ ($CDCl_3$): δ 7.21(s, 1H, Ar-H), 7.36 (m, 7H, Ar-H),7.57(d, 1H, Ar-H J = 10.6 Hz), 7.86 (d, 1H, Ar-H, J = 8.5Hz), 5.42 (s, 1H,-OH), 3.65 (dd, 2H,$CH_2$ J = 18.6Hz).3n: $^1$H NMR 400 MHZ ($CDCl_3$): δ 6.95(d, 2H, Ar-H, J= 8.7Hz), 7.45 (d, 1H, Ar-H J = 6.6 Hz), 7.67(d, 2H, Ar-H, J= 8.8 Hz), 7.82 (d, 1H, Ar-H, J= 10.1Hz), 8.26 (d, 1H, Ar-H, J = 7.6Hz) 3.85 (s, 3H,$OCH_3$) 5.34 (s, 1H,-OH), 3.61(dd, 2H, $CH_2$ J=18.6Hz).3e: m/z 462/464 (10%, $M^+$/ $M^+$+2), 444/446 (10%, $M^+$-$H_2O$), 425/427 (15%, 444-fluorine radical), 340/342 (10%, $M^+$-benzoic acid) 191 (10%, 2,4-dichloro-5-fluorobenzonitrile radical cation),137(15% phenyl cyanide radical cation), 105(100% benzoyl cation), 77 (45% phenyl cation)3i: m/z 522/524 (20%, $M^+$/ $M^+$+2), 504/506 (25%, $M^+$-$H_2O$), 485/487 (15%, 504-fluorine radical), 366/368 (10%, $M^+$-4-chlorobenzoic acid) 191 (10%, 2,4-dichloro-5-fluorobenzonitrile radical cation),163(15% 3,4-dimethoxy phenyl cyanide radical cation), 139(100% benzoyl cation), 111 (45% 3,4-dimethoxy benzyl cation).3r: m/z 497/499 (20%, $M^+$/ $M^+$+2), 481/482 (25%, $M^+$-$H_2O$), 462/464 (15%, 481-fluorine radical), 376/378 (10%, $M^+$-nicotinic acid) 191 (70%, 2,4-dichloro-5-fluorobenzonitrile radical cation),163(30% 2,4-dichloro-5-fluoro phenyl radical cation), 106(100% pyridoyl cation), 78(70% pyridyl cation). |
| 6b | $^1$H NMR 300 MHZ ($CDCl_3$): δ 3.85 (s, 3H, $OCH_3$) 6.97(m, 3H, Ar-H,), 7.25(s, 1H, C4-H |
| pyrazole), | 7.52(d, 1H, Ar-H, J= 6.9Hz), 7.61(m, 2H, Ar-H), 4.34 (s, 1H,-NH). |
| 6f: | $^1$H NMR 300 MHZ ($CDCl_3$): δ 7.17(s, 1H, C4-H pyrazole), 7.35 (dd, 1H, Ar-H, J = 2 Hz), 7.52(d, 1H, Ar-H, J = 6.8Hz), 7.62(d, 1H, Ar-H, J= 9.5 Hz), 7.65(d, 1H, Ar-H, J= 8.5Hz), 4.34 (s, 1H,-NH). |

## RESULTS AND DISCUSSION

In the present anticancer screening program of 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazolines, 1-benzoyl-3-phenyl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3c, 1-4-chlorobenzoyl-3-(4-methoxyphenyl)-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3h, 1-(4-chlorobenzoyl)-3-(4-chlorophenyl)-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3k, 1-(4-chlorobenzoyl)-3-(3,4-dimethoxyphenyl)-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3i, 1-pyridoyl-3-phenyl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3m, 1-pyridoyl-3-(3,4-dimethoxyphenyl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3o, 1-pyridoyl-3-(4-chlorophenyl)-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3q, 1-pyridoyl-3-(3,4-methylinedioxyphenyl)-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazoline 3p and 1H pyrazoles 3-phenyl-5-(2,4-dichloro-5-flurophenyl) pyrazole 6a, 3-(3,4-dimethoxyphenyl)-5-(2,4-dichloro-5-flurophenyl) pyrazole 6c, (3,4-methylinedioxyphenyl)-5-(2,4-dichloro-5-flurophenyl) pyrazole 6d,

