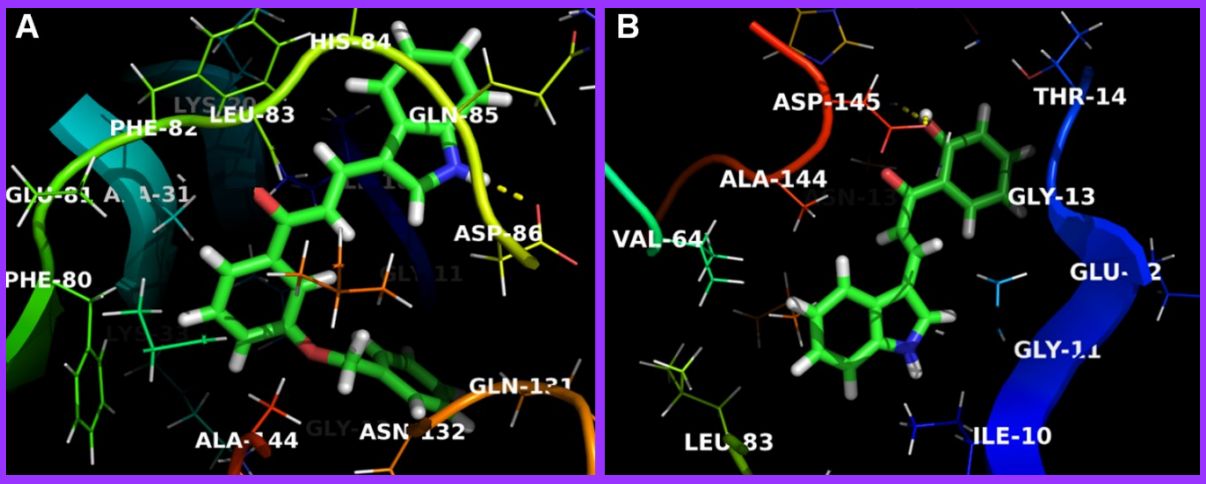

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Chemical synthesis, docking studies and biological effects of functionalized 1,3-diaryl-2-propen-1-ones on human colon cancer cells

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Abstract

A series of 1, 3-diaryl-2-propen-1-ones was synthesised in order to obtain a new type of anti-cancer drug, designed with hybrid features to inhibit colon cancer activated receptor. Based on computational modelling and docking studies, potential inhibitors were synthesised and their biological activity evaluated. The structures of newly synthesized compounds were confirmed by ^{1}HNMR, ^{13}CNMR and Mass spectrometry. All analogues were evaluated for *in vitro* cytotoxicity against human colon (caco-2) cancer cell lines. Compounds **1b**, **1f-1h**, and **2i** showed significant cytotoxicity. Chalcones **1b**, **1f** and **1g** were identified as the most potent and selective anti-cancer agents with IC_{50} values <1 µg/mL and 1.5 µg/mL, against caco-2 cell line, respectively. In conclusion, this finding confirms the suitability of indolyl chalcone analogues as candidates for further investigation towards the management of colon cancer related diseases.

Introduction

Various research groups have focused on chemoprevention and working on development of new lead molecules. Usually early detection and focus on improvement of currently used drugs can be very helpful for curbing the disease. From the available literature it's clear that the quinoline ring system and their fused derivatives are significant structural units and present as substructure in various alkaloids, therapeutics and synthetic analogues, which exhibit good biological activities (Larsen et al., 1996; Roma et al., 2000). Various quinolines derivatives are reported as anti-malarial, anti -inflammatory, antiasthmatic, antibacterial, anti-hypertensive and platelet derived growth factor receptor tyrosine kinase (PDGF-RTK) inhibiting agents (Dube et al., 1998). A large variety of quinolines are reported to exhibit substantial anti-cancer activities (Alkasomi et al., 2010) Quinoline derivative act as anti-cancer agents through a variety of mechanisms for example; cell cycle arrest in the G2 phase (Kim et al., 2005) inhibition of topoisomerase (Ching et al., 2008) and tubulin polymerization inhibition (Alkasomi., 2009) Another mechanism of action is the inhibition of tyrosine kinases (Mulvihill et al., 2008). These results encourage us to design the molecules containing quinoline ring with different functional group to assess their biological activity.

