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Efficient microwave irradiation enhanced stereoselective synthesis and antitumor activity of indolylchalcones and their pyrazoline analogs

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Abstract. 2-Aryl-1*H*-indole-3-carbaldehyde derivatives underwent Claisen–Schmidt condensation with acetophenone derivatives under microwave irradiation condition compared with the conventional heating to afford excellent yields of *trans* substituted indolylchalcones which subjected to condensation reaction with phenylhydrazine to afford their indolylpyrazoline analogs. The antitumor activity of the synthesized compounds was examined and evaluated against human hepatocellular carcinoma cell line (Hep-G2) as well as the half maximal inhibitory concentration (IC_{50}). Most of them showed high potent antitumor activity.

Keywords. Indolylchalcones; indolylpyrazolines; microwave irradiation; stereoselectivity; antitumor; Hep-G2.

1. Introduction

Indole nucleus is an important structural component in many drugs.[1,2] In addition, several indole derivatives have been reported to possess a wide variety of biological and pharmacological properties.[3–7]

Chalcones, 1,3-diarylprop-1-en-2-ones, are a class of compounds consisting of two aryl rings linked by an α,β-unsaturated ketone moiety. Some chalcones are natural products found in various plant species around the world and in the last decade they have been shown to display a wide range of medicinal properties.[8–11] The anticancer activity of certain chalcones is believed to be a result of binding to tubulin and preventing it from polymerising into microtubules.[12]

Pyrazolines are important nitrogen-containing five-membered heterocyclic compounds. Several pyrazoline derivatives possess important pharmacological activities and therefore they are useful materials in drug research.[13] Moreover, pyrazoline derivatives were found to possess antitumor,[14] antibacterial,[15] anti-inflammatory,[16] antidiabetic[17] and antidepressant activities.[18]

On the other hand, microwave assisted organic synthesis (MAOS) has become increasingly popular in recent years to improve the yields and shorten reaction times in a variety of reactions.[19]

Prompted by the above-mentioned biological properties of indoles, chalcones, pyrazolines and the interest with design and synthesis of indole and pyrazoline derivatives,[20–22] as well as the utility of microwave irradiation in organic synthesis,[22,23] a synthesis of novel series of substituted 3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-ones and their substituted 1-phenyl-3-aryl-5-(2-arylindolyl)-pyrazoline analogs *via* both microwave irradiation and the conventional method were presented. Subsequently, investigation of their cytotoxic effect *in vitro* against human hepatocellular carcinoma cell line (Hep-G2) has been illustrated.

2. Experimental

2.1 *Materials, method and instruments*

All melting points were taken on a Stuart scientific melting point apparatus (Stuart Scientific, Stone, and Staffordshire, UK) and were uncorrected. The ^{1}H-NMR spectra were recorded on a Varian Ger-

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mini-2000 (300 MHz) spectrometer (Varian Inc., Palo Alto, CA, USA), using TMS as the internal standard and with DMSO-*d*6 as the solvent; the chemical shifts are reported in ppm (δ) and coupling constants (J) values are given in Hertz (Hz). Signal multiplicities are represented by: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *dd* (double doublet) and *m* (multiplet). IR spectra were obtained on a Nicollet IR 200 FT–IR spectrophotometer using KBr pellets. Elemental analyses (C, H, N) were conducted using the Elemental Analyzer Yanaca CHN Corder MT-3, their results were found to be in good agreement (± 0·3%) with the calculated values. MS spectra were established on GC MS-QP 1000 EX Mass Spectrometer (Shimadzu, Tokyo, Japan). The microwave-induced reactions were carried out in an open Pyrex-glass vessel under atmospheric pressure in domestic Whirl Pool-TALENT oven. The synthesized products and each reaction were carried out under conventionally or microwave (MW) irradiation conditions were monitored on Merck silica gel 60 F254 plates (type E; Merck, Darmstadt, Germany) using UV light (254 and 360 nm) for detection. The required starting material, **1**, was prepared by adopting the earlier reported procedures.[22b] All chemicals and solvents were purchased from E. Merck (Darmstadt, Germany) and Sigma-Aldrich. All diagrams and calculations were performed using maxus (Bruker Nonius, Delft & MacScience, Japan).

2.2 *General procedure for the synthesis of indolylchalcone derivatives* **3a–l**

2.2a *Microwave irradiation procedure:* A mixture of 2-aryl-1*H*-indole-3-carbaldehyde **1** (1 mmol), acetophenone **2** (1 mmol), ethylene glycol (1 mL) and piperidine (0·5 mL) in a Pyrex-glass opened vessel was subjected to a microwave irradiation at 750 W. Irradiation was carried out in successive of 30 s periods (table 1). After cooling to room temperature, water (10 mL) was added to the mixture. The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol to afford the pure product **3a–l**.

2.2b *Conventional heating procedure:* A mixture of 2-aryl-1*H*-indole-3-carbaldehyde **1** (1 mmol), acetophenone **2** (1 mmol), ethylene glycol (5 mL) and piperidine (1 mL) was heated under reflux at 160–180°C for the appropriate time (table 1). After cooling, water (10–20 mL) was added to the flask. The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol to afford the pure product **3a–l**.

