ISSN: 0974 - 7516 Volume 10 Issue 8

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 10(8), 2014 [295-307]

Synthesis and antitumor activity of some novel heterocyclic compounds derived from chalcone analogues

Manal M.Kandeel[1], Nadia A.Abdou[2], Hanan H.Kadry[1]*, Rana M.El-Masry[2]

[1]**Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, 11562, (EGYPT)**

[2]**Organic Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, 6 October City, (EGYPT)**

E-mail: hanankadry2005@yahoo.com

ABSTRACT

A series of chalcones **(3a-c)** was synthesized and cyclocondensed with hydrazine hydrate, hydroxylamine, urea and thiourea to afford novel pyrazoline **(4a-c)**, **(5a-c)**, isoxazoline **(6)**, dihyrdopyrimidinone **(7a**, **b)** and dihyrdopyrimidinethione **(8a**, **b)** derivatives respectively. In addition, a new barbiturate **(9)** and thiobarbiturate **(10)** derivatives were synthesized by reaction of chalcone derivatives with barbituric and thiobarbituric acid. Also, the novel pyrazolyl chalcone **(13)** was synthesized, reacted with hydrazine hydrate in ethanol and acetic acid respectively, to afford new pyrazoline derivative **(14)** and its N-acetyl derivative **(15)**. Twelve of the synthesized derivatives were selected by NCI to be evaluated for their antitumor activity by *in-vitro* disease-oriented human cells screening panel assay. All the tested compounds exhibited a broad spectrum of antitumor activity especially against renal cancer UO-31. Compound **(5c)** is considered to be the most active member identified in this study with a broad spectrum of activity against most cell lines.

KEYWORDS

Antitumor activity;
Chalcones;
Indole derivatives;
Pyrazoline;
Synthesis.

INTRODUCTION

Cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is supposed to become the primary cause of death within the coming years[1]. The identification of novel structures that can be potentially useful in designing new, potent, selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers[2]. Many clinically successful anticancer drugs are either natural products or have been developed from naturally occurring lead compounds, such as taxol, topotecan and irinotecan[3]. Beside the compounds previously mentioned, chalcones constitute an important group of natural products and serve as precursors for the synthesis of different classes of flavonoids and isoflavonoids, which are abundant in edible plants[4]. Chalcone derivatives are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. Natural and synthetic chalcones have shown broad spectrum of biological activities such as cytotoxic[5], antimalarial[6], antileishmanial[7], anti-inflammatory[8], anti-HIV[9], antifungal[10] and as tyrosine kinase inhibitors[11]. Recent development of anticancer agents involve structural modification of chalcones to improve

their bioavailability and to study the role of various substituent on aryl or heteroaryl rings[12].

Chalcones were recognized to have synthetic utility in the preparation of pharmacologically interesting heterocyclic systems like pyrazoline derivatives which are attracting interest of many researchers, because of their bioactivity such as antimicrobial[13], anti-amoebic\, antinociceptive[15], anticancer[16] and anti-inflammatory[17]. As a consequence, a large number of 2-pyrazolines have been described in the chemical literature, using different synthetic methods for their preparation. An especially popular procedure is based on the reaction of α, β-unsaturated aldehydes and ketones with hydrazines.

Also, Pyrimidine nucleus is a pharmacophoric scaffold that could be synthesized from α, β unsaturated ketones and represents a class of heterocyclic compounds with a wide range of biological applications. Many of them are widely used as anticonvulsant[18] and analgesic[19]. Some compounds containing pyrimidine moiety were reported to possess antitumor activities[20] and many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use.

As a part of our ongoing studies aiming to developing new chalcone analouges as anticancer agents, we were interested in analogs in which a phenyl ring of chalcone is replaced by an indole nucleus. 3-Substituted indole is one of the 'privileged medicinal scaffold' found in many biologically active compounds, especially with anticancer and anti-tumour[21]. Besides being biologically active, they are also used extensively as synthons in organic synthesis that possesses a potentially reactive site for a variety of chemical reactions.

Encouraged by these observations and also in continuation of our search for potent anticancer agents[22] targeting the development of new chalcone analouges as anticancer agents, we underwent the synthesis of substituted 1-indolyl-3-phenylpropenones and their conversion to other heterocycles like pyrazoline isoxazoline and dihydropyrimidine derivatives. Also, the synthesis of some pyrazoline derivatives derived from chalcone of p-hydroxyacetophenone was carried out to get some new biodynamic compounds, which may be used as potent anticancer agents. The *in vitro* antitumor activity of the newly synthesized compounds was evaluated according to the current one-dose protocol of the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay.

EXPERIMENTAL

Chemistry

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England). Microanalysis was carried out at Micro-analytical Unit, Faculty of Science, Cairo University and the regional center for microbiology and biotechnology, Al-Azhar University. Analyses indicated were within ± 0.4 % of the theoretical values. Infrared Spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wave number (cm^{-1}) using potassium bromide discs. The NMR spectra were recorded on a Varian Gemini 200 MHz and Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR spectra were run at 300 MHz and ^{13}C NMR spectra were run at 100.40 MHz in dimethylsulfoxide(DMSO-d_6). Chemical shifts were quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC was carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany). The developing solvents were benzene/acetone (4:1) and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Compounds **(3a)**[23], **(3b)**,**(3c)**[24], **(11)** and **(12)**[25] were obtained according to the reported procedures.

General procedure for the synthesis of (4a-c)

A mixture of chalcone **(3a-c)** (0.001mol) and 99% hydrazine hydrate (0.1 mL, 0.002mol) in ethanol (5 mL) was refluxed for 5 h. The reaction mixture was poured onto ice–water (10 mL). The formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

4-[5-(*1H*-Indol-3-yl)-4,5-dihydro-*1H*-pyrazol-3-yl]phenol (4a)

Yield: 37 % ; m.p. 290-291 ^{o}C; IR ν_{max}/cm^{-1}:

3510.00(OH), 3394.72, 3217.27 (NH), 3059.10 (CH aromatic), 2989.23 (CH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm: 2.876 (dd,1H, *J*= 8.2, 3.6 Hz, pyrazoline H-4), 3.416 (dd,1H, *J*=8.6, 3.6 Hz pyrazoline H-4), 4.365 (t, 1H, *J*= 3.8 Hz pyrazoline H-5), 7.037-7.173 (m, 3H, indole H-5, H-6 and H-7), 7.439 (d, 2H, *J*=7.6 Hz, H-3, H-5 of 4-OH C_6H_4), 7.779 (br s, 1H, indole H-4), 7.856 (d, 2H, *J*=7.8 Hz, H-2 and H-6 of 4-OH C_6H_4), 8.212 (s, 1H, indole H-2), 8.248 (s, 1H, OH, exch. D_2O), 11.185 (s, 1H, NH of indole, exch. D_2O), 11.576 (s, 1H, NH of pyrazole, exch.D_2O); Anal. Calcd for $C_{17}H_{15}N_3O$ (277.30): C, 73.63; H, 5.45; N, 15.15. Found: C, 74.01; H, 4.99; N, 15.32.

3-[3-(4-Chlorophenyl)-4, 5-dihydro-*1H*-pyrazol-5-yl]-*1H*-indole (4b)

Yield: 21%; m.p. 240-241 °C; IR ν_{max}/cm^{-1}: 3394.72, 3217.27 (NH), 3059.10 (CH aromatic), 2967.22 (CH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm :3.621(dd,1H, *J*=8.5, 3.9 Hz, pyrazoline H-4), 3.782 (dd, 1H, *J*= 8.2,4.0 Hz, pyrazoline H-4), 5.921(t, 1H, J= 4.5 Hz pyrazoline H-5), 7.096-7.186 (m, 3H, indole H-5, H-6 and H-7), 7.435 (d, 2H, *J*=7.8 Hz, H-3, H-5 of 4-Cl C_6H_4), 7.785 (br s, 1H, indole H-4), 7.853 (d, 2H, *J*=7.8 Hz, H-2, H-6 of 4-Cl C_6H_4), 8.201 (s, 1H, indole H-2), 11.190 (s, 1H, NH of indole, exch. D_2O), 11.580 (s, 1H, NH of pyrazole, exch.D_2O); Anal. Calcd for $C_{17}H_{14}ClN_3$ (295.50): C, 69.03; H, 4.77; N, 14.21. Found: C, 69.38; H, 4.41; N, 14.46.