Medicinal chemists are tirelessly exploring for a better and more suitable cancer therapeutics. Chal-cones (1, 3-diaryl-2-propen-1-ones), constituting an enone system between two aromatic rings are an important class of natural products which are considered as precursors for various flavonoids and exhibit interesting pharmacological activities (Stu et al., 1971). Chalcones, originating from natural and synthetic routes possess several biological activities, such as cytotoxic (Modzelewska et

al., 2006) anti-malarial (Dominguez et al., 2005), anti-leishmanial (Boeck et al., 2006) anti-inflammatory (Yang et al., 2007), anti-HIV (Cheenpracha et al., 2006), anti-fungal (Svetaz et al., 2004) and as tyrosine kinase inhibitors (Neryr et al., 2004). Because of very high pharmacological interest, these molecules have attracted medicinal chemists to design and synthesize further large number of chalcones with different functional groups. In the recent years, the development of anti-cancer agents was achieved by structural modification of chalcones to increase their bioavailability and to study the effect of various substituents on aryl or heteroaryl rings (Meng et al., 2007).

The heteroaryl rings are widely distributed in nature and possess a variety of significant biological activities. The indole ring is an important moiety in many pharmacologically active compounds in which some studies related reported for anti-cancer effectiveness (Grugni et al., 2006). Some of the individual anti-cancer compounds in which the indole ring is responsible for the activity are panobinostat (Prince et al., 2009), cediranib (Nikolinakos et al., 2008), indole-3-carbinol (Aggarwal et al., 2005) (Figure 1). Basically, indolyl chalcones are not much explored for their anti-cancer potential (Aggarwal et al., 2005). In the present study, we have synthesized two different series Figure 2 of novel indolyl chalcones 1a-j (Scheme 1) and 2a-k (Scheme 2) and evaluated their anti-cancer activity *in vitro* against four human cancer cell lines. Compounds 1b, 1f-1h, and 2i showed significant cytotoxicity. Chalcones 1b, 1f and 1g were identified as the most potent and selective anti-cancer agents with IC_{50} values 7.4 µg/mL and 7.8 µg/mL, against human colon (Caco-2) cell line, respectively. Based on computational modelling and docking studies, potential inhibitors were synthesised and their biological activity evaluated.

Materials and Methods

Chemistry and instrument

Melting point was determined on a Toshniwal melting point apparatus and is uncorrected. IR spectra were recorded on a PerkinElmer 1719 FT-IR spectrophotometer. NMR spectra were obtained in acetone-d_6, DMSO-d_6 and pyridine-d_5 on a Bruker Avance, 300 MHz instrument using TMS as internal standard. The chemical shift values are reported in ppm and coupling constants in Hz. ESI-MS spectra were recorded on a Perkin Elmer Turbo Mass/Shimadzu LC-MS. TLC analyses were carried out on precoated silicage l60 F_{254} plates (Merck) using solvent system, hexane: ethyl acetate (7:3). The compounds were visualized by either exposure of TLC plates to I_2 vapours or by spraying with vanillin- sulphuric acid reagent, followed by heating at 110°C for 15 min. Si-gel, 60-120 mesh (spectrochem) was used in the column chromatography for the purification of metabolites. HPLC analyses were carried out on waters spherisorb ODS2 (250 x 4.6 mm i.d., 10 µm) column using binary gradient elution with acetonitrile and water mobile