2.2c *(E)-3-(1-Allyl-2-phenyl-1H-indol-3-yl)-1-phenyl-prop-2-en-1-one* (**3a**): Yellow solid; m.p. 66–68°C; IR ν_{max} (KBr) 3054 (CH=CH), 1649 (C=O); ^{1}H–NMR (DMSO–d_6) δ (ppm): 4·71–4·73 (*m*, 1H, =CH_2), 4·81 (*d*, 2H, NCH_2), 5·10 (*d*, 1H, =CH_2), 5·83–5·96 (*m*, 1H, CH=), 7·34–8·25 (*m*, 14H, ArH), 7·51 (*d*, 1H, J = 15 Hz), 7·62 (*d*, 1H, J = 15 Hz); MS (EI), *m*/*z* 363 (M^+, 44). Anal. Calcd. for $C_{26}H_{21}NO$: C, 85·92; H, 5·82; N, 3·85. Found: C, 85·99; H, 6·07; N, 3·94.

2.2d *(E)-3-(1-Allyl-2-phenyl-1H-indol-3-yl)-1-(4-nitrophenyl)-prop-2-en-1-one* (**3b**): Pale red crystals; m.p. 188–190°C; IR ν_{max} (KBr) 3052 (CH=CH), 1648 (C=O); ^{1}H–NMR (DMSO-d_6) δ (ppm): 4·73–4·74 (*m*, 1H, =CH_2), 4·81 (*d*, 2H, NCH_2), 5·11 (*d*, 1H, =CH_2), 5·86–5·96 (*m*, 1H, CH=), 7·36–8·34 (*m*, 13H, ArH), 8·25 (*d*, 1H, J = 15·3 Hz), 8·34 (*d*, 1H, J = 15·3 Hz); MS (EI), *m*/*z* 408 (M^+, 46). Anal. Calcd. for $C_{26}H_{20}N_2O_3$: C, 76·45; H, 4·94; N, 6·86. Found: C, 76·50; H, 5·19; N, 6·97.

2.2e *(E)-3-(1-Allyl-2-phenyl-1H-indol-3-yl)-1-biphenyl-4-yl-prop-2-en-1-one* (**3c**): Yellow crystals; m.p. 110–112°C; IR ν_{max} (KBr) 3063 (CH=CH), 1644 (C=O); ^{1}H–NMR (DMSO–d_6) δ (ppm): 4·72–4·74 (*m*, 1H, =CH_2), 4·81 (*d*, 2H, NCH_2), 5·11 (*d*, 1H, =CH_2), 5·84–5·96 (*m*, 1H, CH=), 7·36–8·25 (*m*, 18H, ArH), 7·83 (*d*, 1H, J = 15·3 Hz), 8·14 (*d*, 1H, J = 15·3 Hz); MS (EI), *m*/*z* 439 (M^+, 45). Anal. Calcd. for $C_{32}H_{25}NO$: C, 87·44; H, 5·73; N, 3·19. Found: C, 87·51; H, 5·98; N, 3·30.

2.2f *(E)-3-(1-Allyl-2-p-tolyl-1H-indol-3-yl)-1-phenyl-prop-2-en-1-one* (**3d**): Yellow solid; m.p. 110–112°C; IR ν_{max} (KBr) 3029 (CH=CH), 1650 (C=O); ^{1}H–NMR (DMSO–d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 4·72–4·73 (*m*, 1H, =CH_2), 4·81 (*d*, 2H, NCH_2), 5·10 (*d*, 1H, =CH_2), 5·83–5·96 (*m*, 1H, CH=), 7·33–8·27 (*m*, 13H, ArH), 7·77 (*d*, 1H, J = 15·6 Hz), 7·83 (*d*, 1H, J = 15·6 Hz); MS (EI), *m*/*z* 377 (M^+, 48). Anal. Calcd. for $C_{27}H_{23}NO$: C, 85·91; H, 6·14; N, 3·71. Found: C, 85·98; H, 6·40; N, 3·60.

2.2g *(E)-3-(1-Allyl-2-p-tolyl-1H-indol-3-yl)-1-(4-nitrophenyl)-prop-2-en-1-one* (**3e**): Yellow solid; m.p. 130–132°C; IR ν_{max} (KBr) 3029 (CH=CH),

1649 (C=O); ^{1}H–NMR (DMSO–d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 4·72–4·73 (*m*, 1H, =CH_2), 4·81 (*d*, 2H, NCH_2), 5·10 (*d*, 1H, =CH_2), 5·83–5·96 (*m*, 1H, CH=), 7·25–8·27 (*m*, 12H, ArH), 7·70 (*d*, 1H, J = 15 Hz), 8·05 (*d*, 1H, J = 15 Hz); MS (EI), *m*/*z* 422 (M^+, 46). Anal. Calcd. for $C_{27}H_{22}N_2O_3$: C, 76·76; H, 5·25; N, 6·63. Found: C, 76·83; H, 5·51; N, 6·52.