3-[3-(4-Bromophenyl)-4,5-dihydro-*1H*-pyrazol-5-yl]-*1H*-indole (4c)

Yield: 58%; m.p. 250-251 °C ; IR ν_{max}/cm^{-1}: 3394.72, 3217.27 (NH), 3059.10 (CH aromatic), 2981.2(CH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm : 3.615 (dd,1H, *J*=8.6, 3.8 Hz, pyrazoline H-4), 3.862 (dd, 1H, *J*= 8.2, 4.0 Hz, pyrazoline H-4), (m, 2H, pyrazoline H-4), 5.910 (t,1H, *J*= 3.5 Hz pyrazoline H-5), 7.096-7.129 (m, 2H, indole H-5 and H-6), 7.404 (d, 2H, *J*=7.6 Hz, H-3, H-5 of 4-Br C_6H_4), 7.558-7.752 (m, 2H, indole H-7, H-4), 7.821 (d, 2H, *J*=7.6 Hz, H-2, H-6 of 4-Br C_6H_4), 8.183 (s, 1H, indole H-2), 11.164 (s, 1H, NH of indole, exch. D_2O), 11.320, 11.550 (2s, 1H, NH of pyrazole, exch.D_2O); MS m/z 340.2 (M^+, 2.05%), 342.3(M+2, 1.86%) ;Anal. Calcd for $C_{17}H_{14}BrN_3$ (340.20): C, 60.02; H, 4.15; N, 12.35. Found: C, 60.34; H, 3.85; N, 12.53.

General procedure for the synthesis of (5a-c)

A mixture of chalcone **(3a-c)** (0.001 mol) and 99% hydrazine hydrate (0.1 ml, 0.002 mol) in glacial acetic acid (5 mL) was refluxed for 5 h. The reaction mixture was poured onto ice- water (10 mL), the formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

1-[3-(4-Hydroxyphenyl)-5-(*1H*-indol-3-yl)-4,5-dihydropyrazol-1-yl]ethanone (5a)

Yield: 32%; m.p.140-141°C; IR ν_{max}/cm^{-1}: 3402.43 (NH), 3055.24 (CH aromatic), 2927.94 (CH aliphatic), 1643.35 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 2.225 (s, 3H, CH_3), 3.693 (dd,1H, *J*=7.5, 3.3 Hz, pyrazoline H-4), 3.825 (dd, 1H, *J*= 7.6, 3.3 Hz, pyrazoline H-4), 5.585(t, 1H, *J*=4.1Hz pyrazoline H-5), 7.102-7.242 (m, 2H, indole H-5 and H-6), 7.140 (d, 2H, *J*=7.2 Hz, H-3, H-5 of 4-OH C_6H_4), 7.495-8.018 (m, 2H, indole H-7 and H-4), 8.078 (s, 1H, indole H-2), 8.330 (d, 2H, *J*=7.2 Hz, H-2, H-6 of 4-OH C_6H_4), 9.919 (s, 1H, OH, exch.D_2O), 11.505 (s, 1H, NH of indole, exch. D_2O); Anal. Calcd for $C_{19}H_{17}N_3O_2$ (319.34): C, 71.46; H, 5.37; N, 13.16. Found: C, 71.28; H,5.12; N,12.85.

1-[3-(4-Chlorophenyl)-5-(*1H*-indol-3-yl)-4,5-dihydropyrazol-1-yl]ethanone (5b)

Yield: 20%; m.p. 100-101 °C; IR ν_{max}/cm^{-1}: 3402.43 (NH), 3055.24 (CH aromatic), 2924.09 (CH aliphatic), 1666.50 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 2.220 (s, 3H, CH_3), 3.781(dd,1H, *J*=8.9, 3.6 Hz, pyrazoline H-4), 3.831 (dd, 1H, *J*= 8.6, 3.6 Hz, pyrazoline H-4), 4.160(t, 1H, 4.2 Hz Pyrazoline H-5), 6.918-7.527 (m, 4H, indole H-5, H-6, H-7 and H-4),7.689 (d, 2H, *J*= 7.1 Hz, H-3, H-5 of 4-ClC_6H_4), 8.339(d, 2H, *J*= 7.2 Hz, H-2, H-6 of 4-ClC_6H_4), 8.897 (br s, 1H, indole H-2), 11.696 (s, 1H, NH of indole, exch. D_2O) ; MS m/z 337.05 (M^+, 19.98%), 339.10 (M+2, 6.54%) ;Anal. Calcd for $C_{19}H_{16}ClN_3O$ (337.50): C, 67.56; H, 4.77; N, 12.44. Found: C,67.96; H, 4.38; N,12.67.

1-[3-(4-Bromophenyl)-5-(*1H*-indol-3-yl)-4,5-dihydropyrazol-1-yl]ethanone (5c)

Yield: 40%; m.p. 65-66 °C; IR ν_{max}/cm^{-1}: 3402.43 (NH), 3055.24 (CH aromatic), 2924.09 (CH aliphatic),

1674.21 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm : 2.210 (s, 3H, CH_3), 3.821 (dd, 1H, *J*= 8.8, 3.8 Hz, pyrazoline H-4), 4.084(dd, 1H, *J*= 8.8, 3.7 Hz, pyrazoline H-4), 5.798(t, 1H, *J*= 4.1 Hz, pyrazoline H-5), 7.037 (t, 1H, *J*=8.4, indole H-5), 7.372 (t, 1H, *J*=8.4, indole H-6), 7.471 (d, 2H, *J*=7.6 Hz, H-3, H-5 of 4-Br C_6H_4), 7.641 (d, 1H, *J*=8.4, indole H-7), 8.137 (d, 2H, *J*=7.6 Hz, H-2, H-6 of 4-Br C_6H_4), 8.293 (d, 1H, *J*=8.4 Hz, indole H-4), 8.370 (s, 1H, indole H-2), 11.653 (s, 1H, NH of indole, exch. D_2O); Anal. Calcd for $C_{19}H_{16}BrN_3O$ (382.24): C, 59.70; H, 4.22; N, 10.99. Found: C,59.74; H,4.27, N, 10.95

Procedure for the synthesis of 4-[5-(*1H*-Indol-3-yl)-4,5-dihydroisoxazol-3-yl]phenol (6)

A mixture of chalcone **(3a)** (0.26 g, 0.001 mol), hydroxylamine hydrochloride (0.13 gm, 0.002 mol) and sodium acetate (0.16 g, 0.002 mol) in ethanol (5 mL) was refluxed for 5 h. The reaction mixture was poured onto ice- water (15 mL), the formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

Yield: 54%; m.p. 195-196 °C; IR ν_{max}/cm^{-1}: 3387.00 (NH), 3051.39 (CH aromatic); ^{1}H NMR (DMSO-d_6) δ ppm: 2.660 (dd,1H, *J*= 8.9, 3.2 Hz, isooxazole H-4), 3.44(dd, 1H, *J*= 8.8, 3.2 Hz, isoxazole H-4), 4.358(t, 1H, *J*= 5.3Hz, isoxazole H-5), 7.143-7.230 (m, 3H, indole H-5, H-6 and H-7), 7.469 (d, 2H, *J*=7.6 Hz, H-3, H-5 of 4-OH C_6H_4), 7.904 (br s, 2H, indole H-2 and H-4), 8.338 (d, 2H, *J*=7.2 Hz, H-2, H-6 of 4-OH C_6H_4), 8.890 (s, 1H, OH, exch.D_2O), 11.678 (s, 1H, NH of indole, exch. D_2O); Anal. Calcd for $C_{17}H_{14}N_2O_2$(278.29): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.76; H, 4.71; N, 10.22.

General procedure for the synthesis of (7a,b)

A cold solution of chalcone **(3a,c)** (0.001 mol) and urea (0.06 g, 0.001 mol) in absolute ethanol (10 mL) and few drops of sulphuric acid was heated under reflux for 5 h. The reaction mixture was set aside at room temperature for 24 h, neutralized with 10% sodium carbonate (10 mL). The separated solid was filtered, dried and crystallized from ethanol.