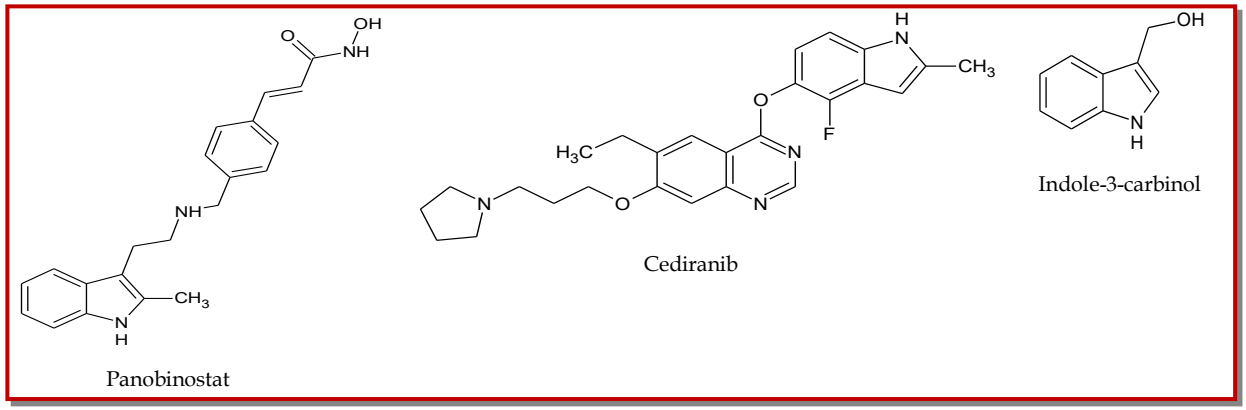


Figure 1: Anti-cancer drugs having indole ring

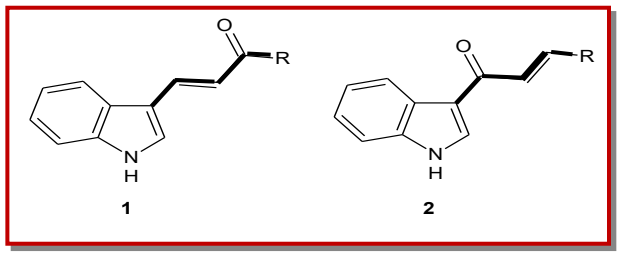
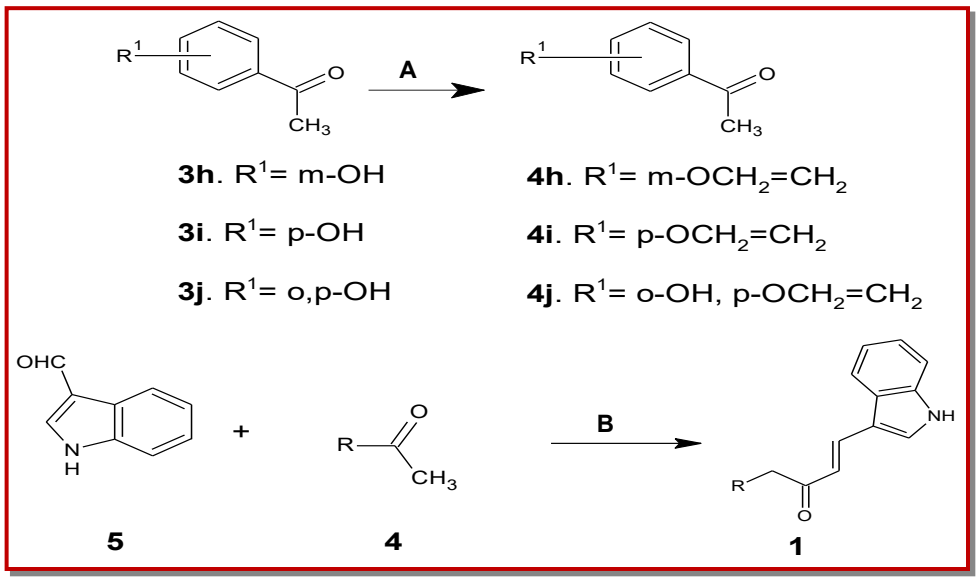


Figure 2: Skeleton of chalcone (α, β-unsaturated ketone) responsible for its biological activity

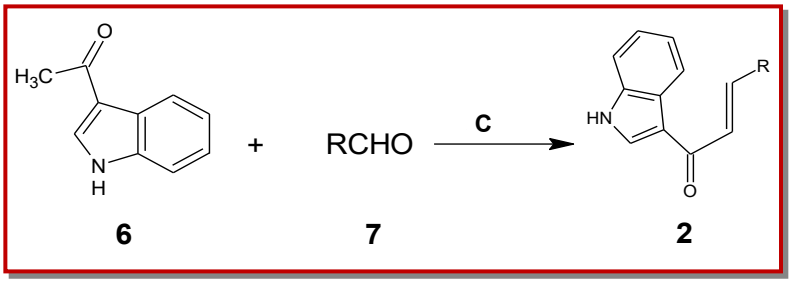
phase (70:30) at a flow rate of 0.6 mL /min, column temperature of 25° and UV detection at λ230 nm. The compounds were identified by their spectral IR, ID (^{1}H, ^{13}C, DEPT) and 2D (COSY, HSQC, HMBC) ESIMS) NMR and ESI/MS analysis.

Synthesis of indolyl chalcones, series 1

Indolyl chalcones **1a-j** were prepared by the reaction of indol-3-carboxaldehyde **5** with appropriate acetophenone **4** in presence of NaOH at RT (Scheme 1) (Jeong et



Scheme 1: Reagents and conditions: A) allyl bromide, acetone, 60°C; B) NaOH, methanol, 1-15 hours, RT



Scheme 2: Reagents and conditions: c) $SOCl_2$, methanol, 1-2 hours, RT

al., 2004).

The contents of reaction mixture were poured into ice-cold water and neutralized with dilute hydrochloric acid. The solid so obtained was filtered, column chromatographed and recrystallized from ethanol to afford pure compounds.