2.2h *(E)-3-(1-Allyl-2-p-tolyl-1H-indol-3-yl)-1-biphenyl-4-yl-prop-2-en-1-one* (**3f**): Yellow solid; m.p. 112–114°C; IR ν_{max} (KBr) 3041 (CH=CH), 1643 (C=O); ^{1}H-NMR (DMSO–d_6) δ (ppm): 2·45 (*s*, 3H, CH_3), 4·72–4·74 (*m*, 1H, =CH_2), 4·82 (*d*, 2H, NCH_2), 5·10 (*d*, 1H, =CH_2), 5·84–5·96 (*m*, 1H, CH=), 7·35–8·26 (*m*, 17H, ArH), 7·82 (*d*, 1H, J = 15 Hz), 8·15 (*d*, 1H, J = 15 Hz); MS (EI), *m*/*z* 453 (M^+, 49). Anal. Calcd. for $C_{33}H_{27}NO$: C, 87·38; H, 6·00; N, 3·09. Found: C, 87·45; H, 6·26; N, 2·98.

2.2i *(E)-3-(1-Benzyl-2-phenyl-1H-indol-3-yl)-1-phenyl-prop-2-en-1-one* (**3g**): Pale orange solid; m.p. 156–158°C; IR ν_{max} (KBr) 3081 (CH=CH), 1653 (C=O); ^{1}H-NMR (DMSO–d_6) δ (ppm): 5·39 (*s*, 2H, NCH_2), 6·90–8·25 (*m*, 19H, ArH), 7·71 (*d*, 1H, J = 15·3 Hz), 8·06 (*d*, 1H, J = 15·3 Hz); MS (EI), *m*/*z* 413 (M^+, 80). Anal. Calcd. for $C_{30}H_{23}NO$: C, 87·14; H, 5·61; N, 3·39. Found: C, 87·21; H, 5·86; N, 3·53.

2.2j *(E)-3-(1-Benzyl-2-phenyl-1H-indol-3-yl)-1-(4-nitrophenyl)-prop-2-en-1-one* (**3h**): Orange crystals; m.p. 166–168°C; IR ν_{max} (KBr) 3066 (CH=CH), 1654 (C=O); ^{1}H–NMR (DMSO–d_6) δ (ppm): 5·41 (*s*, 2H, NCH_2), 7·34–8·35 (*m*, 18H, ArH), 7·66 (*d*, 1H, J = 15 Hz), 7·75 (*d*, 1H, J = 15 Hz); MS (EI), *m*/*z* 458 (M^+, 17). Anal. Calcd. for $C_{30}H_{22}N_2O_3$: C, 78·59; H, 4·84; N, 6·11. Found: C, 78·66; H, 5·09; N, 6·22.

2.2k *(E)-3-(1-Benzyl-2-phenyl-1H-indol-3-yl)-1-biphenyl-4-yl-prop-2-en-1-one* (**3i**): Yellow solid; m.p. 140–142°C; IR ν_{max} (KBr) 3055 (CH=CH), 1653 (C=O); ^{1}H-NMR (DMSO-d_6) δ (ppm): 5·40 (*s*, 2H, NCH_2), 7·31–8·30 (*m*, 23H, ArH), 7·83 (*d*, 1H, J = 15·3 Hz), 8·15 (*d*, 1H, J = 15·3 Hz); MS (EI), *m*/*z* 489 (M^+, 35). Anal. Calcd. for $C_{36}H_{27}NO$: C, 88·31; H, 5·56; N, 2·86. Found: C, 88·38; H, 5·81; N, 2·94.

2.2l *(E)-3-(1-Benzyl-2-p-tolyl-1H-indol-3-yl)-1-phenyl-prop-2-en-1-one* (**3j**): Yellow solid; m.p. 122–124°C; IR ν_{max} (KBr) 3053 (CH=CH), 1655 (C=O); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 5·38 (*s*, 2H, CH_2), 7·20–8·26 (*m*, 18H, ArH), 7·66 (*d*, 1H, J = 15 Hz), 7·74 (*d*, 1H, J = 15 Hz); MS (EI); *m*/*z* 427 (M^+, 28). Anal. Calcd. for $C_{31}H_{25}NO$: C, 87·09; H, 5·89; N, 3·28. Found: C, 87·15; H, 6·15; N, 3·17.

2.2m *(E)-3-(1-Benzyl-2-p-tolyl-1H-indol-3-yl)-1-(4-nitrophenyl)-prop-2-en-1-one* (**3k**): Orange solid; m.p. 132–134°C; IR ν_{max} (KBr) 3055 (CH=CH), 1649 (C=O); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 5·40 (*s*, 2H, NCH_2), 7·33–8·35 (*m*, 17H, ArH), 7·65 (*d*, 1H, J = 15·6 Hz), 7·77 (*d*, 1H, J = 15·6 Hz); MS (EI), *m*/*z* 472 (M^+, 46). Anal. Calcd. for $C_{31}H_{24}N_2O_3$: C, 78·79; H, 5·12; N, 5·93. Found: C, 78·86; H, 5·38; N, 5·82.