Table 4: Preliminary *in vitro* anticancer screening a data of Active 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazolines (3) and 3-aryl-5-(2,4-dichloro-5-flurophenyl)-1(H)-pyrazoles (6)

| | | | Growth percentage[b] | | |
|---|---|---|---|---|---|
| Compounds | NSC No. | Sample conc.×$10^{-4}$(M) [a] | Lung NCI-H 460 | Breast MCF 7 | CNS SF-268 |
| 3k | 713322 | 1.00 | -8 | 8 | 12[c] |
| 3h | 713324 | 1.00 | 48 | 30 | 81[c] |
| 3c | 713325 | 1.00 | 43 | 38 | -36[c] |
| 3i | 713326 | 1.00 | 16 | 10 | 48[c] |
| 3m | 715684 | 1.00 | -53 | -54 | -56[c] |
| 3o | 715685 | 1.00 | -44 | -57 | -68[c] |
| 3q | 715686 | 1.00 | -23 | -18 | -40[c] |
| 3p | 715687 | 1.00 | -60 | -41 | -63[c] |
| 6a | 715688 | 1.00 | 69 | 54 | 24[c] |
| 6c | 715689 | 1.00 | 21 | -66 | -27[c] |
| 6d | 715690 | 1.00 | -3 | -37 | -71[c] |

[a]: Fixed concentration assay (100 μM; standard NCI protocol), [b]: Percent cell growth reduction following 48 h incubation with test compounds (optical density, sulforhodamine procedure), [c]: Active when growth percentage is <32% for any of the three cell lines

Table 5: Sixty cell line *in vitro* anticancer screening of (3) and (6) ($GI_{50}$, μM)