6-(4-Hydroxyphenyl)-4-(*1H*-indol-3-yl)-3,4-dihydropyrimidin-2(*1H*)-one (7a)

Yield: 40%; m.p. 190-191 °C; IR ν_{max}/cm^{-1}: 3470.00-3315.00 (OH, NHs), 3055.24 (CH aromatic), 1670,73 (C=O) ; ^{1}H NMR (DMSO-*d6*) δ ppm: 3.832 (d, 1H, pyrimidine H-4), 6.943 (d, 1H, pyrimidine H-5), 7.015 (d, 2H, *J*=7.8 Hz, H-3, H-5 of 4-OH C_6H_4), 7.173 (d, 1H, *J*=8.1 Hz, indole H-7), 7.213 (d, 2H, *J*=7.8 Hz, H-2, H-6 of 4-OH C_6H_4), 7.327-7.668 (m, 2H, indole H-5 and H-6), 8.146 (d, 1H, *J*=8.1 Hz, indole H-4), 8.745 (s, 1H, indole H-2), 10.478 (s, 1H, OH, D_2O exch.), 11.025 (s, 1H, NH, D_2O exch.), 11.593 (s, 1H, NH, D_2O exch.), 12.062 (s, 1H, NH, D_2O exch.); MS m/z 305.1(M^+, 15.5%), 306.1(M+1, 15.5%); Anal. Calcd for $C_{18}H_{15}N_3O_2$ (305.12): C, 70.81; H, 4.95; N, 13.76. Found: C, 70.88; H, 4.92; N, 13.89.

6-(4-Bromophenyl)-4-(*1H*-indol-3-yl)-3,4-dihydropyrimidin-2(*1H*)-one (7b)

Yield: 45%; m.p. 132-133 °C; IR ν_{max}/cm^{-1}: 3440.00-3320.00 (NHs), 3055.24 (CH aromatic), 1674.21 (C=O), 1612.49 (C=N); ^{1}H NMR (DMSO-*d6*) δ ppm: 4.310 (d, 1H, pyrimidine H-4), 6.912 (d, 1H, pyrimidine H-5), 7.103 (d, 2H, *J*=6.9 Hz, H-3, H-5 of 4-Br C_6H_4), 7.235 (d, 2H, *J*=7.2 Hz, H-2, H-6 of 4-Br C_6H_4), 7.313-7.341 (m, 2H, indole H-5 and H-6), 7.846 (d, 1H, *J*=8.4 Hz, indole H-7), 8.109 (d, 1H, *J*=8.7 Hz, indole H-4), 8.716 (s, 1H, indole H-2), 10.428 (s, 1H, NH, D_2O exch.), 11.025 (s, 1H, NH, D_2O exch.), 12.085 (s, 1H, NH, D_2O exch.); Anal. Calcd for $C_{18}H_{14}BrN_3O$ (367.03): C, 58.71; H, 3.83; N, 11.41. Found: C, 58.69; H, 3.89; N, 11.58.

General procedure for the synthesis of (8a,b)

A cold solution of chalcone **(3a,c)** (0.001 mol) and thiourea (0.08 gm, 0.001 mol) in absolute ethanol (10 mL) and few drops of sulphuric acid was heated under reflux for 5 h. the reaction mixture was set aside at room temperature for 24 h, neutralized with 10% sodium carbonate (10 mL) and the separated solid was filtered and crystallized from ethanol.

6-(4-Hydroxyphenyl)-4-(*1H*-indol-3-yl)-3,4-dihydropyrimidine-2(*1H*)-thione (8a)

Yield: 38%; m.p.181-182 °C; IR ν_{max}/cm^{-1}: 3413.39-3315.46 (OH, NHs), 3052.76 (CH aromatic), 1613.16 (C=N),1323.01(C=S); ^{1}HNMR (DMSO-*d6*) δ ppm: 4.200 (d, 1H, pyrimidine H-4), 6.938 (d, 1H, pyrimidine H-5), 7.002 (d, 2H, *J*=8.7

Hz, H-3, H-5 of 4-OHC_6H_4), 7.223 (dd, 1H, *J*=7.2 Hz, indole H-5), 7.291 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-OH C_6H_4), 7.511 (dd, 1H, *J*=7.5 Hz, indole H-6), 8.132 (d, 1H, *J*=7.2 Hz, indole H-7), 8.182 (d, 1H, *J*=7.8 Hz, indole H-4), 8.756(s, 1H, indole H-2),10.445 (s, 1H, OH, D_2O exch.), 10.494 (s, 1H, NH, D_2O exch.), 11.597 (s, 1H, NH, D_2O exch.), 12.076 (s, 1H, NH, D_2O exch.); Anal. Calcd for $C_{18}H_{15}N_3OS$ (321.09): C, 67.27; H, 4.70; N, 13.07.Found: C, 67.32; H, 4.77, N, 13.07.

6-(4-Bromophenyl)-4-(*1H*-indol-3-yl)-3,4-dihydropyrimidine-2(*1H*)-thione (8b)

Yield: 40%; m.p. 100-101 °C; IR ν_{max}/cm^{-1}: 3445.00-3360.00 (NHs), 3055.24 (CH aromatic), 1616.35 (C=N), 1350.21(C=S); ^{1}H NMR (DMSO-*d6*) δ ppm: 4.300 (d, 1H, pyrimidine H-4), 7.007 (d, 1H, pyrimidine H-5), 7.238 (d, 2H, *J*=6.9 Hz, H-3, H-5 of 4-Br C_6H_4), 7.352 (d, 2H, *J*=6.6 Hz, H-2, H-6 of 4-Br C_6H_4), 7.409 (d, 1H, *J*=7.8 Hz, indole H-7), 7.658-7.741 (m, 2H, indole H-5and H-6), 8.000 (d, 1H, *J*=7.8 Hz, indole H-4), 8.746 (s, 1H, indole H-2), 10.476 (s, 1H, NH, D_2O exch.), 11.612 (s, 1H, NH, D_2O exch.), 12.092 (s, 1H, NH, D_2O exch.); MS m/z383.1(M^+, 1.51%), 385.1 (M+2.0.99%) Anal. Calcd for $C_{18}H_{14}BrN_3S$(383.01): C, 56.26; H, 3.67; N, 10.93.Found: C, 56.30; H, 3.68, N, 11.04

5-(4-(3-(*1H*-Indol-3-yl)acryloyl)phenyl)pyrimidine-2,4,6(*1H,3H,5H*)-trione (9)

A mixture of chalcone **(3a-c)** (0.01mol) in ethanol (15 mL), barbituric acid (1.28 gm, 0.01mol) in dioxane (15 mL) and triethylamine (3 drops) was refluxed with stirring for 3 h. The formed precipitate was filtered, washed several times with ethanol and dried.

Yield: 69 % ; m.p. >300 °C; IR ν_{max}/cm^{-1}: 3360.00, 3271.27, 3159.40 (NH), 3078.39 (CH aromatic), 2804.50 (CH aliphatic), 1724.36, 1685.79, 1639.49 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 7.335-7.365 (m, 4H, indole H-5, H-6 and H-7, pyrimidine H-5), 7.591 (d, 2H, H-3, H-5 of C_6H_4), 7.898 (d, 2H, H-2, H-6 of C_6H_4), 8.738 (br s, 2H, indole H-4 and =CH), 9.559 (br s, 2H, indole H-2 and =CH), 11.056 (s, 1H, NH, exch.D_2O), 11.139 (s, 1H, NH, exch.D_2O), 12.752 (s, 1H, NH, exch.D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ : 61.42, 108.62, 111.41, 113.18, 117.66, 121.71, 122.69, 123.70, 129.16, 136.42, 138.03, 139.77, 143.69, 150.40, 163.25,164.55 ; Anal. Calcd for $C_{21}H_{15}N_3O_4$ (373.36): C, 67.56; H, 4.05; N, 11.25. Found: C, 67.62; H, 4.11; N, 11.37.

5-(4-(3-(*1H*-indol-3-yl)acryloyl)phenyl)-2-thioxo-dihydropyrimidine-4,6(*1H,5H*)-dione (10)

A mixture of chalcone **(3a-c)** (0.01mol) in ethanol (70 mL), thiobarbituric acid (1.44 gm, 0.01 mol) in dioxane (15 mL) and triethylamine (3 drops) was refluxed with stirring for 3 h. The formed precipitate was filtered, washed several times with ethanol and dried.

Yield: 40%; m.p. >300 °C; IR ν_{max}/cm^{-1}: 3630.03, 3437.15, 3155.54 (NH), 3066.82 (CH aromatic), 2908.65 (CH aliphatic), 1685.79, 1631.78 (C=O),1334.05(C=S); ^{1}H NMR (DMSO-d_6) δ ppm 7.332-7.363 (m, 4H, indole H-5, H-6 and H-7, pyrimidine H-5), 7.589 (d, 2H, H-3, H-5 of C_6H_4), 7.880 (d, 2H, H-2, H-6 of C_6H_4), 8.714 (br s, 2H, indole H-4 and =CH), 9.485 (br s, 2H, indole H-2 and =CH), 12.210 (s, 1H, NH, exch.D_2O), 12.256 (s, 1H, NH, exch.D_2O), 12.955 (s, 1H, NH, exch.D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 66.11, 108.79, 112.31, 113.38, 117.86, 123.10, 124.04, 128.91, 136.63, 141.07, 144.58, 149.71, 161.00, 162.92, 177.73, 201.10; MS m/z 389.3 (M^+, 6.08%), 390.3 (M+1, 24.14%);Anal. Calcd for $C_{21}H_{15}N_3O_3S$ (389.43): C, 64.77; H, 3.88; N, 10.79. Found: C, 64.75; H, 3.91; N, 10.88.