2.2n *(E)-3-(1-Benzyl-2-p-tolyl-1H-indol-3-yl)-1-biphenyl-4-yl-prop-2-en-1-one* (**3l**): Pale yellow solid; m.p. 126–128°C; IR ν_{max} (KBr) 3023 (CH=CH), 1649 (C=O); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 5·39 (*s*, 2H, NCH_2), 7·32–8·29 (*m*, 22H, ArH), 7·82 (*d*, 1H, J = 15 Hz), 8·15 (*d*, 1H, J = 15 Hz); MS (EI), *m*/*z* 503 (M^+, 20). Anal. Calcd. for $C_{37}H_{29}NO$: C, 88·24; H, 5·80; N, 2·78. Found: C, 88·31; H, 6·06; N, 2·67.

2.3 *General procedure for the synthesis of Indolylpyrazoline derivatives* **4a–l**

2.3a *Microwave irradiation procedure:* A mixture of substituted (*E*)-3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-one **3a–x** (1 mmol), phenylhydrazine (1 mmol), ethyl alcohol (2 mL) and acetic acid (0·5 mL) in a Pyrex-glass opened vessel was subjected to a microwave irradiation at 350W in a successive of 30 s periods (table 2). After cooling to room temperature, crystals that deposited were filtered off, washed with ethanol and dried to afford the pure product **4a–l**.

2.3b *Conventional heating procedure:* A mixture of substituted (*E*)-3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-one **3a–l** (1 mmol), phenylhydrazine (1 mmol) in ethyl alcohol (10–15 mL) with acetic acid (0·5 mL) was boiled under reflux (table 2). After cooling to room temperature, crystals that deposited were filtered off, washed with ethanol and dried to afford the pure product **4a–l**.

2.3c *1-Allyl-3-(2,5-diphenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-phenyl-1H-indole* (**4a**): Pale yellow crys-

tals; m.p. 168–170°C; IR ν_{max} (KBr) 2929 (CH), 1594 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3·40–3·49 (*m*, 1H, CH_2 pyrazoline), 3·96 (*dd*, 1H, CH_2 pyrazoline, J = 12·9 Hz), 4·64–4·70 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·10 (*d*, 1H, $=CH_2$ allyl), 5·29 (*dd*, 1H, CH pyrazoline, J = 8·1 Hz), 5·81–5·91 (*m*, 1H, CH = allyl), 6·59–7·80 (*m*, 19H, ArH); MS (EI), *m*/*z* 453 (M^+, 72). Anal. Calcd. for $C_{32}H_{27}N_3$: C, 84·74; H, 6·00; N, 9·26. Found: C, 84·82; H, 6·26; N, 9·36.

2.3d *1-Allyl-3-[5-(4-nitrophenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-2-phenyl-1H-indole* (**4b**): Dark red crystals; m.p. 200–202°C; IR ν_{max} (KBr) 2918 (CH), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4·00 (*dd*, 2H, CH_2 pyrazoline, J = 13·4 Hz), 4·62–4·67 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·00 (*d*, 1H, $=CH_2$ allyl), 5·40–5·48 (*m*, 1H, CH pyrazoline), 5·80–5·89 (*m*, 1H, CH = allyl), 6·65–8·28 (*m*, 18H, ArH); MS (EI), *m*/*z* 498 (M^+, 100). Anal. Calcd. for $C_{32}H_{26}N_4O_2$: C, 77·09; H, 5·26; N, 11·24. Found: C, 77·16; H, 5·51; N, 11·26.

2.3e *1-Allyl-3-(5-biphenyl-4-yl-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-phenyl-1H-indole* (**4c**): Yale yellow crystals; m.p. 192–194°C; IR ν_{max} (KBr) 2976 (CH), 1595 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4·10 (*dd*, 2H, CH_2 pyrazoline, J = 13·1 Hz), 4·64–4·70 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·10 (*d*, 1H, $=CH_2$ allyl), 5·28–5·35 (*m*, 1H, CH pyrazoline), 5·82–5·91 (*m*, 1H, CH = allyl), 6·61–7·89 (*m*, 23H, ArH); MS (EI), *m*/*z* 529 (M^+, 85). Anal. Calcd. for $C_{38}H_{31}N_3$: C, 86·17; H, 5·90; N, 7·93. Found: C, 86·25; H, 6·10; N, 8·04.

2.3f *1-Allyl-3-(2,5-diphenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-p-tolyl-1H-indole* (**4d**): Pale yellow crystals; m.p. 160–162°C; IR ν_{max} (KBr) 2915 (CH), 1593 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·40 (*s*, 3H, CH_3), 3·41–3·52 (*m*, 1H, CH_2 pyrazoline), 3·88–4·07 (*m*, 1H, CH_2 pyrazoline), 4·63–4·69 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·04 (*d*, 1H, $=CH_2$ allyl), 5·24–5·39 (*m*, 1H, CH pyrazoline), 5·81–5·90 (*m*, 1H, CH = allyl), 6·58–7·80 (*m*, 18H, ArH); MS (EI), *m*/*z* 467 (M^+, 23). Anal. Calcd. for $C_{33}H_{29}N_3$: C, 84·76; H, 6·25; N, 8·99. Found: C, 84·83; H, 6·51; N, 8·88.

