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Synthesis and cellular cytotoxicities of new *N*-substituted indole-3-carbaldehyde and their indolylchalcones

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Abstract. A simple and efficient method for *N*-alkylation of indole-3-carbaldehyde derivatives using a mixture of different bases in DMF under conventional and microwave irradiation conditions to afford *N*-substituted indole-3-carbaldehyde derivatives **3a–o** is reported. These derivatives which undergo Claisen–Schmidt condensation with 1-biphenyl-4-yl-ethanone yielded the corresponding indolylchalcone derivatives **5a–h**. A comparative study showed that the microwave irradiation condition afforded excellent yield and shorten reaction time of all the synthesized indole derivatives which possess promising antitumor activity as well as interchelation bioactivity of indolylchalcones **5a–h** with DNA.

Keywords. *N*-alkylation; indolylchalcones; antitumor; microwave irradiation.

1. Introduction

The indole nucleus is an important structure in numerous natural or synthetic alkaloids,[1] and in medicinal chemistry.[2] The diversity of the structures encountered, as well as their biological and pharmaceutical relevance, have motivated research aimed at the development of new economical, efficient and selective synthetic strategies, particularly for the synthesis of substituted indole rings.[3–4] The substituted indoles have been referred to as privileged structures since they are capable of binding to many receptors with high affinity.[5] Therefore, the synthesis and selective functionalization of indoles have been the focus of active research.[6–8]

Chalcones (α, β-unsaturated ketones) are important intermediate products in organic synthesis;[9,10] they also exhibit versatile biological activity.[11,12] Recent studies on biological evaluation of chalcones revealed some to be anti-cancer,[13] nitric oxide regulation modulatory[14] and anti-hyperglycemic agents.[15] These compounds are usually synthesized by the Claisen–Schmidt condensation of aromatic aldehydes with methyl ketones in the presence of bases such as KOH,[16] LiHDMS[17] and calcined $NaNO_3$/natural phosphates.[18] The acid catalysed methodologies include the use of Zeolites,[19] K_3PO_4[20] and BF_3–Et_2O.[21]

The indole derivatives have been widely studied, α, β-unsaturated ketones of chalcone types containing this heterocycle in which products of crotonic condensation of 3-formyl indole derivatives with different acetophenones were described previously in basic media using mostly piperidine as a catalyst.[22–25]

Microwave assisted organic synthesis (MAOS) has become increasingly popular in recent years to improve the yields and shorten reaction time in a variety of reactions.[26,27]

Hence, in continuation of our interest on indole derivatives,[28] the utility of microwave irradiation in organic synthesis and the evaluation of different classes of heterocyclic nucleus as anti cancer bioactive compounds,[29,30] we presented in this context an efficient and facile microwave *N*-alkylation of indole-3-carbaldehyde derivatives and their Claisen–Schmidt condensation with 1-biphenyl-4-yl-ethanone to afford novel indolylchalcones and investigation of their antitumor activity as well as their binding capability to genomic DNA extracted from bacteria.

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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 Germini-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* (quadruplet), *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 Analyser Yanaca CHN Corder MT-3, their results were found to be in good agreement (± 0·3%) with the calculated values. MS spectra were run 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 presser in domestic Whirl Pool-TALENT oven. The synthesized products and each reaction carried out under conventionally or microwave (MW) irradiation condition were monitored on Merck silica gel 60 F254 plates (type E; Merck, Darmstadt, Germany) using UV light (254 and 360 nm) for detection. All chemicals and solvents were purchased from E· Merck (Darmstadt, Germany) and Sigma-Aldrich.

2.2 *General procedure for the synthesis of N-substituted indole-3-carbaldehyde derivatives **3a–o***

2.2a *Microwave irradiation procedure:* A mixture of indole-3-carbaldehyde **1a–e** (1 mmol), the appropriate alkylating reagent **2a–c** (1 mmol), KOH (4 mmol), anhydrous K_2CO_3 (4 mmol) and dimethylformamide (1 mL) in an open Pyrex-glass vessel was subjected to microwave irradiation at 350 W (table 1). Irradiation was carried out in successive 30 s periods to avoid overheating of the solvent and the reaction mixture left another 30 sec. at room temperature as a time gap between every successive irradiation period. After completion of the reaction as monitored by TLC, the reaction mixture was cooled, and poured onto water· The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol.

