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Synthesis and biological evaluation of indolyl chalcones as antitumor agents

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ABSTRACT

A series of indolyl chalcones were synthesized and evaluated in vitro for their anticancer activity against three human cancer cell lines. Compounds **3b–d**, **3h**, **3j**, **3l**, **3m**, **4g**, and **4j** showed significant cytotoxicity, particularly, indolyl chalcones **3l** and **3m** were identified as the most potent and selective anticancer agents with IC_{50} values 0.03 and 0.09 μM, against PaCa-2 cell line, respectively.

Chalcones (1,3-diaryl-2-propen-1-ones) with an enone system between two aromatic rings constitute an important class of natural products which serve as precursors for the preparation of various flavonoids and exhibit interesting pharmacological activities.[1,2] Natural and synthetic chalcones have shown broad spectrum of biological activities such as antiinflammatory,[3] antituberculosis,[4] antifungal,[5] antimalarial,[6] antileish-manicidal,[7] and anticancer.[8–12] Recent development of anticancer agents involve structural modification of chalcones to improve their bioavailability and to study the role of various substituents on aryl or heteroaryl rings.[13] Chalcones, in which both the 1,3-diaryl rings are separated by three-carbon α,β-unsaturated carbonyl system, are structurally similar to indolyl heterocycles **1** (Fig. 1) having 2,5-diaryl rings separated by three atoms and connected to each other via a five-membered heterocyclic unit.

A large number of 5-(3′-indolyl)azoles isolated from different microorganisms are reported to display interesting biological activities.[14–16] The natural bis(indole) alkaloids such as topsentin and nortopsentins have demonstrated significant in vitro cytotoxicity against P388 cells.[17] Recently, we have reported indolyl-1,3,4-oxadiazoles **2** as potent anticancer agents.[18] There are many indole-based compounds found to be effective as tubulin assembly inhibitors such as recently reported 3-arylthioindoles which induce significant cellular apoptosis.[19] In the recent past, many diarylazoles are reported as analogs of chalcones with reduced activity. Replacement of a five-membered heterocyclic ring with an enone moiety has been proven to be beneficial for the biological activity.[20,21] Indole-based chalcones are largely unexplored for their anticancer potential.[3b,6,22] In continuation of our efforts to design indole-based novel and selective antitumor agents, we report herein the synthesis and anticancer activity of indolyl chalcones **3** and **4**.

In the present study we have synthesized two different series of indolyl chalcones **3** and **4** with various structural modifications.

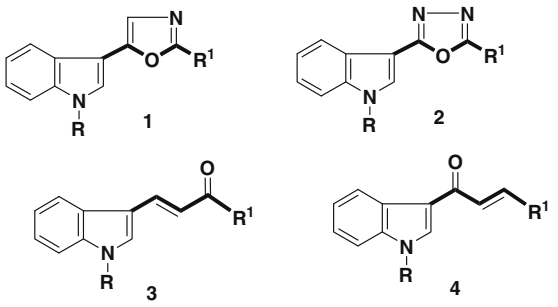
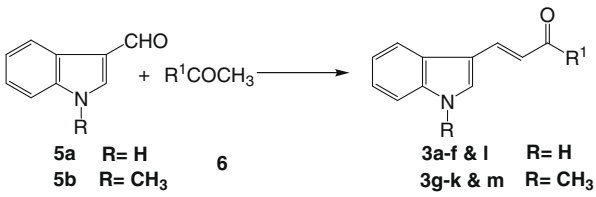


Figure 1.



Scheme 1. Reagents and conditions: piperidine, ethanol, reflux, 80 °C, 20 h.

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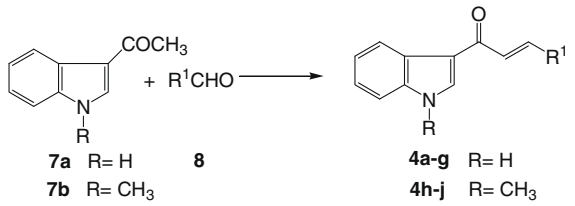

Scheme 2. Reagents and conditions: NaOH, ethanol, reflux 80 °C, 15 h.