| | $GI_{50}$, μM | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Panel/cell line | 3k | 3h | 3c | 3i | 3m | 3o | 3q | 3p | 6a | 6c | 6d |
| **Leukemia** | | | | | | | | | | | |
| CCRF-CEM | 18.8 | 50.2 | >100 | 1.99 | 24.6 | 11.4 | 13.3 | 19.0 | 82.3 | 11.6 | 35.0 |
| HL-60 (TB) | 21.2 | 51.5 | >100 | 0.49 | 21.4 | 12.8 | 15.1 | 20.2 | 28.9 | 15.6 | >100 |
| K-562 | 7.25 | 40.7 | >100 | 5.83 | 21.4 | 17.4 | 16.6 | 27.9 | 0.49 | 46.1 | 5.05 |
| MOLT-4 | 9.26 | 12.8 | 8.61 | 4.13 | 14.5 | 8.97 | 6.46 | 15.3 | 16.9 | 14.5 | >100 |
| RPMI-8226 | 8.22 | 22.8 | 38.0 | 6.03 | 18.2 | 13.4 | 12.2 | 27.2 | 30.9 | 17.2 | 3.50 |
| SR | - | - | - | - | 22.5 | 7.28 | 8.84 | 14.7 | >100 | 11.1 | 9.08 |
| **Non-small cell lung cancer** | | | | | | | | | | | |
| A549/ATCC | 19.6 | 49.2 | 93.7 | - | 27.6 | 20.4 | 25.0 | 28.7 | >100 | 40.7 | 2.93 |
| EKVX | 8.59 | 6.05 | 32.6 | 27.7 | 21.8 | 19.6 | 17.5 | 20.5 | 61.5 | 22.2 | 2.09 |
| HOP-62 | 32.2 | >100 | 17.2 | 99.9 | 16.2 | 19.0 | 21.5 | 18.0 | 64.4 | 39.9 | 2.36 |
| HOP-92 | 11.6 | >100 | 21.4 | 13.2 | 20.8 | 14.1 | 17.4 | 20.8 | >100 | 20.6 | 2.07 |
| NCI-H226 | 50.6 | >100 | >100 | >100 | 15.6 | 12.8 | 11.6 | 16.2 | 50.7 | 20.8 | 1.55 |
| NCI-H23 | 12.2 | 37.6 | 42.3 | 47.1 | 27.0 | 20.4 | 18.6 | 25.0 | >100 | 30.0 | 2.26 |
| NCIH322M | 17.8 | 47.5 | 23.6 | 32.6 | 32.2 | 23.8 | 31.4 | 21.7 | >100 | 28.9 | 2.73 |
| NCI-H460 | 21.7 | 56.5 | 83.3 | 9.13 | 18.6 | 15.2 | 21.5 | 17.4 | >100 | 32.1 | 3.12 |
| NCI-H522 | 12.9 | >100 | 56.1 | 6.68 | 26.1 | 13.8 | 19.5 | 17.0 | >100 | 20.2 | 3.12 |
| **Colon cancer** | | | | | | | | | | | |
| COLO-205 | 18.4 | 93.5 | >100 | - | 14.1 | 12.3 | 9.97 | 15.1 | >100 | 19.2 | 1.84 |
| HCC-2998 | 15.5 | 43.4 | 30.9 | 8.25 | - | - | - | - | - | - | - |
| HCT-116 | 5.66 | 23.4 | 50.0 | 6.14 | 13.8 | 14.8 | 8.31 | 16.3 | >100 | 25.7 | 2.23 |
| HCT-15 | 15.5 | 28.1 | >100 | 8.28 | 26.2 | 14.8 | 13.6 | 18.0 | >100 | 21.2 | 6.64 |
| HT29 | 8.96 | 17.3 | 23.5 | 4.29 | 19.6 | 12.3 | 14.8 | 17.7 | >100 | 22.1 | 4.69 |
| KM12 | 6.96 | 24.5 | >100 | 8.15 | 25.7 | 19.1 | 28.8 | 26.9 | >100 | 26.4 | 4.33 |
| SW-620 | 17.4 | - | >100 | 6.97 | 28.3 | 18.0 | 22.2 | 23.8 | >100 | 29.6 | 5.38 |
| **CNS cancer** | | | | | | | | | | | |
| SF-268 | 47.4 | >100 | 55.5 | 21.2 | 30.6 | 17.3 | 19.2 | 19.9 | >100 | 29.2 | 2.40 |
| SF-295 | 20.6 | >100 | 29.5 | 26.0 | 16.3 | 15.5 | 19.5 | 19.1 | >100 | 18.3 | 2.64 |
| SF-539 | 69.3 | >100 | 43.1 | >100 | 15.4 | 16.1 | 12.2 | 16.7 | 23.0 | 15.9 | 1.49 |
| SNB-19 | 14.6 | >100 | 36.4 | - | 40.0 | 29.8 | 41.3 | 32.6 | >100 | 49.5 | 3.19 |
| SNB-75 | 10.0 | 15.0 | - | - | 16.4 | 23.5 | 5.71 | 15.2 | >100 | 23.3 | 1.80 |
| U251 | 15.5 | >100 | 32.4 | 19.2 | 16.4 | 13.8 | 14.3 | 16.7 | 51.5 | 26.9 | 1.90 |
| **Melanoma** | | | | | | | | | | | |
| LOX IMVI | 15.2 | 77.9 | 97.1 | 37.9 | 15.7 | 13.9 | 15.1 | 16.5 | 99.2 | 18.4 | 2.90 |
| MALME-3M | 30.6 | >100 | 57.9 | - | 29.3 | 22.6 | 33.3 | 23.4 | >100 | 30.4 | 4.94 |
| M14 | 28.7 | 44.8 | 73.6 | 12.5 | 22.0 | 15.9 | 22.0 | 31.8 | >100 | 75.0 | 6.42 |
| SK MEL-2 | 17.0 | 21.7 | 29.0 | 78.7 | 19.4 | 14.8 | 22.0 | 20.7 | >100 | 79.6 | 17.0 |
| SK MEL-28 | 44.3 | >100 | 49.8 | >100 | 25.1 | 19.5 | 23.6 | 25.5 | >100 | 48.7 | 3.23 |