2.3g *1-Allyl-3-[5-(4-nitrophenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-2-p-tolyl-1H-indole* (**4e**): Red crystals; m.p. 198–200°C; IR ν_{max} (KBr) 2916 (CH), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·40 (*s*, 3H, CH_3), 3·92–4·12 (*m*, 2H, CH_2 pyrazoline), 4·63–4·81 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·03 (*d*, 1H, $=CH_2$ allyl), 5·43–5·58 (*m*, 1H, CH pyrazoline), 5·82–5·89 (*m*, 1H, CH = allyl), 6·50–8·28 (*m*, 17H, ArH); MS (EI), *m*/*z* 512 (M^+, 45). Anal. Calcd. for $C_{33}H_{28}N_4O_2$: C, 77·32; H, 5·51; N, 10·93. Found: C, 77·39; H, 5·77; N, 10·82.

2.3h *1-Allyl-3-(5-biphenyl-4-yl-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-p-tolyl-1H-indole* (**4f**): Pale yellow crystals; m.p. 170–172°C; IR ν_{max} (KBr) 2916 (CH), 1595 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·41 (*s*, 3H, CH_3), 3·42–3·51 (*m*, 1H, CH_2 pyrazoline), 3·93–4·08 (*m*, 1H, CH_2 pyrazoline), 4·64–4·76 (*m*, 1H, CH = allyl; *d*, 2H, NCH_2), 5·05 (*d*, 1H, $=CH_2$ allyl), 5·30–5·42 (*m*, 1H, CH pyrazoline), 5·79–5·91 (*m*, 1H, CH = allyl), 6·57–7·89 (*m*, 22H, ArH); MS (EI), *m*/*z* 543 (M^+, 100). Anal. Calcd. for $C_{39}H_{33}N_3$: C, 86·15; H, 6·12; N, 7·73. Found: C, 86·22; H, 6·38; N, 7·62.

2.3i *1-Benzyl-3-(2,5-diphenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-phenyl-1H-indole* (**4g**): Pale yellow crystals; m.p. 162–164°C; IR ν_{max} (KBr) 2920 (CH), 1593 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4·03–4·92 (*m*, 2H, CH_2 pyrazoline), 5·31 (*s*, 2H, NCH_2), 5·34–5·35 (*m*, 1H, CH pyrazoline), 6·61–7·79 (*m*, 24H, ArH); MS (EI), *m*/*z* 503 (M^+, 65). Anal. Calcd. for $C_{36}H_{29}N_3$: C, 85·85; H, 5·80; N, 8·34. Found: C, 85·92; H, 6·06; N, 8·43.

2.3j *1-Benzyl-3-[5-(4-nitrophenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-2-phenyl-1H-indole* (**4h**): Dark red crystals; m.p. 184–186°C; IR ν_{max} (KBr) 2923 (CH), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3·97–4·07 (*m*, 2H, CH_2 pyrazoline), 5·30 (*s*, 2H, NCH_2), 5·50–5·52 (*m*, 1H, CH pyrazoline), 6·63–8·28 (*m*, 23H, ArH); MS (EI), *m*/*z* 548 (M^+, 38). Anal. Calcd. for $C_{36}H_{28}N_4O_2$: C, 78·81; H, 5·14; N, 10·21. Found: C, 78·87; H, 5·39; N, 10·31.

2.3k *1-Benzyl-3-(5-biphenyl-4-yl-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-phenyl-1H-indole* (**4i**): Pale yellow solid; m.p. 168–170°C; IR ν_{max} (KBr) 2916 (CH), 1595 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4·07–4·96 (*m*, 2H, CH_2 pyrazoline), 5·32 (*s*, 2H, NCH_2), 5·38–5·39 (*m*, 1H, CH pyrazoline), 6·63–7·87 (*m*, 28H, ArH); MS (EI), *m*/*z* 579 (M^+, 43). Anal. Calcd. for $C_{42}H_{33}N_3$: C, 87·01; H, 5·74; N, 7·25. Found: C, 87·10; H, 5·80; N, 7·36.

2.3l *1-Benzyl-3-(2,5-diphenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-p-tolyl-1H-indole* (**4j**): Pale yellow crystals; m.p. 160–162°C; IR ν_{max} (KBr) 2918 (CH), 1594 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·38 (*s*, 3H, CH_3), 3·46–3·59 (*m*, 1H, CH_2 pyrazoline), 3·97 (*dd*, 1H, CH_2 pyrazoline, J = 12·9 Hz), 5·31 (*s*, 2H, NCH_2), 5·36–5·44 (*m*, 1H, CH pyrazoline), 6·62–7·79 (*m*, 23H, ArH); MS (EI), *m*/*z* 517 (M^+, 22). Anal. Calcd. for $C_{37}H_{31}N_3$: C, 85·85; H, 6·04; N, 8·12. Found: C, 85·92; H, 6·30; N, 8·01.