General methods for the preparation of chalcones involve Claisen–Schmidt condensation of appropriate aryl methyl ketones and aldehydes in presence of acid or base.[23] The chalcones **3** were prepared by the reaction of indol-3-carboxaldehyde **5** with appropriate acetophenone **6** in presence of piperidine under refluxing conditions (Scheme 1).[23–25] However, under similar reaction conditions, synthesis of compounds **4** was unsuccessful. Further, the reaction of 3-acetylindole **7** with appropriate aldehyde **8** in presence of dilute sodium hydroxide under refluxing condition resulted in the formation of chalcones **4** in good yield (Scheme 2).[26,27] Since we were successful in our initial efforts to prepare both types of chalcones (**3**, **4**) using aforementioned conditions in good yields, further reaction conditions were not optimized.

Indolyl chalcones **3** and **4** were assayed for their in vitro cytotoxicity against three human cancer cell lines: epithelial (A-549), pancreatic carcinoma (PaCa-2) and androgen-independent human prostatic adenocarcinoma (PC-3).[28] The IC_{50} values were used to determine the growth inhibition in the presence of indolyl chalcones **3** and **4** against A-549, PaCa-2 and PC-3 cancer cell lines. From the IC_{50} values summarized in Table 1, the compounds **3** have shown significant cytotoxicity, especially **3c**, **3l**, and **3m**.

The compound **3b** with *p*-methoxy group is moderately cytotoxic against all the three cell lines without any selectivity. Introduction of second methoxy group in the aryl ring is beneficial for the activity (compound **3c** vs compound **3b**). The compound **3c** exhibited improved cytotoxicity against all the cancer cell lines when compared to the compound **3b**. However, replacement of 3,4-dimethoxy groups with methylenedioxy moiety led to the compound **3d** with reduced activity. Further, compounds **3l** and **3m** bearing 3,4,5-trimethoxy groups in the aromatic ring are most active in this series, the compound **3l** is selectively cytotoxic against PaCa-2 cancer cell line with an IC_{50} value of 0.03 μM. The N-methylation of indole ring nitrogen led to the compound **3m** with reduced activity (IC_{50} = 0.09 μM) against PaCa-2 cancer cell line. In other series, compounds **4g** and **4j** have shown good activity against A-549 and PaCa-2 cancer cell lines (Table 2). Compound **4g** has displayed significant cytotoxicity against PaCa-2 with an IC_{50} values 4.4 μM. Indolyl chalcone **4j** with a *N*,*N*-dimethyl substituent at para position was moderately active and selective against A-549 with an IC_{50} value of 8.7 μM.

In summary, we have prepared two series of indolyl chalcones that inhibit the growth of A-549, PaCa-2, and PC-3 cancer cell lines at micromolar concentration. Compounds **3c**, **3h**, **3l**, and **3m** were

Table 1
In vitro cytotoxicity data of indolyl chalcones (**3a**–**m**)

Compound	R	R^1	Anticancer activity (IC_{50} μM)[a]					
			A-549		PaCa-2		PC-3	
			24 h	48 h	24 h	48 h	24 h	48 h
3a	H	4-OHC_6H_4	ND	ND	>100	ND	ND	ND
3b	H	4-$OCH_3C_6H_4$	58.2	41.5	48.0	46.5	ND	56.2
3c	H	3,4-$(OCH_3)_2C_6H_3$	9.5	4.9	7.6	4.5	50.6	27.9
3d	H	3,4-$(OCH_2O)C_6H_3$	15.2	10.8	15.1	7.9	>100	34.2
3e	H	4-FC_6H_4	ND	ND	ND	ND	ND	ND
3f	H	2-C_5H_4N	ND	ND	ND	ND	ND	ND
3g	CH_3	4-FC_6H_4	ND	ND	>100	ND	ND	ND
3h	CH_3	3,4-$(OCH_3)_2C_6H_3$	8.5	8.3	8.9	3.9	ND	34.3
3i	CH_3	4-$OCH_3C_6H_4$	58.4	39.7	56.8	40.3	>100	66.6
3j	CH_3	3,4-$(OCH_2O)C_6H_3$	21.6	16.2	24.8	13.4	>100	>100
3k	CH_3	4-OHC_6H_4	92.8	37.1	83.7	36.1	ND	65.6
3l	H	3,4,5-$(OCH_3)_3C_6H_2$	0.20	0.10	ND	0.03	ND	0.10
3m	CH_3	3,4,5-$(OCH_3)_3C_6H_2$	0.21	0.15	0.28	0.09	ND	0.15