***Trans-3-(1H-indol-3-yl)-1-(4′-flouro-3′-methylphenyl)-2-propen-1-one* (1a)**

Orange powder; 20% yield; mp 59-60°C; IR ν^{max}(KBr): 3422, 1548, 1154, 737 (NH), 1653 (chalcone C=O), 1520, 1491, 1440(aromatics) cm^{-1};^{1}H NMR (300 MHz, acetone-d6): δ 2.35 (3H, s, CH_3), 7.19 (1H, d, J=9.3 Hz, H-5′′), 7.27 (2H, m, H-5′, H-6′), 7.54 (1H, dd, J=8.1, 1.2 Hz, H-4′), 7.70 (1H, d, J=15.6 Hz, H-2), 7.98 (1H, d, J=2.7 Hz, H -2′′), 8.04 (1H, dd, J=8.7, 2.7 Hz, H-6′′), 8.08 (1H, brs, H-2′), 8.11 (1H, d, J=15.6 Hz, H-3), 8.10 (1H, d, J=8.1, 1.2 Hz, H-7′), 10.90 (1H, brs, NH); ^{13}C NMR(75 MHz, acetone-d_6): δ 14.35 (CH_3), 113.01 (C-4′), 114.36 (C-1′), 115.49[a] (C-5′′), 115.79[a] (C-2), 121.16 (C-7′), 121.93 (C-6′), 123.61 (C-5′), 126.34 (C-3′), 128.99 (C-6′′), 132.66[b] (C-2′′), 132.72[b] (C-2′), 136.17 (C-1′′), 138.65 (C-8′), 139.36 (C-3), 162.74[c] (C-3′′), 166.03[c] (C-4′′), 188.37 (C-1) (a,b,c=interchangable); ESI-MS, MeOH (Positive): m/z 280 $[M+H]^+$, 302 $[M+Na]^+$, (Negative): 278$[M-H]^-$, $C_{18}H_{14}FNO$.

***Trans-3-(1H-indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one* (1b)**

Yellow solid; 60%, yield; mp 74-75°C; IR ν^{max} (KBr): 3448, 1562, 1120, 735 (NH), 1654(chalcone C=O), 1523, 1495, 1437 (aromatics) cm^{-1};^{1}H NMR (300 MHz, acetone-d_6): δ 5.29 (2H, s, H_2-7′′), 7.24 (2H, m, H-5′, H-6′), 7.27 (1H, m, H-4′′), 7.35 (3H, m, H-10′′, H-11′′, H-12′′), 7.49 (2H, m, H-4′, H-5′′), 7.53(2H, m H-9′′, H-13′′), 7.68 (1H, d, J=15.6 Hz, H-2), 7.70 (1H, s, H-2′′), 7.72 (1H, d, J=7.8, 2.1 Hz, H-7′), 8.01 (1H, d, J=2.7 Hz, H-2′), 8.08 (1H, m, H -6′′), 8.10 (1H, d, J=15.6 Hz, H-3), 10.92 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 70.16 (C-7′′), 112.70 (C -4′), 114.05 (C-1′), 114.28 (C-2′′), 116.85 (C-2), 119.35 (C-4′′), 120.82 (C-6′′), 121.18 (C-7′), 121.65 (C-6′), 123.29 (C-5′), 126.04 (C-3′), 128.00 (C-9′′, (C-13′′), 128.22 (C-5′′), 128.85 (C-10′′, C-12′′), 130.04 (C-11′′), 132.38 (C-2′), 137.74 (C-8′), 138.33 (C-1′′), 139.62 (C-3), 141.10 (C-8′′), 159.36 (C-3′′), 189.09 (C-1); ESI-MS, MeOH (Positive): m/z 354 $[M+H]^+$, (Negative): 352$[M-H]^-$, $C_{24}H_{19}NO_2$.

***Trans-3-(1H-indol-3-yl)-1-(anthracenyl)-2-propen-1-one* (1c)**

Yellow powder; 80% yield; mp 108-109°C; IR ν^{max}(KBr): 3395, 1561, 1164, 746 (NH), 1649 (chalcone C=O), 1515,

1483, 1430 (aromatics) cm^{-1};^{1}H NMR (300 MHz, acetone-d_6): δ 7.16 (2H, d, J=7.8 Hz, H-4′′, H-12′′), 7.23 (2H, m, H -5′, H-6′), 7.26 (4H, m, H-5′′, H-6′′, H-10′′, H-11′′), 7.44 (1H, d, J=2.1 Hz, H-8′′), 7.51 (1H, dd, J=8.1, 2.1 Hz, H-4′), 7.59 (2H, d, J=7.8 Hz, H-3′′, H-13′′), 7.87 (1H, d, J=15.6 Hz, H-2), 7.89 (1H, brs, H-2′), 8.21 (1H, dd, J=8.1, 2.1 Hz, H-7′), 8.55 (1H, d, J=15.6 Hz, H-3), 13.05 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 111.65 (C-5′′, C-11′′), 112.53 (C-4′′, C-12′′), 113.06 (C-4′), 114.23 (C-1′), 116.45 (C-3′′, C-13′′), 121.11[a] (C-7′), 121.89[a] (C-2, C-6′), 123.15[b] (C-6′′, C-10′′), 123.48[b] (C-5′), 124.15 (C-8′′), 126.40 (C-3′), 128.15 (C-1′′), 133.50 (C-2′), 138.88 (C-8′), 139.30 (C-3), 155.35[c] (C-7′′, C-9′′), 155.91[c] (C-2′′, C-14′′), 179.72 (C-1) (a,b,c=interchangable); ESI-MS, MeOH (Positive): m/z 348 $[M+H]^+$, 346$[M-H]^-$, $C_{25}H_{17}NO$.