2.2b *Conventional heating procedure:* A mixture of indole-3-carbaldehyde **1a–e** (10 mmol), the appropriate alkylating reagent **2a–c** (10·85 mmol), anhydrous K_2CO_3 (1·4 g) and dimethylformamide (10 mL) was stirred vigorously and refluxed for 6 h. After completion of the reaction as monitored by TLC, work-up was performed as described above.

2.2c *1-(1-Allyl-2-p-tolyl-1H-indole-3-yl)-ethanone* (**3g**)*:* Colourless crystals; IR (KBr): ν 3044, 2830, 2362, 1650, 1546, 1452, 913, 837 cm^{-}; ^{1}H-NMR: δ 3·41 (*s*, 3H, CH_3), 4·72 (*m*, 1H, = CH_2), 4·74 (*d*, 2H, NCH_2), 5·11 (*d*, 1H, = CH_2), 5·89 (*m*, 1H, CH=), 7·50–7·29 (*m*, 7H, Ar-H), 8·21 (*m*, 1H, H-4), 9·59 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 275 (M^+, 100); Anal. calc. for $C_{19}H_{17}NO$: C, 82·88; H, 6·22; N, 5·09%. Found: C, 82·73; H, 6·32; N, 4·95%.

2.2d *1-(1-Benzyl-2-p-tolyl-1H-indole-3-yl)-ethanone* (**3h**)*:* Yellow crystals; IR (KBr): ν 3048, 2827, 2362, 1649, 1536, 1451, 826 cm^{-1}; ^{1}H-NMR: δ 3·42 (*s*, 3H, CH_3), 5·41 (*d*, 2H, N-CH_2), 7·50–6·92 (*m*, 7H, Ar-H), 8·26 (*m*, 1H, H-4), 9·63 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 325 (M^+, 88); Anal. calc. for $C_{23}H_{19}NO$: C, 84·89; H, 5·89; N, 4·30%. Found: C, 84·74; H, 5·99; N, 4·16%.

2.2e *1-(1-Ethyl-2-p-tolyl-1H-indole-3-yl)-ethanone* (**3i**)*:* Colourless crystals; IR (KBr): ν 3044, 2821, 2362, 1650, 1536, 1458, 1376, 838 cm^{-1}; ^{1}H-NMR: δ 1·21 (*t*, 3H, CH_3), 3·38 (*s*, 3H, CH_3), 4·15 (*q*, 2H, N-CH_2), 7·69–7·30 (*m*, 7H, Ar-H), 8·22 (*m*, 1H, H-4), 9·54 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 263 (M^+, 85); Anal. calc. for $C_{18}H_{17}NO$: C, 82·10; H, 6·51; N, 5·32%· Found: C, 81·96; H, 6·61; N, 5·18%.

2.2f *1-[1-Allyl-2-(4-chlorophenyl)-1H-indole-3-yl]-ethanone* (**3j**)*:* Pale Yellow crystals; IR (KBr): ν 3054, 2843, 2359, 1651, 1531, 1486, 937, 756 cm^{-1}; ^{1}H-NMR: δ 4·77 (*m*, 1H, =CH_2), 4·84 (*d*, 2H, NCH_2), 5·10 (*d*, 1H, =CH_2), 5·89 (*m*, 1H, CH=), 7·65–7·30 (*m*, 7H, Ar-H), 8·52–8·22 (*m*, 1H, H-4), 9·60 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 295 (M^+, 85); Anal. calc. for $C_{18}H_{14}ClNO$: C, 73·10; H, 4·77; N, 4·74%. Found: C, 72·95; H, 4·87; N, 4·60%.

2.2g *1-[1-Benzyl-2-(4-chlorophenyl)-1H-indole-3-yl]-ethanone* (**3k**)*:* Colourless crystals; IR (KBr): ν 3060, 3834, 2362, 1649, 1519, 1453, 750 cm^{-1}; ^{1}H-NMR: δ 5·42 (*d*, 2H, N-CH_2), 7·62–6·91 (*m*, 7H, Ar-H), 8·22 (*m*, 1H, H-4), 9·63 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 345 (M^+, 59); Anal. calc. for

Table 1. Comparative study of the microwave irradiation (MW) and conventional reactions with the melting points (m.p.) of the synthesized compounds.