ND: not detectable.
[a] Data expressed in terms of IC_{50} values were obtained by dose dependent response.

Table 2
In vitro cytotoxicity data of indolyl chalcones (**4a**–**j**)

Compound	R	R^1	Anticancer activity (IC_{50} μM)[a]					
			A-549		PaCa-2		PC-3	
			24 h	48 h	24 h	48 h	24 h	48 h
4a	H	4-OHC_6H_4	—	—	—	—	—	—
4b	H	4-$OCH_3C_6H_4$	ND	ND	ND	ND	ND	ND
4c	H	3,4-$(OCH_3)_2C_6H_3$	—	—	—	—	—	—
4d	H	3,4-$(OCH_2O)C_6H_3$	ND	ND	ND	ND	ND	ND
4e	H	4-$N(CH_3)_2C_6H_4$	ND	ND	ND	ND	ND	ND
4f	H	3-Indolyl	ND	ND	ND	ND	ND	ND
4g	H	4-C_5H_4N	12.9	6.0	7.2	4.4	19.1	9.0
4h	CH_3	4-$OCH_3C_6H_4$	—	—	—	—	—	—
4i	CH_3	3,4-$(OCH_3)_2C_6H_3$	—	—	—	—	—	—
4j	CH_3	4-$N(CH_3)_2C_6H_4$	19.5	8.7	17.1	15.0	ND	ND

ND: not detectable; —: not soluble in buffer.
[a] Data expressed in terms of IC_{50} values were obtained by dose dependent response.

significantly cytotoxic against all three cancer cell lines with selectivity towards PaCa-2. In other series, compounds **4g** and **4j** exhibited good cytotoxicity. The preliminary anticancer activity study of both the series of indolyl chalcones reveals that 3,4,5-trimethoxyphenyl, 4-pyridyl and *N,N*-dimethylphenyl moieties are beneficial for anticancer activity and selectivity.

Further, N-methylation of indole ring nitrogen does not improve the activity. Initial anticancer activity results of indolyl chalcones **3** and **4** have generated further interest to optimize their activity and investigate specific biological targets of these compounds at molecular level.