Table 5: Continued

| Panel/cell line | $GI_{50}$, μM | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3k | 3h | 3c | 3i | 3m | 3o | 3q | 3p | 6a | 6c | 6d |
| SK MEL-5 | 62.8 | >100 | >100 | 33.1 | 13.9 | 12.1 | 11.3 | 15.0 | >100 | 17.1 | 2.87 |
| UACC-257 | 25.7 | 49.9 | 60.4 | 31.3 | 23.6 | 13.7 | 18.5 | 21.1 | 100 | 32.8 | 9.79 |
| UACC-62 | 21.5 | >100 | 45.7 | - | - | - | - | - | - | - | - |
| **Ovarian cancer** | | | | | | | | | | | |
| IGRVOI | 17.9 | >100 | 40.1 | 35.1 | 10.6 | 8.11 | 1.65 | 11.4 | 1.77 | 19.3 | 1.47 |
| OVCAR-3 | 21.8 | >100 | 20.4 | 41.8 | 7.31 | 11.5 | 6.51 | 11.0 | >100 | 15.2 | 1.92 |
| OVCAR-4 | 12.8 | 8.61 | 18.5 | 18.0 | 27.0 | 15.6 | 15.8 | 22.4 | >100 | 32.4 | 2.08 |
| OVCAR-5 | 28.2 | >100 | 71.3 | >100 | 32.8 | 30.2 | 33.7 | 22.4 | >100 | >100 | 3.64 |
| OVCAR-8 | 21.3 | >100 | 48.5 | 58.1 | 25.6 | 14.5 | 25.1 | 18.8 | >100 | 34.4 | 1.86 |
| SK-OV-3 | 62.4 | >100 | 20.9 | >100 | 23.6 | 26.1 | 30.2 | 27.8 | >100 | 31.9 | 1.94 |
| **Renal cancer** | | | | | | | | | | | |
| 786-O | 18.9 | >100 | 30.3 | 17.8 | 22.1 | 22.1 | 17.9 | 18.6 | >100 | 19.6 | 1.88 |
| A498 | 13.6 | 71.6 | 32.0 | 5.20 | - | - | - | - | - | - | - |
| ACHN | 21.3 | >100 | 32.5 | 14.9 | 23.2 | 21.3 | 18.8 | 17.8 | 6.35 | 7.76 | 1.93 |
| CAKI-1 | 13.8 | 27.4 | 27.5 | 18.6 | 33.3 | 24.1 | 28.5 | 28.7 | >100 | 50.8 | 5.35 |
| RXF-393 | 23.9 | >100 | 18.9 | 6.16 | 16.7 | 15.5 | 7.05 | 12.7 | >100 | 15.1 | 1.89 |
| SN12C | 14.5 | 39.1 | 94.4 | - | 17.2 | 15.3 | 14.1 | 18.5 | >100 | 22.7 | 2.48 |
| TK-10 | 52.7 | >100 | 35.9 | 86.8 | 41.0 | 30.4 | 41.1 | 39.1 | >100 | 43.4 | 2.82 |
| UO-31 | 9.05 | 16.9 | 30.9 | 3.08 | 25.6 | 17.4 | 15.9 | 17.3 | >100 | 85.3 | 2.92 |
| **Prostate cancer** | | | | | | | | | | | |
| PC-3 | 10.4 | 62.0 | 31.4 | 6.44 | 20.5 | 12.6 | 12.9 | 21.5 | >100 | 21.2 | 2.92 |
| Du-145 | 27.3 | >100 | 39.0 | 8.51 | 32.1 | 27.9 | 16.9 | 20.9 | >100 | 33.1 | 3.34 |
| **Breast cancer** | | | | | | | | | | | |
| MCF7 | 7.15 | 19.0 | 32.1 | 4.61 | - | - | - | - | - | - | - |
| NCI/ADR-RES | 18.4 | 48.3 | 62.1 | 9.77 | 23.5 | 14.8 | 16.0 | 18.5 | >100 | 25.1 | 3.63 |
| MDA-MB-231/ATCC | 34.2 | >100 | 42.2 | >100 | 15.7 | 13.9 | 15.1 | 17.3 | 55.4 | 30.4 | 1.58 |
| HS-578T | 39.0 | >100 | 34.5 | 24.0 | 27.0 | 19.4 | 29.4 | 33.0 | >100 | 43.7 | 4.15 |
| MDA-MB-435 | 16.7 | 18.6 | 31.8 | 13.2 | 26.9 | 15.2 | 15.9 | 27.1 | >100 | 46.2 | 4.91 |
| MDA-N | 13.6 | 22.4 | 57.6 | 15.5 | 28.6 | 21.3 | 16.7 | 29.3 | >100 | 52.8 | 6.10 |
| BT-549 | 62.2 | >100 | >100 | - | 20.3 | 19.3 | 24.6 | 21.0 | >100 | 30.2 | 3.99 |
| T-47D | 9.96 | 57.7 | 50.8 | 6.27 | 12.3 | 24.0 | 2.39 | 3.59 | >100 | 28.6 | 2.20 |