			Thermal		Microwave		
Compound	R	R_1	Time (h)	Yield (%)	Time (min)[a]	Yield (%)	m.p. (°C)
3a	H	Allyl	6	80·0	4	91	73–74 [b]
3b	H	Benzyl	6	79	4	95	105–107 [b]
3c	H	Ethyl	6	78·6	6	90	99–101 [b]
3d	Phenyl	Allyl	6	83·2	4	93	118–120 [b]
3e	Phenyl	Benzyl	6	82	6	96	176–178 [b]
3f	Phenyl	Ethyl	6	81	6	90	100–102 [b]
3g	4-Methylphenyl	Allyl	6	79·5	6	91	98–100
3h	4-Methylphenyl	Benzyl	6	80	10	95	138–140
3i	4-Methylphenyl	Ethyl	6	82	6	94	98–100
3j	4-Chlorophenyl	Allyl	6	81	6	93	138–140
3k	4-Chlorophenyl	Benzyl	6	79·8	8	94	135–137
3l	4-Chlorophenyl	Ethyl	6	78·9	8	92	128–130
3m	4-Fluorophenyl	Allyl	6	83	8	96	80–82
3n	4-Fluorophenyl	Benzyl	6	83	8	96	116–120
3o	4-Fluorophenyl	Ethyl	6	79·7	10	94	110–112
5a	4-Chlorophenyl	H	0·5	85	6	89	252–254
5b	4-Chlorophenyl	Allyl	0·5	85	8	89	90–92
5c	4-Chlorophenyl	Benzyl	0·5	85·3	6	91	80–82
5d	4-Chlorophenyl	Ethyl	0·5	84·5	8	90	126–128
5e	4-Fluorophenyl	H	0·5	85	6	90	204–206
5f	4-Fluorophenyl	Allyl	0·5	87	8	91	146–148
5g	4-Fluorophenyl	Benzyl	0·5	86	8	90	75–77
5h	4-Fluorophenyl	Ethyl	0·5	85	8	92	122–124

[a]Compounds **3a–o** were irradiated at 350 W and compounds **5a–h** were irradiated at 750 W. [b]Reported melting points of derivatives **3a–f** were in complete accordance with the obtained products[32–34]

$C_{22}H_{16}ClNO$: C, 76·41; H, 4·66; N, 4·05%. Found: C, 76·26; H, 4·76; N, 3·91%.

2.2h *1-[2-(4-Chlorophenyl)-1-ethyl-1H-indol-3-yl]-ethanone* (**3l**)*:* Pale yellow crystals; IR (KBr): ν 3060, 2834, 2362, 1650, 1529, 1457, 1381, 750 cm^{-1}; ^{1}H-NMR: δ 1·21 (*t*, 3H, CH_3), 4·16 (*q*, 2H, N-CH_2), 7·68–7·31 (*m*, 7H, Ar-H), 8·22 (*m*, 1H, H-4), 9·55 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 283 (M^+, 88); Anal. calc. for $C_{17}H_{14}ClNO$: C, 71·96; H, 4·97; N, 4·94%. Found: C, 71·81; H, 5·07; N, 4·80%.

2.2i *1-[1-Allyl-2-(4-fluorophenyl)-1H-indole-3-yl]-ethanone* (**3m**)*:* Pale Yellow crystals; IRs (KBr): ν 3060, 2834, 2363, 1649, 1535, 1454, 1224, 937 cm^{-1}; ^{1}H-NMR: δ 4·78 (*m*, 1H, =CH_2), 4·84 (*d*, 2H, NCH_2), 5·13 (*d*, 1H, =CH_2), 5·89 (*m*, 1H, CH=), 7·70–7·31 (*m*, 7H, Ar-H), 8·21 (*m*, 1H, H-4), 9·59 (*s*, 1H, CHO) ppm; MS (EI) m/z (%): 279 (M^+, 80); Anal. calc. for $C_{18}H_{14}FNO$: C, 77·40; H, 5·05; N, 5·01%· Found: C, 77·25; H, 5·15; N, 4·89%.

2.2j *1-[1-Benzyl-2-(4-fluorophenyl)-1H-indole-3-yl]-ethanone* (**3n**)*:* Pale yellow crystals; IR (KBr): ν 3095, 2892, 2363, 1649, 1517, 1461, 1231 cm^{-1}; ^{1}H-NMR: δ 5·41 (*d*, 2H, N-CH_2), 7·66–6·92 (*m*, 7H, Ar-H), 8·22 (*m*, 1H, H-4), 9·61 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%): 329 (M^+, 100); Anal. calc. for $C_{22}H_{16}FNO$: C, 80·23; H, 4·90; N, 4·25%. Found: C, 80·08; H, 5·00; N, 4·11%.