Acknowledgment

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24. *Synthesis of indolyl chalcones* **3a–m**: A mixture of indol-3-carboxaldehyde **5** (1 mmol) and appropriate acetophenone **6** (1 mmol) in anhydrous ethanol (30 mL) was refluxed in presence of piperidine (0.5 mL) for 20 h. The reaction mixture was poured onto crushed ice, neutralized with acetic acid to afford solid compound which was filtered and recrystallized from ethanol to obtain pure **3a–m**.
25. *Data for selected compounds*: **3a**: yield 65%, dark brown solid, mp 147–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.10 (br s, 1H, OH), 6.54 (d, 1H, J = 8.76 Hz, Ar-H), 7.20–7.25 (m, 2H, Ar-H), 7.49 (d, 1H J = 8.32 Hz, Ar-H), 7.59 (d, 1H, J = 14.64 Hz, H_α), 7.64–7.75 (m, 1H, Ar-H), 8.06–8.08 (m, 3H, Ar-H), 8.09 (d, 1H, J = 14.48 Hz, H_β). 8.37 (d, 1H, J = 3.32 Hz, Ar-H), 9.92 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3275–3197 (OH), 3178 (NH) 1658 (C=O), 1577, 1520, 1487, 972, 837. 752; (FAB) m/z calcd for $C_{17}H_{13}NO_2$ (M)$^+$, 263.0960, obsd 263.0940. **3b**: yield 70%, yellow solid, mp 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 3H, OCH_3), 7.01 (d, 2H, J = 9.56 Hz, Ar-H), 7.25–7.28 (m, 2H, Ar-H), 7.46–7.50 (m, 2H, Ar-H), 7.57 (d, 1H, J = 15.44 Hz, H_α), 7.65 (d, 1H, J = 2.84 Hz, Ar-H), 7.98–8.01 (m, 1H, Ar-H), 8.05 (d, 1H, J = 4.92 Hz), 8.09 (d, 1H, J = 15.44 Hz, H_β), 11.12 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3387 (NH), 1653 (C=O), 1600, 1585, 1523, 1448, 1261, 829, 740; HRMS (FAB) m/z calcd for $C_{18}H_{15}NO_2$ (M+H)$^+$ 278.1136, obsd 278.1178. **3c**: yield 75%, yellow solid, mp 189–191 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (s, 6H, OCH_3), 6.94 (d, 1H, J = 8.56 Hz, Ar-H), 7.28 (d, 1H, J = 14.16 Hz, H_α), 7.43–7.59 (m, 3H, Ar-H), 7.72 (s, 1H, Ar-H), 7.84 (d, 1H, J = 8.24 Hz, Ar-H), 8.08 (d, 1H, J = 15.36 Hz, H_β). 8.30 (d, 1H, J = 4.4 Hz, Ar-H), 8.55 (s, 1H, Ar-H), 10.08 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3219 (NH), 1645 (C=O), 1593, 1554, 1516, 1417, 1228, 800, 740; HRMS (FAB) m/z calcd for $C_{19}H_{17}NO_3$ (M+H)$^+$ 308.1242, obsd 308.1292. **3d**: yield 65%, pale yellow solid, mp 184–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.08 (s, 2H, CH_2), 6.93 (d, 1H, J = 8.12 Hz, Ar-H), 7.24–7.28 (m, 2H, Ar-H), 7.48 (d, 2H, J = 9.52 Hz, Ar-H), 7.53 (d, 1H, J = 15.84 Hz, H_α), 7.66–7.70 (m, 2H, Ar-H), 7.96–7.98 (m, 1H, Ar-H), 8.08 (d, 1H, J = 15.40 Hz, H_β). 11.24 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3190 (NH), 1650 (C=O), 1585, 1558, 1527, 1445, 1248, 830, 735; HRMS (FAB) m/z calcd for $C_{18}H_{13}NO_3$ (M+H)$^+$ 292.0929, obsd 292.0970. **3e**: yield 70%, off-white solid, mp 185–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16–7.21 (m, 2H, Ar-H), 7.31–7.33 (m, 2H, Ar-H), 7.45–7.47 (m, 1H, Ar-H), 7.56 (d, 1H, J = 15.52 Hz, H_α), 7.63 (d, 1H, J = 2.68 Hz), 8.01–8.03 (m, 1H, Ar-H), 8.09 (d, 1H, J = 15.40 Hz, H_β). 8.10–8.13 (m, H, Ar-H), 10.24 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3242 (NH), 1649 (C=O), 1593, 1595, 1537, 1433, 815, 740; HRMS (FAB) m/z calcd for $C_{17}H_{12}FNO$ (M+H)$^+$ 266.0936, obsd 266.0981.
26. *Synthesis of indolyl chalcones* **4a–j**: To a solution of 3-acetylindole **7** (1 mmol) and appropriate aldehyde **8** (1 mmol) in ethanol (20 mL) was added 10% sodium hydroxide (2 mL) and refluxed the reaction mixture for 15 h. The contents of reaction mixture were poured into ice-cold water and neutralized with dilute hydrochloric acid. The solid so obtained was filtered, dried and recrystallized from ethanol to afford pure **4a–j**.