1-(4-Hydroxyphenyl)-3-(3-(4-hydroxyphenyl)-1-phenyl-*1H*-pyrazol-4-yl)prop-2-en-1-one (13)

To a solution of 3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde **(12)** (0.26g ; 0.001 mol), p-hydroxyacetophenone **(1_a)** (0.14g ; 0.001 mol) in ethanol (30 mL), a pellet of KOH (0.11g, 0.002 mol)was added. The reaction mixture was stirred at room temperature for 24 h, and then acidified with hydrochloric acid (2 mL). The formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

Yield: 47%; m.p. 210-211 °C; IR ν_{max}/cm^{-1}: 3348.42 (OH), 3109.25 (CH aromatic), 1685.79 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 6.894 (d,1H, *J*=9.6 Hz, olefenic H), 6.889 (d, 2H, *J*=6.9 Hz, H-3, H-5 of 4-OH C_6H_4), 7.401 (d, 2H, *J*=7.5 Hz, H-3, H-5 of 4-OH C_6H_4), 7.434-7.589 (m, 5H, Ar-H),

7.788 (d, 2H, *J*=6.9 Hz, H-2, H-6 of 4-OH C_6H_4), 7.975 (d, 2H, *J*=7.5 Hz, H-2, H-6 of 4-OH C_6H_4), 7.976 (d, 1H, *J*=9.6 Hz, olefenic H), 9.252 (s, 1H, H-5 pyrazole), 9.760 (s, 2OH, exch. D_2O); MS m/z 382.1 (M^+, 0.13 %); Anal. Calcd for $C_{24}H_{18}N_2O_3$ (382.41): C, 75.38; H, 4.74; N, 7.33. Found: C, 75.36; H, 4.82; N, 7.41.

4-[5-(3-(4-Hydroxyphenyl)-1-phenyl-*1H*-pyrazol-4-yl)-4,5-dihydro-*1H*-pyrazol-3-yl]phenol (14)

A solution of 1-(4-hydroxyphenyl)-3-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one **(13)** (0.38 g, 0.001 mol) and 99% hydrazine hydrate (0.1 mL, 0.002 mol) in ethanol (5 mL) was refluxed for 5 h. The reaction mixture was poured into ice –water (10 mL). The formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

Yield: 68%; m.p. 82-83°C; IR ν_{max}/cm^{-1}: 3356.14 (OH), 3286.70 (NH), 3066.82 (CH aromatic); ^1H NMR (DMSO-d_6) δ ppm : 3.378 (dd,1H, *J*=7.5, 3.9 Hz, pyrazoline H-4), 3.445 (dd, 1H, *J*= 7.4,3.9 Hz, pyrazoline H-4), 4.323 (t,1H, *J*= 4.0 Hz, pyrazoline H-5), 6.920(d, 4H, *J*= 8.4, Hz, H-3, H-5 of 4-OH C_6H_4) 7.350-7.583 (m, 6H, Ar-H and NH exch.D_2O), 7.887 (d, 4H, *J*=8.1 Hz, H-2, H-6 of 4-OH C_6H_4), 8.661 (s, 1H, pyrazole H-5), 9.755 (s,2H, 2OH, exch. D_2O); MS m/z 394.15 (M^+,0.12%). Anal. Calcd for $C_{24}H_{20}N_4O_2$ (394.43): C, 72.71; H, 5.08; N, 14.13. Found: C, 72.69; H, 5.14; N, 14.31.

1-[3-(4-Hydroxyphenyl)-5-(3-(4-hydroxyphenyl)-1-phenyl-*1H*-pyrazol-4-yl)-4,5-dihydropyrazol-1-yl]ethanone (15)

A solution of 1-(4-hydroxyphenyl)-3-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one **(13)** (0.38 g, 0.001 mol) and 99% hydrazine hydrate (0.1 mL, 0.002 mol) in glacial acetic acid (5 mL) was refluxed for 5 h. The reaction mixture was poured into ice –water (10 mL). The formed precipitate was filtered, washed with water, dried and crystallized from ethanol.

Yield: 23%; m.p. 155-156 °C; IR ν_{max}/cm^{-1}: 3371.57 (OH), 3066.82 (CH aromatic), 2924.09 (CH aliphatic), 1654.92 (C=O); ^1H NMR (DMSO-d_6) δ ppm : 2.175 (s, 3H, CH_3), 3.375(dd, 1H, *J*=8.6, 4.9 Hz, pyrazoline H-4), 3.409 (dd, 1H, *J*= 8.8, 4.9 Hz, pyrazoline H-4), 4.342 (t,1H, *J*= 4.8 Hz, pyrazoline H-5), 6.791-7.386 (m, 5H, Ar-H), 7.771 (d, 4H, *J*=8.4 Hz, H-3, H-5 of 4-OH C_6H_4), 7.972 (d, 4H, *J*=8.4 Hz, H-2, H-6 of 4-OH C_6H_4), 8.890 (s, 1H, pyrazole H-5), 9.753, 9.809 (2s, 2OH, exch. D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.22, 29.81, 81.38, 115.27, 115.50, 116.39, 116.54, 118.51, 118.61, 122.92, 126.61, 127.45, 129.54, 129.67, 129.74, 130.08, 135.99, 139.08, 151.41, 157.90, 165.05,171.36 ; Anal. Calcd for $C_{26}H_{22}N_4O_3$ (436.46): C, 71.22; H, 5.06; N, 12.78. Found: C, 71.57; H, 4.73; N, 13.17

Antitumor screening

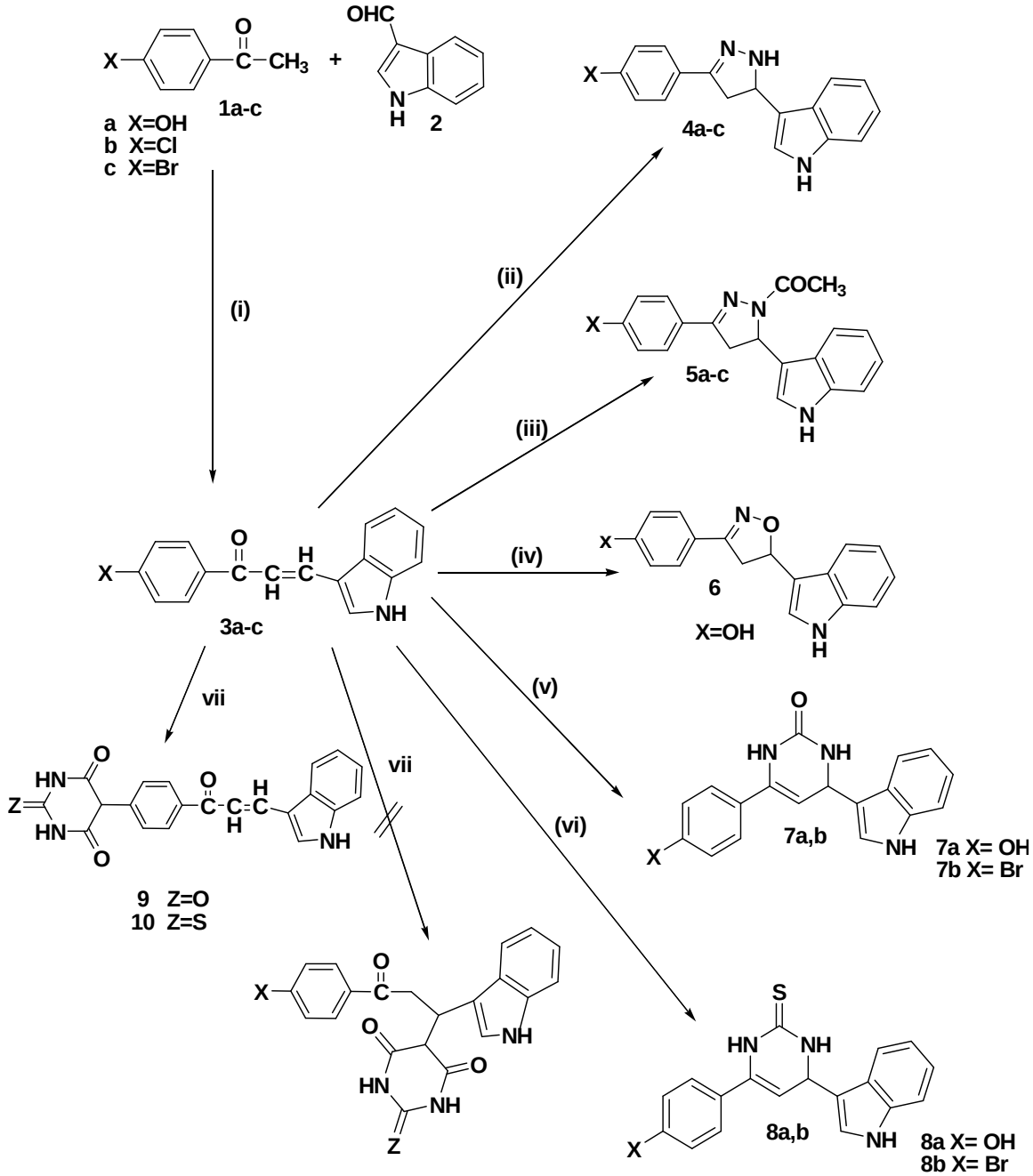
Under a sterile condition, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA), 5 $X10^5$ cells / ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilized in dimethylsulfoxide (DMSO), however, each final dilution contains less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 ml) containing a cell population of 6 X 10^4 cells/ml was pippeted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37°C. The incubator was supplied with 5% CO2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution[26]