2.2k *1-[1-Ethyl-2-(4-fluorophenyl)-1H-indole-3-yl]-ethanone* (**3o**)*:* Pale yellow crystals; IR (KBr): ν 3059, 2829, 2362, 1650, 1532, 1460, 1384, 1222 cm^{-1}; ^{1}H-NMR: δ 1·21 (*t*, 3H, CH_3), 4·15 (*q*, 2H, N-CH_2), 7·73–7·31 (*m*, 7H, Ar-H), 8·20 (*m*, 1H, H-4), 9·53 (*s*, 1H, CHO) ppm; MS (EI) *m/z* (%) 267 (M^+, 100); Anal. calc. for $C_{17}H_{14}FNO$: C, 76·39; H, 5·23; N, 5·24%· Found: C, 76·24; H, 5·38; N, 5·10%.

2.3 *General procedure for the synthesis of indolyl chalcone derivatives* **5a**–**h**

2.3a *Microwave irradiation procedure:* A mixture of indole-3-carbaldehyde derivatives **1d**, **e**, **3j–o** (1 mmol), 1-biphenyl-4-yl-ethanone **4** (1 mmol), ethylene glycol (1 mL) and piperidine (0·5 mL) in an open Pyrex-glass vessel was subjected to micro-

wave irradiation at 750 W (table 1). Irradiation was carried out in successive 30 s periods to avoid overheating of the solvent and the reaction mixture left another 30 s at room temperature as a time gap between every successive irradiation period. After completion of the reaction as monitored by TLC, the reaction mixture was cooled, and poured onto water (10 mL). The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol.

2.3b *Conventional heating procedure:* A mixture of indole-3-carbaldehyde derivatives **1d, e**, **3j–o** (1 mmol), 1-biphenyl-4-yl-ethanone **4** (1 mmol), ethylene glycol (5 mL) and piperidine (1 mL) was refluxed at 160–180°C (table 1). After completion of the reaction as monitored by TLC, work-up was performed as described above.

2.3c *(E)-1-Biphenyl-4-yl-3-[2-(4-chlorophenyl)-1H-indol-3-yl]-prop-2-en-1-one* (**5a**)*:* Pale yellow solid; IR (KBr): ν 3265, 1640, 1604, 1445, 1285, 1046 cm^{-1}; ^{1}H-NMR: δ 7·30–8·23 (*m*, 17H, Ar-H), 7·78 (*d*, 1H, *J* = 15·3 Hz), 8·02 (*d*, 1H, *J* = 15·3 Hz), 12·29 (*br s*, 1H, NH) ppm; MS (EI) *m/z* (%): 433 (M^+, 100); Anal. calc. for $C_{29}H_{20}ClNO$: C, 80·27; H, 4·65; N, 3·23%. Found: C, 80·54; H, 4·70; N, 3·21%.

2.3d *(E)-3-[1-Allyl-2-(4-chlorophenyl)-1H-indol-3-yl]-1-biphenyl-4-yl-prop-2-en-1-one* (**5b**)*:* Pale yellow solid; IR (KBr): ν 3435, 3055, 2978, 1650, 1604, 1550, 1576, 1238, 1299 cm^{-1}; ^{1}H-NMR: δ 4·69 (*m*, 1H, $=CH_2$), 4·69 (*d*, 2H, NCH_2), 6·65 (*d*, 1H, $=CH_2$), 7·37 (*m*, 1H, CH=), 7·39–8·31 (*m*, 17H, Ar-H), 7·86 (*d*, 1H, *J* = 15 Hz), 8·19 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 473 (M^+, 88); Anal. calc. for $C_{32}H_{24}ClNO$: C, 81·09; H, 5·10; N, 2·96%. Found: C, 80·98; H, 5·35; N, 2·94%.

2.3e *(E)-3-[1-Benzyl-2-(4-chlorophenyl)-1H-indol-3-yl]-1-biphenyl-4-yl-prop-2-en-1-one* (**5c**)*:* Pale yellow solid; IR (KBr): ν 3745, 2890, 2363, 1693, 1647, 1516, 1464, 802, 512 cm^{-1}; ^{1}H-NMR: δ 5·41 (*s*, 2H, CH_2), 7·38–8·28 (*m*, 22H, Ar-H), 7·84 (*d*, 1H, *J* = 15 Hz), 8·16 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 524 (M^+, 85); Anal. calc. for $C_{36}H_{26}ClNO$: C, 82·51; H, 5·00; N, 2·67%. Found: C, 82·24; H, 5·25; N, 2·64%.