***Trans-3-(1H-indol-3-yl)-1-(benzofuran)-2-propen-1-one* (1d)**

Dark brown solid; 40%, yield; mp 59-60°C; IR ν^{max} (KBr): 3395, 1151, 1156, 736 (NH), 1654 (chalcone C=O), 1509, 1483, 1427 (aromatics) cm^{-1};^{1}H NMR (300 MHz, acetone-d_6): δ 7.11 (2H, m, H-5′, H-6′), 7.35 (1H, d, J=15.6 Hz, H-2), 7.49 (3H, m, H-4′, H-5′′, H-6′′), 7.53 (1H, d, J=15.6 Hz, H-3), 7.61 (1H, d, J=2.7 Hz, H-2′), 7.92 (1H, dd, J=8.4, 2.1 Hz, H-7′), 8.01 (1H, dd, J=6.9, 2.7 Hz, H-4′′), 8.12 (1H, dd, J=7.2, 2.4 Hz, H-8′′), 8.62 (1H, s, H-2′′), 13.05 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 112.92 (C-4′), 113.31 (C-1′), 120.69[a] (C-2), 121.86[a] (C-7′), 123.42 (C-6′), 124.71 (C-5′), 125.71[b] (C-3′, C-2′′), 125.91[b] (C-7′′), 126.66 (C-4′′), 127.96 (C-5′′), 128.72 (C-3′′), 129.05 (C-6′′), 131.76 (C-8′′) 133.17 (C-2′), 136.65[c] (C-8′), 138.47[c] (C-1′′), 142.74 (C-3), 198.91 (C-1), (a,b,c=interchangable); ESI-MS, MeOH (Positive): m/z 288$[M+H]^+$, Negative: 286$[M-H]^-$, $C_{19}H_{13}NO_2$.

***Trans-3-(1H-indol-3-yl)-1-(4′-chlorophenyl)-2-propen-1-one* (1e)**

Yellow fluffy crystals, 60% yield, obtained and analysed by spectroscopic data as described by an earlier method (Black., et al 1992).

***Trans-3-(1H-indol-3-yl)-1-(2′-chlorophenyl)-2-propen-1-one* (1f)**

Yellow shiny crystals, 85% yield, obtained and analysed by spectroscopic data as described by an earlier method (Black., et al 1992).

***Trans-3-(1H-indol-3-yl)-1-(2′-hydroxyphenyl)-2-propen -1-one* (1g)**

Light brown crystals, 55% yield, obtained and analysed by spectroscopic data as described by an earlier method (Black., et al 1992).

***Trans-3-(1H-indol-3-yl)-1-(3′-allyloxyphenyl)-2-propen-1-one* (1h)**

Light brown powder; 65% yield; mp 90-92°C;IR ν^{max} (KBr): 3389 1564, 1211, 739 (NH), 1653 (chalcone C=O), 1523, 1458, 1420 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 4.64 (2H, d, J=5.1 Hz, H_2-7′′), 5.26 (1H, dd, J=10.5, 0.9 Hz, Ha-9′′), 5.40 (1H, dd, J=17.4, 1.5 Hz, Hb-9′′), 6.05 (1H, m, H-8′′), 7.24 (3H, m, H-5′, H-6′, H-4′′), 7.49 (2H, d, J=8.1 Hz, H-4′, H-5′′), 7.54 (1H, s, H-2′′), 7.60 (1H, d, J=15.6 Hz, H-2), 8.03 (1H, d, J=6.6 Hz, H-7′), 8.06 (1H, d, J=15.6 Hz, H-3), 8.09 (1H, d, J=2.1 Hz, H-2′), 7.70 (1H, d, J=8.1 Hz, H-6′′), 9.90 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 69.20 (C-7′′), 113.42 (C-4′), 113.60 (C-1′), 114.44 (C-2′′), 116.31 (C-2), 118.42 (C-9′′), 119.82 (C-4′′), 121.09 (C-7′), 121.59 (C-6′′), 122.07 (C-6′), 123.59 (C-5′), 126.05 (C-3′), 130.72 (C-5′′), 134.10 (C-2′), 134.39 (C-8′′), 138.44 (C-8′), 140.08 (C-3), 140.90 (C-1′′), 159.25 (C-3′′), 189.60 (C-1); ESI-MS, MeOH (Positive): m/z 304$[M+H]^+$, Negative: 302$[M-H]^-$, $C_{20}H_{17}NO_2$.