RESULTS AND DISCUSSION

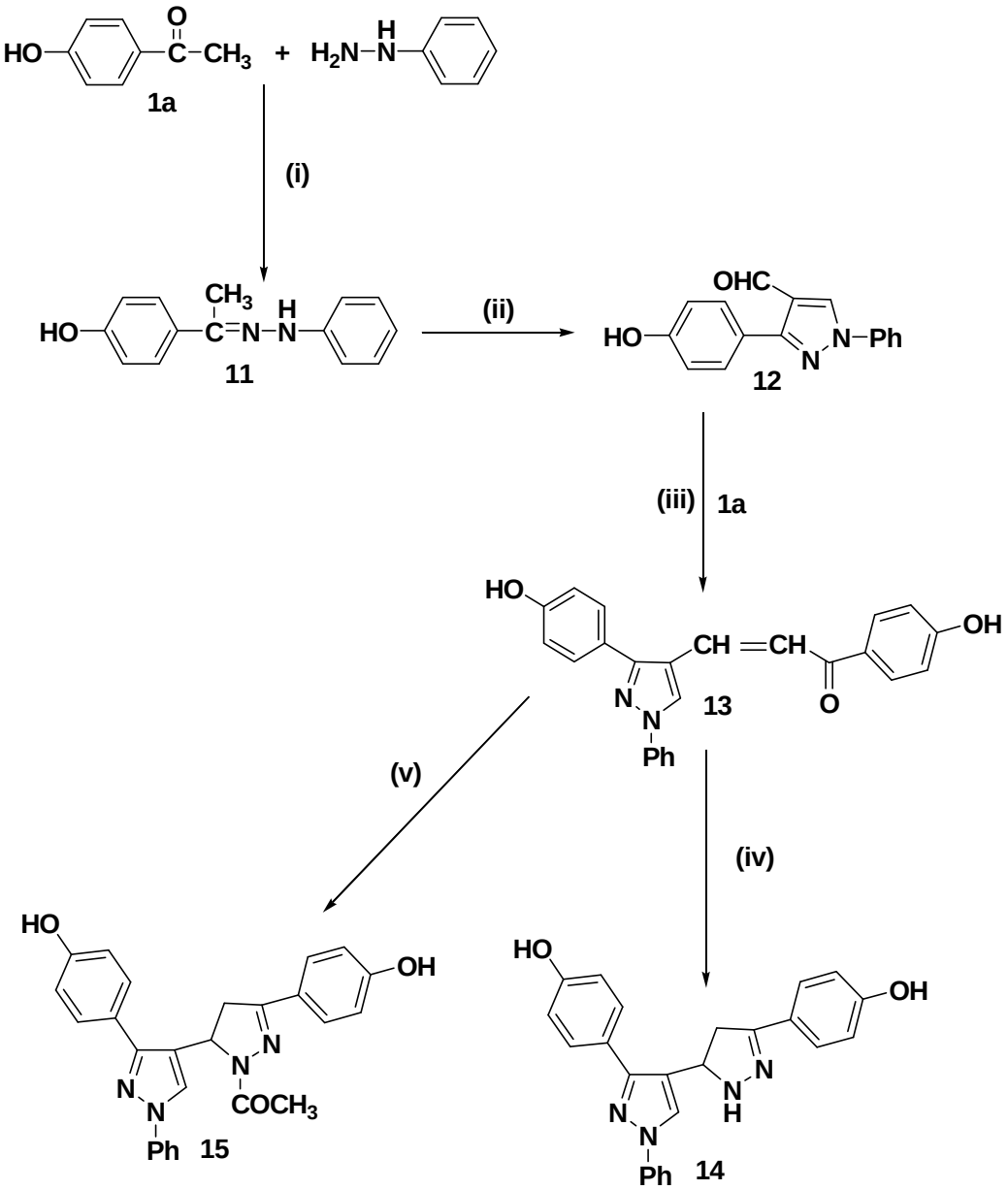
Chemistry

The synthesis of the target compounds was accomplished according to the reaction sequences illustrated in Schemes 1 and 2. Chalcones **(3a)**[23], **(3b,c)**[24] were synthesized by reacting 4-substituted acetophenone with indolyl-3-carboxaldehyde in the presence of potassium hydroxide by conventional Claisen-Schmidt conden-

sation. The reaction between chalcones **(3a–c)** with hydrazine hydrate in ethanol led to synthesis of novel pyrazoline derivatives **(4a-c)**. The synthesized compounds were characterized by their physical and spectral data (IR, ^{1}H-NMR) that confirmed the structures of the novel compounds. The IR spectra of the chalcones **(3a-c)** showed the characteristic band for conjugated C=O at 1635cm^{-1} which disappeared in the pyrazoline derivatives **(4a-c)**. Compounds **(4a-c)** showed an additional sharp band in the region 3394–3217 cm^{-1} due to the NH stretch. The ^{1}HNMR data of **(4a-c)** showed H_4, H'_4 of pyrazoline ring as double doublet centered at δ 2.576-3.416, 3.621-3.782 ppm respectively. While H_5 of pyrazoline nucleus appeared as



Scheme 1 : Reagents and conditions: (i) EtOH, 40% KOH, stir, rt, 24 h; (ii) NH_2NH_2, EtOH, reflux, 5 h; (iii) NH_2NH_2, glacial acetic acid, reflux, 5 h; (iv) $NH_2OH.HCl$, $NaOCOCH_3$, EtOH, reflux, 5 h; (v) Urea, EtOH, conc. H_2SO_4, reflux, 5 h; (vi) Thiourea, EtOH, conc. H_2SO_4, reflux, 5 h; (vii) for 9, barbituric acid, dioxane, EtOH, triethylamine, reflux, 3 h, for 10, thiobarbituric acid, dioxane, EtOH, triethylamine, reflux, 3 h.