2.3m *1-Benzyl-3-[5-(4-nitrophenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-2-p-tolyl-1H-indole* (**4k**) Orange crystals; m.p. 184–186°C; IR ν_{max} (KBr) 2917 (CH), 1594 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·37 (*s*, 3H, CH_3), 3·50–3·59 (*m*, 1H, CH_2 pyrazoline), 4·01 (*dd*, 1H, CH_2 pyrazoline, J = 13·2 Hz), 5·33–5·51 (*m*, 1H, CH pyrazoline), 5·39 (*s*, 2H, NCH_2), 6·67–8·29 (*m*, 22H, ArH); MS (EI), *m*/*z* 562 (M^+, 100). Anal. Calcd. for $C_{37}H_{30}N_4O_2$: C, 78·98; H, 5·37; N, 9·96. Found: C, 79·05; H, 5·63; N, 9·85.

2.3n *1-Benzyl-3-(5-biphenyl-4-yl-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl)-2-p-tolyl-1H-indole* (**4l**): Pale yellow crystals; m.p. 182–184°C; IR ν_{max} (KBr) 2943 (CH), 1593 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2·38 (*s*, 3H, CH_3), 3·47–3·55 (*m*, 1H, CH_2 pyrazoline), 3·99 (*dd*, 1H, CH_2 pyrazoline, J = 12·9 Hz), 5·31 (*s*, 2H, NCH_2), 5·31–5·36 (*m*, 1H, CH pyrazoline), 6·60–7·89 (*m*, 27H, ArH); MS (EI), *m*/*z* 593 (M^+, 56). Anal. Calcd. for $C_{43}H_{35}N_3$: C, 86·98; H, 5·94; N, 7·08. Found: C, 87·05; H, 6·20; N, 6·97.

3. Results and discussion

3.1 *Synthesis and characterization*

Indolylchalcones were reported as antitumor agents, immunosuppressant and therapeutic agents for autoimmune diseases.[24] We have previously reported the synthesis of a series of indolylchalcones with promising *in vitro* antitumor activity.[22b] Thus, in the present study, an extension of research on the design, synthesis and investigation of the cytotoxic effect of a new series of indolylchalcone derivatives against human Hep-G2 was considered.

2-Aryl-1*H*-indole-3-carbaldehyde derivatives **1** underwent Claisen–Schmidt condensation with different acetophenones **2** in ethylene glycol and few drops of piperidine under microwave irradiation (scheme 1). The reaction proceeded within few minutes (3–10 min) with successive period of 30 sec. at 750 W to avoid overheating of the solvent to afford substituted (*E*)-3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-one derivatives **3a–l** in moderate to good yields compared with the conventional method under the same reaction condition (table 1).

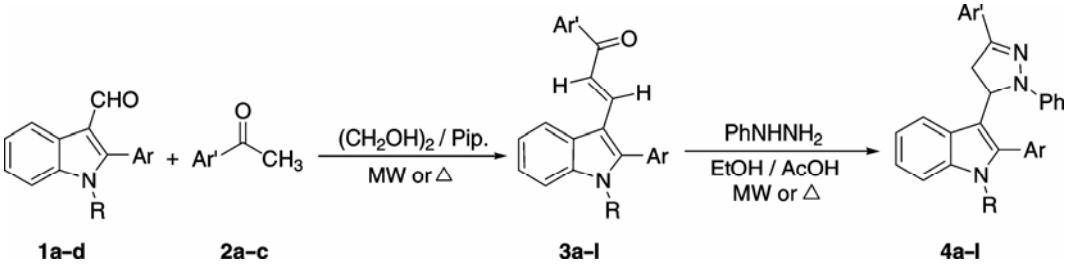
^{1}H-NMR spectra revealed that the indolylchalcone fragments **3a–l** were existed in trans configuration due to appearance of the CH=CH group as an AB system as two doublets at δ = 7·51 and 8·34 ppm with the coupling constant J = 15·3 Hz[25] and the aromatic protons appeared as complex multiplets at δ 6·90–8·52 ppm. IR spectra of compounds **3a–l** showed the appearance of absorption bands at the region 1655–1643 cm^{-1} corresponding to conjugated (C=O) and absorption bands at the region 3081–3023 cm^{-1} corresponding to (CH=CH). All the obtained products **3a–l** both under microwave irradiation and conventional heating were existed in *trans* configuration which showed a clear evidence for the stereoselective of the indolylchalcones under the given condition. Moreover, the X-ray crystallographic analysis[26] of the selected indolylchalcone **3f** confirmed the *trans* configuration (figure 1) as well as it's ^{1}H-NMR spectral data.

Attempts to synthesize substituted 3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-one derivatives **3a–l** in ethyl alcohol with few drops of piperidine and in aqueous ethanolic potassium hydroxide either under microwave irradiation or conventional heating revealed that the reactions took longer time and afforded poor yield.