2.3f *(E)-1-Biphenyl-4-yl-3-[2-(4-chlorophenyl)-1-ethyl-1H-indol-3-yl]-prop-2-en-1-one* (**5d**)*:* Pale yellow solid; IR (KBr): ν 3745, 2362, 1648, 1567, 1298, 1232, 1085 cm^{-1}; ^{1}H-NMR: δ 1·18 (*t*, 3H, CH_3), 4·11 (*q*, 2H, CH_2), 7·38–8·28 (*m*, 17H, Ar-H), 7·83 (*d*, 1H, *J* = 15 Hz), 8·14 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 461 (M^+, 100); Anal. calc. for $C_{31}H_{24}ClNO$: C, 80·59; H, 5·24; N, 3·03%. Found: C, 80·34; H, 5·39; N, 2·99%.

2.3g *(E)-1-Biphenyl-4-yl-3-[2-(4-fluorophenyl)-1H-indol-3-yl]-prop-2-en-1-one* (**5e**)*:* Pale yellow solid; IR (KBr): ν 3746, 3253, 2363, 1741, 1548, 1451, 1288, 844 cm^{-1}; ^{1}H-NMR: δ 7·33–8·22 (*m*, 17H, Ar-H), 7·82 (*d*, 1H, *J* = 14·7 Hz), 8·02 (*d*, 1H, *J* = 14·7 Hz), 12·29 (*br s*, 1H, NH) ppm; MS (EI) *m/z* (%): 417 (M^+, 90); Anal. calc. for $C_{29}H_{20}FNO$: C, 83·43; H, 4·83; N, 3·36%. Found: C, 83·15; H, 4·98; N, 3·33%.

2.3h *(E)-3-[1-Allyl-2-(4-fluorophenyl)-1H-indol-3-yl]-1-biphenyl-4-yl-prop-2-en-1-*one (**5f**)*:* Pale yellow solid; IR (KBr): ν 3445, 3055, 1651, 1583, 1561, 1424, 1292 cm^{-1}; ^{1}H-NMR: δ 4·69 (*m*, 1H, $=CH_2$), 4·69 (*d*, 2H, NCH_2) 5·15 (*d*, 1H, $=CH_2$), 5·87 (*m*, 1H, CH=), 7·36–8·31 (*m*, 17H, Ar-H), 7·86 (*d*, 1H, *J* = 15 Hz), 8·15 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 457 (M^+, 87); Anal. calc. for $C_{32}H_{24}FNO$: C, 84·00; H, 5·29; N, 3·06%. Found: C, 83·74; H, 5·54; N, 3·01%.

2.3i *(E)-3-[1-Benzyl-2-(4-fluorophenyl)-1H-indol-3-yl]-1-biphenyl-4-yl-prop-2-en-1-one* (**5g**)*:* Pale yellow solid; IR (KBr): ν 3745, 2889, 2363, 1647, 1517, 1454, 1289, 1223 cm^{-1}; ^{1}H-NMR: δ 5·40 (*s*, 2H, CH_2), 6·90–8·22 (*m*, 22H, Ar-H), 7·84 (*d*, 1H, *J* = 15 Hz), 8·16 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 507 (M^+, 100); Anal. calc. for $C_{36}H_{26}FNO$: C, 85·18; H, 5·16; N, 2·76%. Found: C, 85·32; H, 5·41; N, 2·74%.

2.3j *(E)-1-Biphenyl-4-yl-3-[2-(4-fluorophenyl)-1-ethyl-1H-indol-3-yl]-prop-2-en-1-one* (**5h**)*:* Pale yellow solid; IR (KBr): ν 3445, 2891, 2363, 1836, 1741, 1606, 1565, 1228 cm^{-1}; ^{1}H-NMR: δ 1·19 (*t*, 3H, CH_3), 4·11 (*q*, 2H, CH_2), 7·37–8·31 (*m*, 17H, Ar-H), 7·83 (*d*, 1H, *J* = 15 Hz), 8·14 (*d*, 1H, *J* = 15 Hz) ppm; MS (EI) *m/z* (%): 445 (M^+, 92); Anal. calc. for $C_{31}H_{24}FNO$: C, 83·57; H, 5·43; N, 3·14%. Found: C, 83·21; H, 5·68; N, 3·12%.

3 Results and discussion

3.1 *Synthesis and characterization*

1*H*-Indole-3-carbaldehyde derivatives **1a**–**e** was alkylated with the appropriate alkylating agent **2a**–**c**

using anhydrous K_2CO_3 in DMF.[31] The reaction proceeded under reflux for 6 h to afford the corresponding *N*-substituted indole-3-carbaldehyde derivatives **3a**–**o** in good yield (scheme 1). The compounds **3a**–**o** were also synthesized in excellent yield within few minutes (4–10 min) under microwave irradiation using mixture of KOH and anhydrous K_2CO_3 in DMF with successive period of 30 s to avoid overheating of the solvent (table 1).