Scheme 2 : Reagents and conditions (i) EtOH, glacial acetic acid, reflux, 1 h (ii) $POCl_3$, DMF, reflux at 70°C, 8 h; (iii)1a:phydroxyacetophenone, EtOH, KOH, stir, rt, 24 h; (iv) NH_2NH_2, EtOH, reflux, 5 h; (v) NH_2NH_2, glacial acetic acid, reflux, 5 h

triplet at 4.365-5.921 ppm. Meanwhile, cyclization of chalcones **(3a-c)** with hydrazine hydrate in presence of glacial acetic acid afforded 1-*N*- acetyl derivatives **(5a-c)**. On the other hand, cyclization of **(3a)** by treatment with hydroxylamine hydrochloride in the presence of sodium acetate in absolute ethanol afforded 4-(5-(*1H*-indol-3-yl)-4,5-dihydroisoxazol-3-yl) phenol **(6)** according to the previously described procedure for the preparation of analogous compounds[27] in a good yield. The structure was supported by its ^{1}H NMR spectrum, which showed double doublets at δ 2.66 and δ 3.44 for CH_2 protons of isoxazoline ring. The CH proton at C-5 of isoxazoline was obtained as triplet at δ 4.358. Thus, disappearance of signals of the olefinic protons and appearance of CH_2 and CH proton signals in the spectrum confirmed the formation of isoxazoline ring. Furthermore, treatment of chalcone **(3a,c)** with urea or thiourea in presence of ethanol containing few drops of sulfuric acid using the method reported by Abd El-Gawad[28] afforded dihydropyrimidine derivatives **(7a,b)** and **(8a,b)** respectively. The IR spectra of compounds **(7a,b)** showed the presence of absorption

bands from 3440 to 3315 cm^{-1} for the NH group and from 1674 to 1662 cm^{-1} for C=O stretching vibrations. The structure of the pyrimidin-2-ones **(7a,b)** was further supported by their ^{1}HNMR spectral data, which showed two doublets from δ 3.8 to 4.3 ppm and from δ 6.9 to 7.01 ppm, respectively, due to methene and olefinic protons of the pyrimidine ring. The two NH protons of the pyrimidine ring were seen as two broad singlets from δ10.43 to 10.49 ppm and from δ 12.06 to 12.09 ppm, respectively.

One of the objectives of this work was the addition of barbituric acid and thiobabrituric acid to the chalcones through Michael addition, according to the procedure previously adopted by Osman and his colleague[29]. Refluxing of **(3a-c)** with barbituric or thiobarbituric acid in dioxane as a high boiling solvent in the presence of triethylamine in an attempt to obtain the addition product was unsuccessful. Instead, unexpected nucleophilic substitution of the chlorine, bromine and hydroxyl atom at 4-position of phenyl ring with barbiturate anion was isolated. This unexpected result can be attributed to the high electron density of indole nucleus which can be extended by conjugation to the β-carbon of substituted propanone decreasing its nucleophilicity and enhance its resistance to nucleophilic attack. At the same time, the presence of electron withdrawing carbonyl group at the p- position to phenyl ring, the presence of good leaving halogen atoms and dioxane as aprotic solvent enhance nucleophilic substitution at 4-position. The structure of compounds **(9)**, **(10)** was supported by analytical and spectral data. IR ^{1}HNMR, ^{13}CNMR and MS spectrum of reaction of **(3a-c)** with barbituric gave the same spectra for the three compounds indicating that the separated product is only one and the same compound. IR of compound **(9)** displayed characteristic absorption band at 1639 due to C=O of chalcone and three peaks at 3360, 3271, 3159 $cm^{-1}$for NH. Also, two sharp peaks at 1724.36, 1685.79 due to the carbonyl groups of barbituric acid. Thiobarbiturate derivative **(10)** gave nearly the same results with the exception that there is no peak for carbonyl function at 1724 cm^{-1} and appearance of a peak at 1334 cm^{-1} for the C=S group.

On the other hand, 3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazole-4-carboxaldehyde **(12)** was synthesized from 4-hydroxy acetophenone phenylhydrazone **(11)** by Vilsmeier-Haack reagent[25]. Subsequently, the Claisen–Schmidt condensation of the obtained aldehyde **(12)** with p-hydroxyacetophenone afforded the corresponding pyrazolyl chalcone **(13)**. The structure elucidation of compound **(13)** was based on the spectral data. The IR spectra of compound **(13)** clearly showed absorption bands at 1685 cm^{-1} assigned to C=O functionality. ^{1}H-NMR spectrum of compound **(13)** was consistent with a *Z*–olefinic structure, the β olefinic proton appeared as doublet signal at 6.89 ppm, while the α- olefinic hydrogen was found along with aromatic region at 7.98 ppm with coupling constant between them of *J*= 9.6 Hz which agrees with *Z* conformation. The appearance of Z conformation not *E* may be attributed to large range of mesomeric effects due to large conjugation present in such compound[30]. Continuing with the synthetic approach, the reaction of a mixture of chalcone and hydrazine in the presence of ethanol as a solvent afforded the desired compound **(14)** in a moderate yield. When the same reaction was carried out using acetic acid instead of ethanol, it gave a different product identified as the corresponding *N*-acetyl derivative **(15)**. This finding suggests that acetic acid acted not only as a solvent but also as acetylating agent. Compounds **(14)** and **(15)** were characterized by detailed spectroscopic data. In the ^{1}HNMR spectra, the two methylenic 4-H protons and the 5-H proton of the pyrazoline moiety form an ABX spin system. Thus, the 4-H_A and 4'- H_B appeared each one as a double- doublet at 3.375 ppm and 3.409-3.440 ppm respectively while 5-H appear as triplet at 4.323-4.342 ppm.

Preliminary in-vitro anticancer screening

Out of the newly synthesized compounds, twelve derivatives **(4a)**, **(4b)**, **(4c)**, **(5a)**, **(5b)**, **(5c)**, **(6)**, **(7a)**, **(9)**, **(13)**, **(14)** and **(15)** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. A single dose (10 μM) of the test compounds was used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells[26]. The data was reported as mean-graph of the percent growth of the treated cells, and was presented

Full Paper

TABLE 1 : Percentage growth inhibition (GI %) of in-vitro subpanel tumor cell lines at 10 µM concentration of tested compounds. Bold values represents to point out the active compounds and lethal effect

Cell Line	Compound											
	4a	4b	4c	5a	5b	5c	6	7a	9	13	14	15
Leukemia												
CCRF-CEM	58.89	37.16	45.25	55.24	17.83	47.77	L	41.85	17.17	10.15	27.04	Nt
HL-60(TB)	14.05	L	19.26	Nt	L	32.80	L	19.25	L	L	L	-
K-562	-	L	11.64	-	-	41.92	L	26.46	-	-	23.70	13.89
MOLT-4	43.36	12.70	29.66	32.05	11.79	48.81	-	48.24	16.31	33.87	33.74	20.78
RPMI-8226	-	-	18.18	-	13.50	17.53	L	24.87	-	-	32.40	Nt
SR	-	L	L	-	-	27.37	-	nt	Nt	11.50	L	15.99
Non-Small ell Lung Cancer												
A549/ATCC	-	-	-	-	-	16.61	L	14.46	-	-	L	-
HOP-62	L	L	L	L	33.72	41.98	L	19.54	-	L	L	L
HOP-92	10.93	23.83	36.77	12.65	20.29	19.06	13.55	-	-	-	13.52	Nt
NCI-H226	-	Nt	-	10.90	Nt	13.71	-	-	L	-	13.19	11.44
NCI-H23	-	L	-	11.29	23.85	23.08	-	12.35	-	-	-	-
NCI-H322M	-	-	-	-	16.44	17.73	-	13.71	L	-	-	11.46
NCI-H460	-	L	-	L	17.74	15.25	-	-	L	L	-	L
NCI-H522	14.34	18.94	L	L	25.17	-	L	-	13.28	14.07	-	L
Colon Cancer												
COLO 205	L	L	L	L	-	14.17	-	27.76	L	L	-	L
HCC-2998	L	L	L	L	L	L	L	nt	Nt	-	22.41	L
HCT-116	-	-	-	14.08	38.34	50.65	-	19.27	-	12.14	-	-
HCT-15	-	-	-	-	19.08	25.77	-	17.40	-	-	11.24	-
HT29	L	L	L	L	-	10.82	L	-	L	L	L	L
KM12	L	-	-	-	15.24	15.63	L	13.80	L	-	18.00	-
SW-620	-	-	-	-	10.97	17.61	-	-	L	-	-	L
CNS Cancer												
SF-268	12.87	-	11.02	-	13.47	27.74	-	15.42	-	11.05	12.61	12.84
SF-295	-	-	-	-	24.72	19.57	-	nt	Nt	L	L	-
SF-539	-	-	-	-	18.27	27.06	-	12.09	-	10.62	17.50	13.68
SNB-19	L	L	-	L	L	15.72	L	21.08	-	26.97	-	12.36
SNB-75	27.43	20.53	nt	17.37	19.06	26.95	L	nt	Nt	17.52	Nt	33.25
U251	-	-	-	-	25.07	44.25	-	30.02	-	11.64	-	-
Melanoma												
LOX IMVI	-	-	10.28	11.97	13.19	30.44	-	23.26	-	12.56	12.44	15.34
MALME-3M	12.28	17.19	-	-	12.75	10.97	L	13.30	-	-	-	-
M14	-	-	10.28	-	23.85	31.10	-	20.94	-	-	-	L
MDA-MB-435	-	-	-	-	11.49	21.74	-	18.87	-	-	-	L
SK-MEL-2	L	-	L	L	-	L	L	11.18	-	L	L	-
SK-MEL-28	-	-	L	-	-	12.54	L	-	L	-	L	L
SK-MEL-5	L	-	L	-	21.17	13.90	L	-	-	L	-	13.86
UACC-257	-	L	-	-	L	10.58	-	11.02	L	-	L	L
UACC-62	-	L	-	-	-	16.06	L	-	L	-	11.57	14.44