possessed growth percentage to less than 32% against all the tested 3 cancer cell lines and were regarded as active compounds. Prescreen results are given in Table 4. These 6 compounds were then passed on for evaluation in the full panel of 60 cell lines derived from seven cancer types namely, Lung, Colon, Melanoma, Renal, Ovarian, CNS and Leukemia. These compounds showed antiproliferative activity on the whole cell panel. The screening data is presented in Table 5. Compound 1H-pyrazole 6d, (3,4-methylinedioxyphenyl)-5-(2,4-dichloro-5-flurophenyl) pyrazole, showed highest activity with Growth Inhibition ($GI_{50}$) value <10 μM against all tested 60 cell lines except for Leukamia CCRF-CEM, HL-60TB, K-562 cell lines. Whereas hydroxypyrazolines 3i, 3k, 3m, 3o, 3p and 3q showed moderate activity with $GI_{50}$ value <50 μM against all tested 60 cell lines. Compounds 3h, 3c, 6c appear to be less active with $GI_{50}$ value >100 μM for some of the tested cell lines. Compound 6a appears to be least active with $GI_{50}$ value >100 μM for almost all the tested cell lines.

The most active compound 6d emerged as most effective against Non-small cell Lung cancer cells A549/ATCC = 2.93 μM, EKVX = 2.09 μM, HOP-62 = 2.36 μM, HOP-92 = 2.07 μM, NCI-H226 = 1.55 μM, NCI-H23 = 2.26 μM, NCIH322M = 2.73 μM, NCI-H460 = 3.12 μM, NCI-H522= 3.12 μM among the tested cell lines.

The Total Growth Inhibition (TGI) and Lethal Concentration ($LC_{50}$) values for the most active compound [6d] is given in Table 6. For leukemia cell lines both parameters are more than 100 μM and for the other cell lines these values remain less than 20 μM and hence proves to be a cytostatic and cytotoxic for these lines. With this information it is immature to comment on structure activity relationship. However, it appears that the presence of 3,4-methylenedioxy and pyridyl moieties may also contribute to their enhanced activity.

Table 6: Total Growth Inhibition (TGI) and Lethal Concentration (LC50) of 3-(3,4-Methylenedioxy phenyl)-5-(2,4-dichloro-5-flurophenyl)-1(H)-pyrazole (6d)