N-substituted indole-3-carbaldehyde derivatives have been characterized on the basis of spectral studies (MS, ^{1}H NMR and IR) and elemental analysis. The IR spectra of the compounds **3a**–**o** showed no absorption in the region 3300–3100 cm^{-1} indicating the disappearance of NH. ^{1}H NMR spectra of the compounds **3a**–**o** showed no signals for the NH protons, whereas, the allyl, benzyl and ethyl groups were easily detected and their chemical shifts described in the experimental part. The values of the elemental analysis were found to be in good agreement ($\pm$ 0·3%) with the calculated values. The *N*-substituted indole-3-carbaldehyde derivatives **3a**–**f** was found to be completely compatible with the reported results.[32–34]

Indolylchalcones were reported as antitumor agents, immunosuppressant, and therapeutic agents for autoimmune diseases.[35] Therefore, our target in this context was the synthesis of new derivatives of indolylchalcones and investigates their antitumor activity and binding capability to DNA.

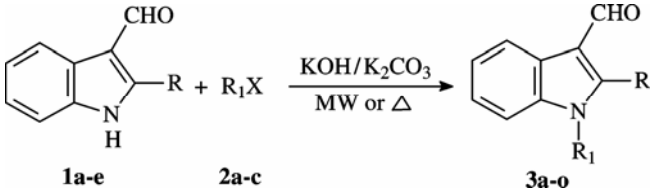
The selection of 1*H*-Indole-3-carbaldehyde derivatives **1d**, **e** and *N*-substituted indole-3-carbaldehyde derivatives **3j**–**o** were to investigate the role and the difference of their behavioiur either chemically or biologically· Moreover, the choose of 1-biphenyl-4-yl-ethanone **4** as the appropriate aryl ketone for Claisen–Schmidt condensation based on that the connection with a freely rotating single bond, two aromatic rings are expected to show dynamic structural change through interaction with DNA[36] and the fact that increasing the aromatic properties might increase the action of the indolylchalcones **5a**–**h** on the tumor cells which might be referred to the chelation between the extended aromatic 6π electrons and the DNA of the tested tumor cells.[37–39] In further investigation based on the recently published article[40] the author and his research group are planning to couple the indolylchalcones with thalidomide moieties which might lead to enhancement of the antitumor bioactivity of the new highly bioactive thalidomide dithiocarbamate derivatives.

Thus, 1*H*-Indole-3-carbaldehyde derivatives **1d**, **e** and *N*-substituted indole-3-carbaldehyde derivatives **3j**–**o** were condensed with 1-biphenyl-4-yl-ethanone **4** in refluxing ethylene glycol and few drops of piperidine at 160–180°C.[41] The reaction proceeded within 30 min afforded their indolylchalcone derivatives **5a**–**h** in good yield (scheme 2). The indolylchalcones **5a**–**h** was also synthesized in excellent yield within few minutes (6–8 min) under microwave irradiation with successive period of 30 s to avoid overheating of the solvent (table 1).

Indolylchalcones **5a**–**h** has been characterized on the basis of spectral studies (MS, ^{1}H NMR and IR) and elemental analysis. The IR spectra of indolylchalcones **5a** and **5e** showed characteristic absorption bands at 3300–3100 cm^{-1} (NH) and 1620 cm^{-1} (C=C–C=O). The structure of compounds **5a**–**h** were evidenced by the disappearance of the formyl proton in ^{1}H NMR spectra and appearance of the pattern CH=CH as an AB system which appears at δ = 7·83 and 8·12 ppm as two doublets with the coupling constant J = 15 Hz. The J of 15 Hz between H-α and H-β proton is consistent with a *trans* relationship for the olefinic double bond of the –CO–CH=CH– group, The values of the elemental analysis were found to be in good agreement ($\pm$ 0·3%) with the calculated values.