Cell Line	Compound											
	4a	4b	4c	5a	5b	5c	6	7a	9	13	14	15
Ovarian Cancer												
IGROV1	-	-	-	18.92	25.05	39.85	-	30.73	-	-	16.84	15.60
OVCAR-3	-	L	-	-	10.01	25.96	-	-	L	L	-	-
OVCAR-4	13.81	-	13.66	-	38.05	44.59	12.14	22.18	-	12.90	-	11.67
OVCAR-5	-	-	-	-	-	11.00	-	-	L	L	-	-
OVCAR-8	-	L	-	-	-	15.70	-	12.41	-	Nt	-	-
NCI/ADR-RES	L	-	L	L	13.64	12.92	-	-	L	-	-	L
SK-OV-3	L	L	L	-	-	-	L	-	L	-	L	-
Renal Cancer												
786-0	L	L	L	L	21.11	24.10	L	-	L	-	L	L
A498	-	19.38	34.88	-	28.45	12.64	25.85	L	18.22	35.03	-	22.60
ACHN	-	-	-	-	21.54	24.77	10.75	20.75	-	-	12.12	19.14
CAKI-1	11.10	14.33	13.26	16.47	38.78	36.88	16.44	20.96	-	-	16.81	16.75
RXF 393	L	L	L	L	19.36	60.90	L	33.89	-	-	-	-
SN12C	-	L	11.49	-	-	22.43	L	11.98	L	-	-	-
TK-10	L	L	L	L	-	L	L	L	L	L	L	L
UO-31	50.45	37.57	42.57	48.29	39.48	57.11	34.93	52.80	39.47	30.00	31.40	40.11
Prostate Cancer												
PC-3	21.28	13.18	22.61	23.65	18.45	28.02	15.87	30.96	13.82	13.62	20.76	-
DU-145	L	L	L	L	-	L	L	L	L	L	20.95	L
Breast Cancer												
MCF7	25.61	23.72	33.24	22.90	28.49	42.91	-	43.31	12.09	-	15.14	16.61
MDA-MB-231/ATCC	14.57	-	32.29	27.50	Nt	52.62	-	34.24	L	19.09	44.77	28.19
HS 578T	10.31	-	11.74	10.94	-	25.41	10.66	11.39	L	-	-	-
BT-549	18.21	-	19.89	18.42	23.20	30.86	12.81	-	-	16.59	L	L
T-47D	-	-	11.80	-	27.31	31.82	L	35.10	-	18.51	29.10	25.59
MDA-MB-468	L	Nt	-	-	Nt	27.09	L	12.29	-	L	-	12.21

a -, GI <10%; nt , not tested; L, compound proved lethal to the cell

as percentage growth inhibition (GI %).

The obtained data revealed that some of the tested subpanel tumor cell lines exhibited variable sensitivity profiles against most of the tested compounds. Regarding the activity towards individual cell lines, the renal cancer UO-31cell line exhibited a wide range of sensitivity towards all of the tested analogues, particularly compound **(5c)** (GI 57.11%). Moreover, compounds **(4a)**, **(4c)**, **(5a)** and **(7a)** showed moderate growth inhibitory activity against the same cell line with GI values of 50.45, 42.57, 48.29, and 52.80%, respectively. Furthermore, regarding the activity against the leukemia subpanel, the growth of the leukemia MOLT-4 cell line was variably affected by the presence of all the tested compounds except the analog **(6)**. Particular effectiveness against this cell line was shown by compounds **(5c)**, **(7a)** with a GI value of 48.81, 48.24%, respectively. Whereas the analogue **(4a)** exhibited moderate activity against the same cell line (GI 43.36). The growth of the CCRF-CEM cell line was found to be affected by the presence of the ten tested compounds with a reliable sensitivity to compounds **(4a)** and **(5a)** (GI 58.89and 55.24%, respectively). Meanwhile, leukemia cell line CCRF-CEM; colon cancer HCT-116; renal cancer RXF 393, UO-31 and breast cancer MDA-MB 231/ATCC proved to be selectively sensitive to **(5c)** with GI values of 47.77, 50.65, 60.90, 57.11 and 52.62%, respectively. It is worth-mentioning that only compounds **(5b)** and **(5c)** were able to inhibit the growth of the non-small cell lung cancer HOP-62 cell line with

GI values of 33.72 and 41.98%, respectively. Regarding to broad spectrum antitumor activity; close examination of the data presented in TABLE 1, revealed that compounds **(5c)** and **(7a)** are the most active members of this study, showing effectiveness toward numerous cell lines belonging to different tumor subpanels. The same analogy indicated that **(4c)**, **(5b)** and **(14)** possess moderate antitumor activity. Compounds **(4a)**, **(5a)** and **(7a)** possess selective potency towards leukemia cell lines, and renal cancer cell lines.

Structure-activity correlation, based on the number of cell lines proved sensitivity towards each of the synthesized individual compounds, revealed that, 1-(3-(4-bromophenyl)-5-(*1H*-indol-3-yl)-4,5-dihydropyrazol-1-yl) ethanone **(5c)** is more active antitumor agent than the other pyrazoline derivatives **(4a-c)**, **(5a,b)**. Counterparts. The replacement of a pyrazoline nucleus by isoxazoline ring (compound **(6)**) causes a dramatic decrease in the antitumor activity. While the replacement of pyrazoline ring with dihydropyrimidine nucleus (compound **(7a)**) shows moderate antitumor activity. Replacement of indole nucleus by 3 (4-hydroxy phenyl) -1-phenyl-1H-pyrazol nucleus produces compounds **(14)** and **(15)** with nearly a mild broad-spectrum antitumor activity.

CONCLUSION

Starting from Chalcones **(3a-c)**, different new cycliclized derivatives such as pyrazoline **(4a-c)**, **(5a-c)**, isoxaline **(6)** and pyrimidine derivatives **(7a,b)**, **(8a,b)**, **(9)** and **(10)** were prepared. Also pyrazolyl chalcone **(13)** was used as a starting compound for the preparation of pyrazoline derivatives **(14)** and **(15)**. Evaluation of cytotoxic activity for the synthesized compounds by *in-vitro* disease-oriented human cells screening panel assay revealed that all the synthesized compounds exhibit moderate cytotoxic activity especially against renal cancer UO-31cell line, particularly compound **(5c)** which shows inhibition 57.11%. The pyrazoline derivatives are more potent than other cyclized derivatives as anticancer agents. While the isoxaline derivative **(6)** show only mild antitumor against renal cancer UO-31 (34.93%). Meanwhile, the pyrimidine derivatives show moderate antitumor activity. These preliminary encouraging results of anticancer screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of new lead compounds in the treatment of cancer.

ACKNOWLEDGEMENTS

We are grateful to the NCI, Bethesda, MD, USA for performing the antitumor testing of the synthesized compounds.

SUPPLEMENTARY INFORMATION

Supplementary data which containing the srectroscopic data IR,NMR and MS associated with some compounds in this article can be found, in the online version.

REFERENCES

[1] J.B.Gibbs; Science, http://doi:10.1126/science.287.5460.1969, **287**, 1969 **(2000)**.

[2] P.Heffeter, M.A.Jakupec, W.Orner, S.Wild, N.G.Keyserlingk, L.Elbling, H.Zorbas, A.Korynevska, S.Knasmuller, H.Sutterluty, M.Micksche, B.K.Keppler, W.Berger; Biochem.Pharmacol., http://dx.doi.org/10.1016/j.bcp.2005.11.009, **71**, 426 **(2006)**.

[3] **(a)** L.U.Charles, R.Komaki, J.S.Lee, D.M.Shin, J.L.Palmer, B.J.Coldman, K.M.Pisters, J.M.Kurie, F.V.Fossella, B.S.Glisson; Clin.Cancer Res., **9**, 2085 **(2003)**; **(b)** G.F.Fleming, J.W.Kugler, P.C.Hoffman, R.Ansari, J.D.Bitran, A.Klepsch, D.Malone, A.A.Fasanmade, M.J.Ratain, E.E.Vokes; J.Clin Oncology, **16**, 2032 **(1998)**.