***Trans-3-(1H-indol-3-yl)-1-(4′-allyloxyphenyl)-2-propen-1-one* (1i)**

Light brown powder; 30%; mp 110-111°C; IR ν^{max} (KBr): 3372 1561, 1205, 736 (NH), 1651 (chalcone C=O), 1511, 1470, 1406 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 4.65 (2H, d, J=5.04 Hz, H_2-7′′), 5.28 (1H, d, J=10.52 Hz, Hb-9′′), 5.42 (1H, d, J=17.21 Hz, Ha-9′′), 6.05 (1H, m, H-8′′), 7.08 (2H, d, J=8.64 Hz, H-3′′, H-5′′), 7.24 (1H, m, H-5′, H-6′), 7.53 (1H, m, H-4′), 7.67 (1H, d, J=15.40 Hz, H-2), 8.06 (1H d, J=15.56 Hz, H-3), 8.09 (2H, m, H-2′, H-7′), 8.13 (1H, d, J=8.72 Hz, H-2′′), 8.13 (1H, d, J=8.72 Hz, H-6′′), 9.91 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 68.86 (C-7′′), 113.00 (C-4′), 113.19 (C-1′), 115.02 (C-3′′, C-5′′), 115.66 (C-2), 118.36 (C-9′′), 120.74 (C-7′), 121.53 (C-6′), 123.07 (C-5′), 125.66 (C-3′), 130.85 (C-6′′), 131.80 (C-1′′), 130.85 (C-2′′), 133.37 (C-2′), 133.65 (C-8′′), 138.06 (C-8′), 138.74 (C-3), 162.05 (C-4′′), 187.76 (C-1); ESI-MS, MeOH (Positive): m/z 304$[M+H]^+$, Negative: 302$[M-H]^-$, $C_{20}H_{17}NO_2$.

***Trans-3-(1H-indol-3-yl)-1-(4′-allyloxy-2′-hydroxyphenyl)-2-propen-1-one* (1j)**

Yellow powder; 30% yield; mp 110-111°C; IR ν^{max} (KBr): 3569 1559, 1229, 735 (NH), 3380 (OH), 1621 (chalcone C=O), 1497, 1439, 1369 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.65 (2H, d, J=4.92 Hz, H_2-7′′), 5.29 (1H, d, J=10.68 Hz, Hb-9′′), 5.42 (1H, d, J=17.32 Hz, Ha-9′′), 6.04 (1H, m, H-8′′),), 6.50 (1H, s, H-3′′), 6.58 (1H, dd, J=8.92, 2.08 Hz, H-5′′), 7.25 (2H, m, H-5′, H-6′), 7.52 (dd, J=7.92, 2.52 Hz, H-4′), 7.54 (1H, dd, J=7.92, 2.52 Hz, H-4′), 7.68 (1H, d, J=15.3 Hz, H-2), 8.11 (1H, dd, J=7.92, 2.52 Hz, H-7′), 8.14 (1H, s, H-2′), 8.17 (1H, d, J=15.3 Hz, H-3), 8.19 (1H, m, H-6′′), 9.45 (1H, brs, NH); ^{13}C NMR (75 MHz,DMSO-d_6): δ 69.00 (C-7′′), 113.10 (C-4′), 113.36 (C-1′), 102.20 (C-3′′), 107.93 (C-5′′), 114.11 (C-2), 114.51 (C-1′′), 118.46 (C-9′′), 120.85 (C-7′), 121.81 (C-6′), 123.30 (C-5′), 125.63 (C-3′), 132.46 (C-6′′), 165.89 (C-2′′), 134.43 (C-2′), 133.47 (C-8′′), 138.14 (C-8′), 139.90 (C-3), 164.43 (C-4′′), 192.08 (C-1); ESI-MS, MeOH (Positive): m/z 320 $[M+H]^+$, Negative: 318$[M-H]^-$, $C_{20}H_{17}NO_3$.

***Synthesis of indolyl chalcones* 2(a–k)**

Further, the reaction of 3-acetylindole **6** with appropriate aldehyde **7** in presence of $SOCl_2$ resulted in the formation of indolyl chalcones **2a-k** (Scheme 2) (Prince et al., 2009). Acetophenones (**4h-4j**) for the preparation of the compounds **1h-1j** were prepared by etherification of o-hydroxy (**3h**), p- hydroxy (**3i**) and o,p -hydroxy (**3j**) acetophenones respectively with allyl bromide in the presence of KBr in acetone using refluxing condition.

The contents of reaction mixture were poured into ice-cold water. The solid so obtained was filtered, dried and recrystallized from ethanol to afford pure **2(a–k)**.