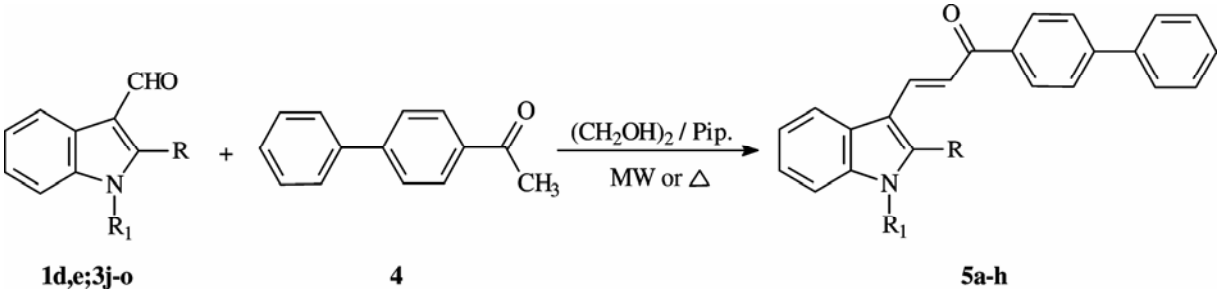
3.2 *Antitumor screening*

3.2a *Cell line:* Ehrlich Ascetic carcinoma (EAC) cell line was purchased from National Cancer institute, Cairo University, and maintained in the peritoneal of female Swiss Albino through serial intraperitoneal (i.p.) injection of 0·2 ml of 2×10^6 cells/ml saline.



	R		R_1	X
1a	H	**2a**	Allyl	Br
1b	Phenyl	**2b**	Benzyl	Cl
1c	4-Methylphenyl	**2c**	Ethyl	Br
1d	4-Chlorophenyl			
1e	4-Fluorophenyl			

Scheme 1. Synthesis of *N*-substituted indole-3-carbaldehyde derivatives **3a–o**.



1d,e;3j-o **4** **5a-h**

Scheme 2. Synthesis of indolylchalcone derivatives **5a–h**.

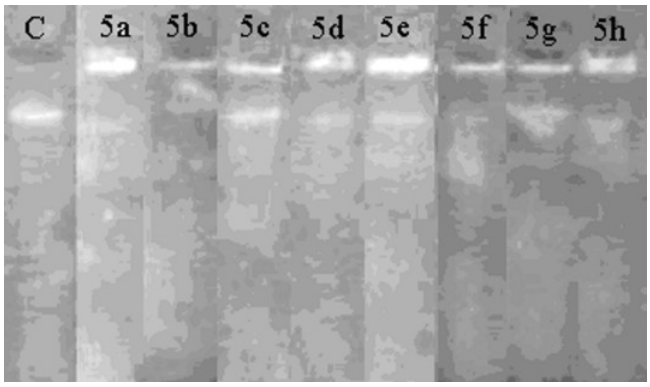


Figure 1. Effect of indolylchalcones on DNA; Lanes from **5a–h** represent DNA incubated for 1 h with the compounds respectively. Lane C is untreated DNA.

3.2b *Antitumor activity:* All the synthesized compounds were screened for *in vitro* antitumor activity by trypan blue exclusion method· Compounds **3a–o** were dissolved in DMSO/H_2O (70 : 30%) to obtain the concentration of 100 mM and the indolylchalcones **5a–h** were dissolved in DMSO (100%)· EAC cells were collected from the peritoneal of infected female Swiss mice on day seven, checked for viability using trypan blue stain.[42] EAC cells were suspended in RPMI1640 (Life Technologies) supplemented with 10% fatal bovine serum, 10 μg/ml streptomycin and 100 U/ml penicillin (Sigma) and titrated in 96 flat-bottomed wells plate (200 μl of 2×10^6/ml). 30 μl of the solution of the compounds was added to each well and was incubated at 37°C for 24 h at 5% CO_2 incubator. Cell viability was checked by using trypan blue staining and the cytotoxicity values were calculated against control EAC cells.[43] The results are expressed as % cell death (table 2).

Three serial concentrations of all the synthesized compounds **3a–o** and **5a–h** were tested *in vitro* cytotoxicity assay against EAC cells line. As shown in table 2, the tested compounds illustrated a wide range of cytotoxic activities against EAC *in vitro* rather than that either EAC control group or thalidomide treated group. The maximal cytotoxic activity of tested compounds was observed at dose 30 μl. Among the all tested compounds the six indolylchalcones **5b–d** and **5f–h** were found to be the most active compounds. This significant increase in cytotoxicity was observed by comparing the cytotoxic effects of compounds **5f**, **5g** and **5h** (*N*-substituted chalcones containing fluorine atom on the phenyl group at position-2 of indole ring) to that of compound **5e** (unsubstituted chalcones containing fluorine atom on the phenyl group at position-2 of indole ring). This indicates that the compounds with *N*-substituted indole moiety by allyl, benzyl or ethyl group lead to an obvious elevation of the antitumor activity of these compounds. This elevation in the antitumor activity was also observed by comparing the cytotoxic effects of compounds **5b, 5c** and **5d** (*N*-substituted chalcones containing chlorine atom on the phenyl group at position-2 of indole ring) to that of compound **5a** (unsubstituted chalcones containing chlorine atom on the phenyl group at position-2 of indole ring).