| Panel/cell line | 6d | |
|---|---|---|
| | TGI (μM) | LC50 (μM) |
| **Leukemia** | | |
| CCRF-CEM | >100 | >100 |
| HL-60 (TB) | >100 | >100 |
| K-562 | >100 | >100 |
| MOLT-4 | >100 | >100 |
| RPMI-8226 | >100 | >100 |
| SR | >100 | >100 |
| **Non-small cell lung cancer** | | |
| A549/ATCC | 8.15 | 57.7 |
| EKVX | 5.93 | 38.4 |
| HOP-62 | 6.53 | 26.8 |
| HOP-92 | 5.53 | 20.8 |
| NCI-H226 | 3.30 | 7.02 |
| NCI-H23 | 6.27 | 30.3 |
| NCIH322M | 6.65 | 32.1 |
| NCI-H460 | 11.5 | >100 |
| NCI-H 522 | 11.2 | 49.1 |
| **Colon cancer** | | |
| COLO-205 | 5.47 | 20.5 |
| HCC-2998 | _ | _ |
| HCT-116 | 5.00 | 13.8 |
| HCT-15 | 6.62 | >100 |
| HT29 | 19.6 | 68.5 |
| KM12 | >100 | >100 |
| SW-620 | 54.0 | >100 |
| **CNS cancer** | | |
| SF-268 | 6.26 | >100 |
| SF-295 | 7.63 | 34.4 |
| SF-539 | 2.97 | 5.91 |
| SNB-19 | 11.5 | 44.9 |
| SNB-75 | 3.68 | 7.53 |
| U251 | 3.54 | 6.58 |
| **Melanoma** | | |
| LOX IMVI | 8.33 | 40.8 |
| MALME-3M | 24.8 | >100 |
| M14 | 27.9 | >100 |
| SK MEL-2 | 39.0 | 89.7 |
| SK MEL-28 | 13.0 | 48.4 |
| SK MEL-5 | 11.5 | 35.5 |
| UACC-257 | 28.2 | 80.0 |
| UACC-62 | - | - |
| **Ovarian cancer** | | |
| IGRVOI | 5.29 | 41.3 |
| OVCAR-3 | 3.97 | 8.23 |
| OVCAR-4 | 5.13 | 27.7 |
| OVCAR-5 | 13.3 | 54.1 |
| OVCAR-8 | 3.60 | 6.98 |
| SK-OV-3 | 4.46 | 10.8 |
| **Renal cancer** | | |
| 786-O | 3.86 | 7.96 |
| A498 | - | - |
| ACHN | 3.77 | 7.35 |
| CAKI-1 | 26.2 | >100 |
| RXF-393 | 4.45 | 11.4 |
| SN12C | 6.96 | 35.2 |
| TK-10 | 5.67 | 15.7 |
| UO-31 | 10.2 | 57.9 |

Table 6: Continued

| Panel/cell line | 6d | |
|---|---|---|
| | TGI (μM) | LC50 (μM) |
| **Prostate cancer** | | |
| PC-3 | 8.49 | 29.3 |
| Du-145 | 13.0 | 63.1 |
| **Breast cancer** | | |
| MCF7 | - | - |
| 1. | NCI/ADR-RES | 16.3>100 |
| MDA-MB-231/ATCC | 3.45 | 7.53 |
| HS-578T | >100 | >100 |
| MDA-MB-435 | 34.3 | >100 |
| MDA-N | 33.1 | >100 |
| BT-549 | 16.8 | 54.0 |
| T-47D | 7.79 | 73.3 |