27. *Data for selected compounds*: **4a**. yield 55%, brown solid, mp 213–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.6 (s, 1H, OH), 6.9–7.26 (m, 4H, Ar-H), 7.27 (d, 1H, J = 15.52 Hz, H_α), 7.30–7.38 (m, 3H, Ar-H), 7.75 (d, 1H, J = 15.5 Hz, H_β), 8.37 (d, 2H, J = 8.08 Hz), 11.02 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3539–3397 (OH), 3180 (NH), 1612 (C=O), 1587, 1518, 1485, 966, 794, 754; HRMS (FAB) m/z calcd for $C_{17}H_{13}NO_2$ (M)$^+$ 263.0946 obsd 263.0870. **4b**: yield 65%, yellow solid, mp 200–202 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16 (s, 3H, OCH_3), 6.94 (d, 2H, J = 8.76 Hz, Ar-H), 7.24–7.29 (m, 2H, Ar-H), 7.35 (d, 1H, J = 15.2 Hz, H_α), 7.46–7.48 (m, 1H, Ar-H), 7.61 (d, 2H, J = 8.76 Hz, Ar-H), 7.75 (d, 1H, J = 15.5 Hz, H_β), 8.08 (d, 1H, J = 3.12 Hz, Ar-H), 8.45–8.48 (m, 1H, Ar-H), 11.24 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3120 (NH), 1639 (C=O), 1604, 1566, 1543, 1442, 1268, 819, 759; HRMS (FAB) m/z calcd for $C_{18}H_{15}NO_2$ (M+H)$^+$ 278.1136, obsd 278.1179. **4c**: yield 75%, pale yellow solid, mp 202–205 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.91 (d, 1H, J = 8.32, Ar-H), 7.16 (d, 1H, J = 1.72 Hz), 7.24 (d, 1H, J = 16.04 Hz, H_α), 7.30–7.36 (m, 3H, Ar-H), 7.43–7.46 (m, 1H, Ar-H), 7.79 (d, 1H, J = 15.5 Hz, H_β), 8.04 (s, 1H, Ar-H), 8.51–8.53 (m, 1H, Ar-H). 10.54 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3140 (NH), 1641 (C=O), 1579. 1556, 1514, 1440, 1269, 794, 748; HRMS (FAB) m/z calcd for $C_{19}H_{17}NO_3$ (M+H)$^+$ 308.1242, obsd 308.1291. **4d**: yield 72%, off-white solid, mp 214–218 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (s, 2H, CH_2), 6.78 (d, 1H, J = 8.0 Hz, Ar-H), 7.05 (d, 1H, J = 1.56 Hz, Ar-H), 7.09 (d, 1H, J = 18.56 Hz, H_α), 7.24–7.38 (m, 4H, Ar-H), 7.69 (d, 1H, J = 15.48 Hz, H_β), 7.93 (d, 1H, J = 2.8 Hz, Ar-H), 8.43–8.46 (m, 1H, Ar-H), 10.51 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3140 (NH), 1637 (C=O), 1602, 1566, 1517, 1489, 1246, 754; HRMS (FAB) m/z calcd for $C_{18}H_{13}NO_3$ (M+H)$^+$ 292.0929, obsd 292.0981. **4e**: yield 80%, light brown solid, mp 191–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.97 (s, 6H, NCH_3), 6.89 (d, 1H, J = 8.28 Hz, Ar-H), 7.15 (d, 1H, J = 1.88 Hz, Ar-H), 7.23 (d, 1H, J = 14.04 Hz, H_α), 7.32–7.37 (m, 5H, Ar-H), 7.77 (d, 1H, J = 15.52 Hz, H_β), 7.89 (s, 1H, Ar-H), 8.50–8.52 (m, 1H, Ar-H), 10.10 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3180 (NH), 1612 (C=O), 1573, 1521, 1494, 1435, 798, 754; HRMS (FAB) m/z calcd for $C_{19}H_{18}N_2O$ (M+H)$^+$ 291.1400, obsd 291.1506. **4f**: yield 55%, pale yellow, mp 180–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.13–7.26 (m, 5H, Ar-H), 7.23 (d, 1H, J = 14.04 Hz, H_α), 7.26 (d, 1H, J = 7.2 Hz), 7.47 (d, 1H, J = 15.98 Hz, H_β), 8.15 (d, 1H, J = 8.00 Hz, Ar-H), 8.28 (d, 2H, J = 8.00 Hz, Ar-H), 9.92 (s, 1H, Ar-H), 11.80 (s, 1H, NH), 11.89 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3197 (NH), 1641 (C=O), 1612, 1577 1529, 1444, 788, 754; HRMS (FAB) m/z calcd for $C_{19}H_{14}N_2O$ (M+H)$^+$ 287.1140, obsd 287.2105. **4g**: yield 55%, dark brown solid, mp 210–213 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.32 (m, 2H, Ar-H), 7.47–7.52 (m, 3H, Ar-H), 7.60 (d, 1H, J = 15.2 Hz, H_α), 7.68 (d, 1H, J = 15.6 Hz, H_β), 8.11 (d, 1H, J = 3.16 Hz), 8.45–8.48 (m, 1H, Ar-H), 8.66 (d, 2H, J = 5.96 Hz, Ar-H), 11.37 (s, 1H, NH); IR (KBr, ν cm^{-1}): 3196 (NH), 1649 (C=O), 1589, 1570, 1517, 1448, 979, 794, 748; HRMS (FAB) m/z calcd for $C_{16}H_{12}N_2O$ (M+H)$^+$ 249.0983, obsd 249.1031. **4h**: yield 60%, yellow solid, mp 126–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H, NCH_3), 3.86 (s, 3H, OCH_3), 6.91 (d, 2H, J = 8.76 Hz, Ar-H), 7.26 (d, 1H, J = 15.56 Hz, H_α), 7.30–7.35 (m, 3H, Ar-H), 7.58 (d, 2H, J = 8.8 Hz, Ar-H), 7.80 (d, 1H, J = 15.52 Hz, H_β), 7.86 (s, 1H, Ar-H), 8.48–8.52 (m, 1H, Ar-H); IR (KBr, ν cm^{-1}): 3040 (C–H), 1644 (C=O), 1569, 1531, 1511, 1454, 1259, 813, 769; HRMS (FAB) m/z calcd for $C_{19}H_{17}NO_2$ (M+H)$^+$ 292.1293, obsd 292.1335.
28. A-549, a human epithelial cell line derived from a lung carcinoma (doubling time; 20–24 h), was obtained from American Type Culture Collection. A-549 were maintained in Dulbecco's modified Eagle medium with high glucose (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) with 100 U/mL penicillin and 100 μg streptomycin. PaCa-2, a human pancreatic