***Trans-1-indolyl-3-(anthracenyl)-2-propen-1-one* (2a)**

Yellow powder; 70% yield; mp 190-191°C, IR ν^{max}(KBr): 3398, 1570, 1234, 728 (NH), 1639 (chalcone C=O), 1518, 1442, 1419, 1381 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.90 (4H, m, H-5′, H-5′′, H-6′′, H-11′), 6.83(2H, m, H-4′, H-12′), 6.95 (2H, m, H-3′, H-13′), 7.16 (1H, d, J=15.6 Hz, H-2), 7.53 (1H, d, J=7.8 Hz, H-4′′), 7.83 (2H, d, J=8.1 Hz, H-6′, H-10′), 7.96 (1H, brs, H-8′), 8.07 (1H, brs, H-2′′), 8.45 (1H, d, J=15.56 Hz, H-3), 8.63 (1H, d, J=7.8 Hz, H-7′′), 12.81 (1H, brs, NH);^{13}C NMR (75 MHz, pyridine-d_5): δ 113.08 (C-4′′), 119.2 (C-1′′), 123.02 (C-6′′, C-7′′), 123.32 (C-5′′), 124.23 (C-4′, C-12′), 126.04 (C-5′, C-11′), 126.10 (C-6′, C-10′), 126.81 (C-3′, C-13′), 127.49 (C-3′′), 128.34 (C-8′), 130.19 (C-1′), 131.67 (C-2′, C-14′), 132.01 (C-7′, C-9′), 134.39 (C-2), 137.57 (C-3), 135.04 (C-2′′), 138.47 (C-8′′), 184.37 (C-1); ESI-MS, MeOH (Positive): m/z 348 [M+H]+, 370 $[M+Na]^+$, $C_{25}H_{17}NO$.

***Trans-1-indolyl-3-(2',4′-dimethoxyphenyl)-2-propen-1-one* (2b)**

Creamish white crystals, 10% yield, obtained and analysed by spectroscopic data as described by an earlier method (Yesuthangan et al., 2011).

***Trans-1-indolyl-3-(3',4′,5′-trimethoxyphenyl)-2-propen-1-one* (2c)**

Light orange crystals;70% yield; mp 191-192°C; IR ν^{max} (KBr): 3448 1581, 1197, 755 (NH), 1642 (chalcone C=O), 1515, 1459, 1426 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (3H, s, $OC\underline{H}_3$), 3.71 (6H, s, 2 x $OC\underline{H}_3$), 7.18 (2H, brs, H-2′, H-6′), 7.24 (2H, m, H-5′′, H-6′′), 7.51 (1H, dd, J=6.3, 2.1 Hz, H-4′′), 7.62 (1H, d, J=15.6 Hz, H-2), 7.78 (1H, d, J=15.6 Hz, H-3), 8.38 (1H, dd, J=6.3, 2.1 Hz, H-7′′), 8.75 (1H, d, J=3 Hz, H-2′′), 12.12 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 56.97 (2 x $O\underline{C}H_3$), 60.99 ($O\underline{C}H_3$), 106.92 (C-2′, C-6′), 113.04 (C-4′′), 118.68 (C-1′′), 122.70 (C-6′′, C-7′′), 123.97 (C-5′′), 124.77 (C-3), 126.81 (C-3′′), 131.69 (C-1′), 135.52 (C-2′′), 137.79 (C-8′′), 140.07 (C-4′), 140.84 (C-2), 154.01 (C-3′, C-5′), 184.54 (C-1); ESI-MS, MeOH (Positive): m/z 338 $[M+H]^+$, $C_{20}H_{19}NO_4$.

***Trans-1-indolyl-3-(2'3',4′-trimethoxyphenyl)-2-propen-1-one*(2d)**

Creamish powder;70% yield; mp 163-164°C; IR ν^{max} (KBr): 3431 1586, 1201, 754 (NH), 1640 (chalcone C=O), 1525, 1493, 1414 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 3.67 (3H, s, $OC\underline{H}_3$), 3.76 (6H, s, 2 x $OC\underline{H}_3$), 6.71 (1H, d, J=9.0 Hz, H-5′), 7.12 (2H, m, H-5′′, H-6′′), 7.40 (1H, d, J=6.6 Hz, H-4′′), 7.60 (1H, d, J=15.6 Hz, H-2), 7.66 (1H, d, J=9.0 Hz, H-6′), 7.74 (1H, d, J=15.56 Hz, H-3), 8.25 (1H, d, J=6.6 Hz, H-7′′), 8.57 (1H, brs, H-2′′), 11.79 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 56.87, 61.32, 62.3 (3 x $O\underline{C}H_3$), 109.28 (C-5′), 113.02 (C-4′′), 118.64 (C-1′′), 122.63 (C-6′′, C-7′′), 122.45 (C-1′), 123.41 (C-6′), 123.91 (C-5′′), 124.01 (C-2), 126.80 (C-3′′), 134.66 (C-3), 135.18 (C-2′′), 137.71 (C-8′′), 142.72 (C-3′), 153.55 (C-2′), 155.85 (C-4′), 184.70 (C-1); ESI-MS, MeOH (Positive): m/z 338 $[M+H]^+$, Negative: 336$[M-H]^+$, $C_{20}H_{19}NO_4$.