## CONCLUSIONS

With the history of pyrazole derivatives as potential bioactive moieties and in a hope to find potential molecules with antiproliferative activity we synthesized fluorine containing hydroxypyrazolines and pyrazoles. The newly synthesized compounds were characterized by analytical and spectral studies. In the present anticancer screening program of 1-aroyl-3-aryl-5-hydroxy-5-(2,4-dichloro-5-flurophenyl) pyrazolines, compounds 3c, 3h, 3k, 3i, 3m, 3o, 3q, 3p and 1H pyrazoles 6a, 6c, 6d were emerged as active compounds. 1H-pyrazole 6d [3,4-methylenedioxy at C 3] showed highest activity with Growth Inhibition ($GI_{50}$) value <10 μM against all tested 60 cell lines except for Leukamia CCRF-CEM, HL-60TB,K-562 cell lines. Whereas hydroxy pyrazoline compounds 3i [3,4-methylenedioxyphenyl at C 3 and 4-chloro phenyl at C 1 substitution], 3k [4-chloro phenyl at C 3 and 4-chloro phenyl at C 1 substitution], 3m[phenyl at C3 and pyridyl at C1 substitution], 3o [3,4-dimethoxyphenyl at C3 and pyridyl at C1 substitution], 3p [3,4-methylenedioxyphenyl at C 3 and pyridyl at C1 substitution] and 3q [4-chloro phenyl at C 3 and pyridyl at C 1 substitution] showed moderate activity with $GI_{50}$ value <50$\mu$M against all tested 60 cell lines. Compounds 3h [4-methoxyphenyl at C 3 and 4-chlorophenyl at C 1 substitution], 3c [3,4-dimethoxy phenyl at C 3 and phenyl at C 1 substitution] and 6c [3,4-dimethoxyphenyl at C 3] appear to be less active with $GI_{50}$ value >100 μM for some of the tested cell lines. Compound 6a [phenyl at C 3 substitution] appears to be least active with $GI_{50}$ value >100 μM for almost all the tested cell lines. The Total growth inhibition (TGI) and Lethal Concentration ($LC_{50}$) values for the most active compound [6d] is given. For leukemia cell lines both parameters are >100 μM and for the other cell lines these values remain <20 μM and hence proves to be a cytostatic and cytotoxic for these lines. It appears that the presence of 3,4-methylenedioxy and pyridyl moieties may contribute to their enhanced activity. However, it is hoped that these pyrazole derivatives may emerge as potential compounds for antiproliferative activity.

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## REFERENCES

Bonacorso, H.G., A.P. Wentz, R.V. Lourega, C.A. Cechinel, T.S. Moraes, H.S. Coelho, N. Zanatta, M.A.P. Martins, M. Hoerner and S.H. Alves, 2006. Trifluoromethyl-containing pyrazolinyl (p-tolyl) sulphpones: The synthesis and structure of promising antimicrobial agents. J. Fluor. Chem., 127: 1066-1072.

Boyd, M.R. and K.D. Paull, 1995. Some Practical Considerations and Application of the National Cancer Institute *in vitro* anticancer drug discovery screen. Drug Dev. Res., pp: 3491.

Elgeuro, J., 1984. Comprehensive Heterocyclic Chemistry. Katritzky, A.R. and C.W. Rees (Eds.), Pergamon Press, New York, 5: 291.

Filler, R. and Y. Kabayashi, 1992. Biomedicinal Aspects of fluorine Chemistry. Elsevier, Amsterdam.

Holla, B.S., K.V. Udupa and K.R. Sridhar, 1989. The reaction of α, β-acetylenic ketones with aroylhydrazines. Bull. Chem. Soc. Japan, 62: 3409-3411.

Holla, B.S., M. Mahalinga, M. Ashok and P. Karegoudar, 2006. Convenient synthesis of some 4'-methtylthio-containing aryl and arylfuryl pyrazolines and their antimicrobial activity studies. Phosphorus, Sulfur and Silicon and the Related Elements, 181: 1427-1436.

Holla, B.S., M. Mahalinga, M.S. Karthikeyan, P.M. Akberali and N.S. Shetty, 2006. Synthesis of some novel pyrazolo [3,4-d] pyrimidine derivatives as potential antimicrobial agents. Bioorg. Med. Chem., 14: 2040-2047.

Holla, B.S., M. Mahalinga, B. Poojary, M. Ashok and P.M. Akberali, 2006. Synthesis of pyrazolines promoted by Amberlyst-15 catalyst. Ind. J. Chem. Section B Org. Med. Chem., 45: 568-571.

Karthikeyan, M.S., B.S. Holla and N.S. Kumari, 2007. Synthesis and antimicrobial studies on novel chloro-fluorine containing hydroxy pyrazolines. Eur. J. Med. Chem., 42: 30-36.

Nowakowska, Z., 2007. A review of anti-infective and antinflamatory chalcones. Eur. J. Med. Chem., 42: 125-137.