carcinoma cell line (doubling time; 24 h), was obtained from Health Science Research Resources Bank (HSRRB) (Osaka, Japan). PaCa-2 were cultured in minimum essential medium with Earle's salts, L-Gln and non-essential amino acids (Nacali Tesque Inc., Kyoto, Japan) containing 10% FBS with 100 U/mL penicillin and 100 μg streptomycin. PC-3, an androgen-independent human prostatic adenocarcinoma cell line (doubling time; 48–60 h), was obtained from HSRRB. PC-3 were maintained in RPMI-1640 (Wako Pure Chem. Ind. Ltd, Osaka, Japan) containing 10% FBS with 100 μg/mL penicillin and 100 μg streptomycin. Cell lines were kept at 37 °C in a humidified atmosphere consisting of air (CO_2 5%). A-549 and PaCa-2 cells were plated 5000 cells per well, and PC-3 cells were plated 1×10^4 cells well in 96-well plates, the day before chalcones (**3** and **4**) treatment. All the compounds were dissolved in dimethylsulfoxide (DMSO) at room temperature. Aliquots of these stock solutions at 100 mM were stored at −20 °C. The cell viability was measured by the cell Counting Kit-8 (Dojin, Kumamoto, Japan) using a spectrophotometer (xMark; Bio-Rad, Hercules, CA, USA) at 450 nm after 24 h and 48 h of chalcones treatment. Final concentrations of the vehicle were 1% DMSO in culture medium. The cell viability of A-549, PaCa-2 and PC-3 human cancer cells was inhibited by the chalcone analogues **3** and **4** for 24–48 h in a dose-dependent manner.