Furthermore, the compounds **5b–d** and **5f–h** demonstrated higher cytotoxicity rather than their precursor's **3j–o** respectively· While the presence of methyl group in compounds **3g**, **3h** and **3i** leads to significant decrease in the cytotoxic values respectively (table 2).

3.3 *Effect of chalcones on DNA*

Genomic DNA was extracted from bacteria.[44] Twenty microliter of indolylchalcones **5a–h** (10 mmol) was incubated with equal volume of DNA (1 μg/μl) at 37°C for 1 h. Agarose gel electrophoresis was done to analyse DNA.

Table 2. Cytotoxic activity of compounds **3a–o** and **5a–h** on (EAC) *in vitro*[a].

Compound	Cytotoxicity (%)		
	10 μl	20 μl	30 μl
Control media	0·0 ± 0·0	0·7 ± 0·6	1·0 ± 1·0
Thalidomide	20·5 ± 2·5	20·5 ± 1·8	56·4 ± 2·1
3a	71·3 ± 0·6	73·7 ± 1·5	72·3 ± 2·1
3b	46·3 ± 2·3	51·7 ± 1·5	52·0 ± 1·0
3c	43·0 ± 1·0	55·3 ± 1·2	55·0 ± 2·6
3d	84·0 ± 2·0	85·3 ± 0·6	86·3 ± 1·5
3e	79·0 ± 1·0	80·3 ± 0·6	83·0 ± 2·6
3f	64·7 ± 1·5	68·3 ± 0·6	71·0 ± 1·0
3g	78·7 ± 1·2	79·7 ± 0·6	81·7 ± 2·1
3h	63·3 ± 2·1	67·0 ± 1·7	73·0 ± 2·6
3i	68·0 ± 2·0	72·0 ± 2·0	73·0 ± 1·7
3j	81·0 ± 6·0	88·7 ± 1·5	89·3 ± 1·5
3k	66·7 ± 1·5	69·0 ± 1·0	65·7 ± 2·1
3l	80·7 ± 0·6	83·7 ± 0·6	84·7 ± 0·6
3m	72·0 ± 2·6	73·3 ± 2·1	75·0 ± 2·6
3n	56·7 ± 1·5	59·7 ± 2·1	60·7 ± 0·6
3o	66·0 ± 1·0	66·7 ± 2·1	68·0 ± 1·0
5a	77·0 ± 1·0	80·0 ± 1·0	84·0 ± 1·0
5b	86·7 ± 0·6	89·7 ± 0·6	92·0 ± 1·0
5c	88·7 ± 1·5	89·0 ± 1·0	95·3 ± 0·6
5d	90·7 ± 0·6	91·0 ± 1·0	94·0 ± 1·0
5e	77·3 ± 1·2	81·0 ± 2·6	84·0 ± 1·0
5f	89·0 ±1·0	91·0 ± 1·0	93·7 ± 0·6
5g	90·7 ± 1·2	92·7 ± 0·6	95·0 ± 1·0
5h	92·0 ± 0·0	94·3 ± 1·2	95·7 ± 1·5

[a]The concentration for all tested compounds was 1 mM/well.

The agarose gel was analysed qualitatively by examination of the presence of DNA bands that migrate slower or farther down on the gel than the control DNA. Treatment of DNA with compounds **5a**–**h** resulted in relaxation of the DNA that prevents the band from migrating down the gel as compared to the untreated DNA (figure 1). Slight degradation in DNA was observed after treatment with these compounds.

4. Conclusion

N-alkylation of indole-3-carbaldehyde derivatives using a mixture of different bases in DMF under conventional and microwave irradiation conditions afforded *N*-substituted indole-3-carbaldehyde derivatives **3a–o**.

Under Claisen–Schmidt condensation condition, indole derivatives **3a–o** reacted with 1-biphenyl-4-yl-ethanone to yield the corresponding indolylchalcone derivatives **5a–h**. A comparative study showed that the microwave irradiation condition is the preferred condition for such synthesis which afforded excellent yield, clean and green synthesis and shortened reaction time of all the synthesized indole derivatives which possess promising antitumor activity as well as interchelation bioactivity of indolylchalcones **5a–h** with DNA.

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