In comparison with the reported methods,[27–30] the synthesis of the indolylchalcones in ethylene glycol and piperidine turned out to be an efficient media in terms of reaction times, manipulation and yields under microwave irradiation condition.

Several pyrazoline derivatives possess important pharmacological activities and some indolylpyrazolines showed moderate to good activity as cycloxygenese-2 (COX-2) and lipoxygenase (LOX) inhibitors.[31] Therefore, as a part of our effort to explore a new anticancer agent, new series of indolylpyrazolines was synthesized and evaluated for their antitumor activity.

Thus, reacting (*E*)-3-(2-aryl-1*H*-indol-3-yl)-prop-2-en-1-one derivatives **3a–l** with phenylhydrazine in absolute ethanol and few drops of glacial acetic acid under microwave irradiation at 350 W with success-



1a–d	Ar	R	2a–c	Ar'
1a	C_6H_5	Allyl	**2a**	C_6H_5
1b	4-MeC_6H_4	Allyl	**2b**	3-$NO_2C_6H_4$
1c	C_6H_5	Benzyl	**2c**	4-PhC_6H_4
1d	4-MeC_6H_4	Benzyl		

Scheme 1. Synthesis of indolylchalcones **3a–l** and indolylpyrazolines **4a–l**.

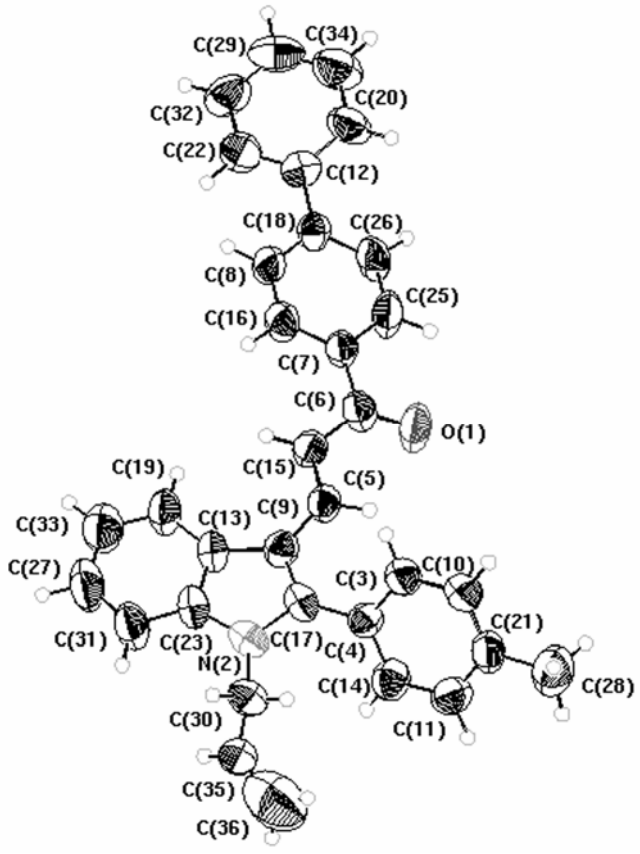


Figure 1. X-ray crystal structure of indolylchalcone (**3f**).

sive period of 30 s, the corresponding indolylpyrazoline analogs **4a–l** were obtained (scheme 1). The yields of the indolylpyrazolins **4a–l** under microwave irradiation condition were high and quantitative, moreover, the reaction time was also shortened compared with the conventional heating (table 2).

All the indolylpyrazolines **4a–l** have a chiral center at 5th position, usually the hydrogen on the chiral carbon atom interacted with the hydrogens adjacent to the chiral center. If the carbon adjacent to the chiral center has one hydrogen atom, then the resulting two enantiomers will have these two hydrogen atoms in *cis* or *trans* position and can easily be distinguished by ^{1}H-NMR spectral analysis. But in this case, the carbon atom next to the chiral center has two hydrogens and would not be distinguished as *cis* or *trans* forms and could not be distinguished by ^{1}H-NMR spectral data.[31]

^{1}H-NMR spectra of **4a–l** fragments showed disappearance of the doublet of doublet at the average value 7·51 and 8·34 ppm and the appearance of the pyrazoline methine proton at 5·45 ppm as well as the methylene protons at 3·82 ppm as doublet of doublet and/or as multiplet.

IR spectra of indolylpyrazolines **4a–l** showed no absorption in the region of 1655–1643 cm^{-1} indicating the disappearance of the conjugated keto group (C=O), the appearance of stretching vibration in the region of 1595–1592 cm^{-1} due to (C=N) and at 2978–2907 cm^{-1} corresponding to the methine (CH) and the methylene (CH_2) of pyrazoline ring. The values of the elemental analyses of compounds **3a–l** and **4a–l** were found to be in good agreement (± 0·3%) with the calculated values.

Trials to synthesis indolylpyrazolines **4a–l** under reflux in glacial acetic acid revealed that there are many by-products were formed and a poor yield of the indolylpyrazoline derivatives was isolated.