[4] H.P.Avila, E.F.A.Smania, F.D.Monache, A.S.Junior; Bioorg.Med.Chem., http://dx.doi.org/10.1016/j.bmc.2008.09.064, **16**, 9790 **(2008)**.

[5] **(a)** A.Modzelewska, C.Pettit, G.Achanta, N.E.Davidson, P.Huang, S.R.Khan; Bioorg.Med.Chem., http://dx.doi.org/10.1016/j.bmc.2006.01.003, **14**, 3491 **(2006)**; **(b)** R.J.Anto, K.Sukumaran, G.Kuttana, M.N.A.Rao, V.Subbaraju, R.Kuttana; Cancer Lett., http://dx.doi.org/10.1016/0304-3835(95)03945-S, **97**, 33 **(1995)**; **(c)** Y.L.Hsu, P.L.Kuo, W.S.Tzeng, C.C.Lin; Food Chem.Toxicol., http://dx.doi.org/10.1016/j.fct.2005.10.003, **44**, 704 **(2006)**; **(d)** N.J.Lawrence, R.P.Patterson, L.L.Ooi, D.Cook,

S.Ducki; Bioorg.Med.Chem.Lett., http://dx.doi.org/10.1016/j.bmcl.2006.08.065, **16**, 5844 **(2006)**.

[6] A.Valla, B.Valla, D.Cartier, R.L.Guillou, R.Labia, L.Florent; Eur.J.Med.Chem., http://dx.doi.org/10.1016/j.ejmech.2005.05.008, **41**, 142 **(2006)**.

[7] S.F.Nielsen, S.B.Christensen, G.Cruciani, A.Kharazmi, T.Liljefors; J.Med.Chem., http://dx.doi:10.1021/jm980410m, **41**, 4819 **(1998)**.

[8] S.H.Lee, G.S.Seo, J.Y.Kim, X.Y.Jin, H.D.Kim, D.H.Sohn; Eur.J.Pharmacol., http://dx.doi.org/10.1016/j.ejphar.2006.01.005, **532**, 178 **(2006)**.

[9] S.Cheenpracha, C.Karalai, C.Ponglimanont, S.Subhadhirasakul, S.Tewtrakul; Bioorg.Med.Chem., http://dx.doi.org/10.1016/j.bmc.2005.10.019, **14**, 1710 **(2006)**.

[10] L.Svetaz, A.Tapia, S.N.Lopez, R.L.E.Furlan, E.Petenatti, R.Pioli; J.Agric.Food.Chem., http://dx.doi:10.1021/jf035213x, **52**, 3297 **(2004)**.

[11] O.Nerya, R.Musa, S.Khatib, S.Tamir, J.Vaya, Phytochemistry, http://dx.doi.org/10.1016/j.phytochem.2004.04.016, **65**, 1389 **(2004)**.

[12] Q.C.Meng, L.Ni, K.J.Worsencroft, J.Ye, M.D.Weingarten, J.M.Simpson, J.W.Skudlarek, E.M.Marino, K.Suen, C.Kunsch, A.Souder, R.B.Howard, C.L.Sundell, M.A.Wasserman, J.A.Sikorski; J.Med.Chem., http://dx.doi.10.1021/jm0614230, **50**, 1304 **(2007)**.

[13] K.Manna, Y.K.Agrawal; Bioorg.Med.Chem.Lett., http://dx.doi.org/10.1016/j.bmcl.2009.03.161, **19**, 2688 **(2009)**.

[14] M.Abid, A.R.Bhat, F.Athar, A.Azam; Eur.J.Med.Chem., http://dx.doi..org/10.1016/j.ejmech.2007.10.032, **44**, 417 **(2009)**.

[15] Z.A.Kaplancikli, G.Turan-Zitouni, A.Özdemir, O.D.Can, P.Chevallet; Eur.J.Med.Chem., http://dx.doi.org/10.1016/j.ejmech.2008.09.002, **44**, 2606 **(2009)**.

[16] D.Havrylyuk, B.Zimenkovsky, O.Vasylenko, L.Zaprutko, R.Lesyk; Eur.J.Med.Chem., http://dx.doi.org/10.1016/j.ejmech.2008.09.032, **44**,1396 **(2009)**.

[17] O.M.Khalil, H.M.Refaat; Orient.J.Chem., **27(4)**, 1581 **(2011)**.

[18] J.Easmon, G.Purstinger, G.Heinisch, T.Roth, H.H.Fiebig, W.Holzer, W.Jäger, M.Jenny, J.Hofmann; J.Med.Chem., http://dx.doi:10.1021/jm000979z, **44**, 2164 **(2001)**.

[19] Y.Dai, K.Hartandi, Z.Ji, A.A.Ahmed, D.H.Albert, J.L.Bauch, J.J.Bouska, P.F.Bousquet, G.A.Cunha, K.B.Glaser, C.M.Harris, D.Hickman, J.Guo, J.Li, P.A.Marcotte, K.C.Marsh, M.D.Moskey, R.L.Martin, A.M.Olson, D.J.Osterling, L.J.Pease, N.B.Soni, K.D.Stewart, V.S.Stoll, P.Tapang, D.R.Reuter, S.K.Davidsen, M.R.Michaelides; J.Med.Chem., http://dx.doi. 10.1021/jm061280h, **50**, 1584 **(2007)**.

[20] **(a)** S.J.Tangeda, A.Garlapati; Eur.J.Med.Chem., http://dx.doi:org/10.1016/j.ejmech. 2009.12.050, **45**, 1453 **(2010)**; **(b)** C.Mugnaini, E.Petricci, M.Botta, F.Corelli, P.Mastromarino, G.Giorgi; Eur.J.Med.Chem., http://dx.doi.org/10.1016/j.ejmech.2006.09.002, **42**, 256 **(2007)**; **(c)** D.Raffa, M.C.Edler, G.Daidone, B.Maggio, M.Merickech, S.Plescia, D.Schillaci, R.Bai; Eur.J.Med.Chem., http://dx.doi.org/10.1016/j.ejmech.2003.12.009, **39**, 299 **(2004)**.

[21] S.Zhu, S.Ji, X.Su, C.Sun, Y.Liu; Tetrahedron Lett., http://dx.doi.org/10.1016/j.tetlet.2008.01.054, **49**, 1777 **(2008)**.

[22] M.M.Kandeel, N.A.Abdou, H.H.Kadry, R.M.El-Masry; Int.J.Chem.Tech.Res., **5**, 401 **(2013)**.

[23] D.Kumar, N.M.Kumar, K.Akamatsu, E.Kusaka, H.Harada, T.Ito; Bioorg.Med.Chem.Lett., http://dx.doi:10.1016/j.bmcl.2010.05.016, **20**, 3916 **(2010)**.

[24] M.N.Bhatia, K.Mahadik; Sci.Pharma., http://dx.doi:10.3797/scipharm.0803-36, **76**, 259 **(2008)**.

[25] S.C.Shetty, V.C.Bhagat; Asian J.Chem., **20**, 5037 **(2008)**.

[26] **(a)** M.R.Grever, S.A.Schepartz, B.A.Chabner; Semin.Oncol., **19**, 622 **(1992)**; **(b)** A.Monks, D.Scudiero, P.Skehan; J.Natl.Cancer Inst., **83**, 757 **(1991)**; **(c)** M.R.Boyd, K.D.Paull; Drug Dev.Res., http://dx.doi.org/10.1002/ddr.430340203, **34**, 91 **(1995)**; **(d)** P.Skehan, R.Storeng, D.Scudiero, A.Monks, J.McMahon, D.Vistica, J.R.Warren, H.Bokesch, S.Kenney, M.R.Boyd; J.Natl.Cancer Inst., http://dx.doi.org/10.1093/jnci/82.13.1107, **82**, 1107 **(1990)**.

[27] M.Amir, S.A.Javed, H.Kumar; Med.Chem.Res., http://dx.doi:10.1007/s00044-009-9194-8, **19**, 299 **(2010)**.

[28] N.M.A.El-Gawad; Bull.Fac.Pharm.Cairo Univ., **37**, 47 **(1999)**.

[29] A.N.Osman, A.A.El-Gendy, M.M.Kandeel, E.M.Ahmad, H.A.A.El-Latif; Bull.Fac.Pharm. Cairo Univ., **41**, 51 **(2003)**.

[30] F.A.Omran, R.M.Mohareb, A.A.El-Khair; Molecules, **16**, 6129 **(2011)**.