All the indolylchalcones **3a–l** and their pyrazoline analogs **4a–l** were obtained both by the microwave

Table 1. Comparative study for the synthesis of indolylchalcones **3a–l**.

			Thermal		Microwave[b]	
Compound	Aldehyde	Acetophenone	Time (min)	Yield[a] (%)	Time (min)	Yield[a] (%)
3a	**1a**	**2a**	25	79	10	88
3b	**1a**	**2b**	15	84	4	95
3c	**1a**	**2c**	30	85	6	93
3d	**1b**	**2a**	25	80	8	89
3e	**1b**	**2b**	25	82	3	92
3f	**1b**	**2c**	30	81	6	91
3g	**1c**	**2a**	25	82	8	90
3h	**1c**	**2b**	15	82	3	93
3i	**1c**	**2c**	20	76	8	87
3j	**1d**	**2a**	30	78	10	90
3k	**1d**	**2b**	25	81	4	91
3l	**1d**	**2c**	30	82	8	90

[a]Isolated yield. [b]Irradiation power was 750 W.

Table 2. Comparative study for the synthesis of indolylpyrazolines **4a–l**.

	Thermal		Microwave[b]	
Compound	Time (h)	Yield[a] (%)	Time (min)	Yield[a] (%)
4a	3	71	10	88
4b	4	76	5	89
4c	2.5	75	8	89
4d	2.5	79	8	89
4e	2.5	78	5	91
4f	2.5	78	10	92
4g	2.5	77	10	91
4h	3	85	4	92
4i	4	75	8	89
4j	2	77	8	90
4k	3	79	4	85
4l	2	78	10	90

[a]Isolated yield. [b]All the compounds were irradiated at 350 W.

irradiation and conventional heating; irrespective of these reaction conditions the IR spectra of each product are identical.

3.2 *Biological activity*

The cytotoxicity of all the synthesized compounds **3a–l** and **4a–l** was screened for their biological activities *in vitro* against Human hepatocellular carcinoma cell line (Hep-G2) using the MTT Cell viability assay.[32] Data were expressed as the percentage of relative viability compared with the untreated cells, the vehicle control and the cytotoxicity indicated by <100% relative viability. The relative inhibitory ratios (%) against Human Hep-G2 were listed in table 3.

From the reported results in table 3 it was clear that, the treatment of Hep-G2 cells with the indolylpyrazoline **4e** exhibited the highest cytotoxic effect with the lowest IC_{50} value of 5·09 μM, while the compounds **4h**, **4b**, and **4k**, demonstrated a moderately high inhibition in the cell viability as concluded from the IC_{50} values of 32·75, 20·51 and 26·58 μM respectively, which revealed a moderate anti-tumor activity against hepatic carcinoma as shown in table 3. On the other hand, the rest of the compounds exhibited no cytotoxic effect due to their high IC_{50} values (> 50 μM).

4. Conclusions

In search of potentially active molecules containing indole moiety and their pyrazoline analogs, a series of new derivatives was synthesized with an efficient microwave irradiation protocol and their antitumor activity analysed. This protocol offers several

Table 3. *In vitro* cytotoxic activity of indolylchalcones **3a–l** and indolylpyrazolines **4a–l** against Hep-G2 by MTT assay.

	Concentration (μM) % of cell viability (Hep-G2 cells)				
Compound	12·5 μM	25 μM	50 μM	100 μM	IC_{50}
3a	107·48	97·73	78·78	63·44	121·59
3b	70·15	61·22	47·23	36·66	57·23
3c	96·5	82·54	72·34	58·34	115·45
3d	101·34	99·41	87·77	81·35	226·48
3e	69·85	60·43	53·22	40·45	65·98
3f	79·41	68·83	56·02	51·32	93·67
3g	93·33	88·762	78·23	63·6	138·03
3h	98·451	83·42	77·91	61·84	128·16
3i	100·07	93·4	88·44	78·76	223·32
3j	109·3	100·1	93·25	86·87	251·69
3k	71·1	64·87	51·89	46·66	78·60
3l	80·8	72·76	59·32	43·22	80·25
4a	99·525	82·36	74·43	66·121	139·94
4b	62·29	42·2	29·435	14·76	20·51
4c	99·9	91·11	78·78	61·344	123·46
4d	83·98	74·43	61·41	56·56	111·94
4e	59·26	32·43	19·18	11·09	5·09
4f	84·63	74·19	62·94	54·32	105·82
4g	101·77	102·33	91·05	85·76	273·56
4h	76·1	42·55	31·72	19·1	32·75
4i	100·81	81·68	73·34	50·11	95·22
4j	92·8	76·998	69·68	56·54	112·15
4k	58·99	49·34	36·8	21·1	26·58
4l	96·5	87·71	81·675	72·32	183·22

advantages such as mild reaction conditions, short reaction times, easy isolation and good yields. The indolylchalcones and their pyrazoline analogs showed high cytotoxic effect with the lowest IC_{50} for indolylpyrazoline **4e** while the compounds **4h**, **4b** and **4k**, showed a moderately high inhibition against human hepatocellular carcinoma cell line (Hep-G2).

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