***Trans-1-indolyl-3-(3'-ethoxy-4-hydroxyphenyl)-2-propen-1-one* (2e)**

Light brown crystals;70% yield; mp 154-155°C; IR ν^{max} (KBr): 3535 1557, 1204, 746 (NH), 3394 (OH), 1641 (chalcone C=O), 1512, 1479, 1403 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 1.17 (3H, t, J=6.9 Hz, CH_3), 3.99 (2H, q, J=6.9 Hz, $OC\underline{H}_2$-), 7.35 (1H, d, J=8.1 Hz, H-5′), 7.39 (1H, d, J=8.1 Hz, H-6′), 7.47 (2H, m, H-5′′, H-6′′), 7.55 (1H, d, J=1.5 Hz, H-2′), 7.67 (1H, d, J=7.5 Hz, H-4′′), 7.85 (1H, d, J=3.0 Hz, H-2′′), 8.06 (1H, d, J=15.3 Hz, H-2), 8.35 (1H, d, J=15.3 Hz, H-3), 9.21 (1H, d, J=7.8 Hz, H-7′′), 13.23 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 16.07 ($\underline{C}H_3$), 65.89 ($O\underline{C}H_2$-), 113.90 (C-4′′), 114.33 (C-2′, C-14′), 118.24 (C-5′, C-11′), 120.84 (C-1′′), 123.47 (C-2), 123.76 (C-6′′), 124.59 (C-7′′), 124.77 (C-5′′), 125.02 (C-6′, C-10′), 128.78 (C-3′′), 129.10 (C-1′), 135.34 (C-2′′), 139.48 (C-8′′), 142.96 (C-3), 149.57 (C-3′, C -13′), 152.04 (C-4′, C-12), 186.47 (C-1); ESI-MS, MeOH (Positive): m/z 308 $[M+H]^+$, Negative: 306$[M-H]^+$, $C_{19}H_{17}NO_3$.

***Trans-1-indolyl-3-(3',5′-dimethoxy-4′-hydroxyphenyl)-2-propen-1-one* (2f)**

Creamy crystals;70% yield; mp 210-211°C; IR ν^{max}(KBr): 3445, 1580, 1191, 740 (NH), 3445 (OH), 1640 (chalcone C=O), 1522, 1491, 1404 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51 (6H, brs, 2x $OC\underline{H}_3$), 6.49 (2H, br s, H-2′), 6.61 (2H, m, H-5′′, H-6′′), 6.90 (1H, dd, *J*=6.9, 1.5 Hz, H-4′′), 6.99 (1H, d, *J*=15.3Hz, H-2), 7.04 (1H, d, *J*=15.3 Hz, H-3), 7.70 (1H, dd, *J*=8.1, 1.8 Hz, H-7′′), 8.06 (1H, d, *J*=2.7 Hz, H-2′′), 11.43 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.01 (2 x $O\underline{C}H_3$), 107.03 (C-2′, C-6′), 113.08 (C-4′′), 118.56 (C-1′′), 122.47 (C-2), 122.61 (C-7′′), 122.81 (C-6′′), 124.08 (C-5′′), 126.42 (C-1′), 126.64 (C-3′′), 135.29 (C-2′′),137.67 (C-8′′), 138.62 (C-4′), 141.83 (C-3), 148.90 (C-3′, C-5′), 185.13 (C-1); ESI-MS (Positive): m/z 324 $[M+H]^+$, Negative: 322 $[M-H]^-$, $C_{19}H_{17}NO_4$.

***Trans-1-indolyl-3-(3',5′-dimethoxy-4′-benzyloxyphenyl)-2-propen-1-one* (2g)**

Creamish crystals;70% yield; mp 209-210°C; IR ν^{max} (KBr): 3440 1576, 1203, 739 (NH), 1655 (chalcone C=O), 1742(ester CO), 1512, 1466, 1426 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 7.24 (1H, dd, J=7.5, 1.5 Hz, H-10′), 7.31 (2H, d, J=7.5 Hz, H-3′, H-6′), 7.54 (2H, dd, J=8.4, 2.7 Hz, H-9′, H-11′), 7.58 (2H, m, H-5′′, H-6′′), 7.68 (1H, d, J=15.3 Hz, H-2), 8.70 (1H, br s, H-2′′), 7.72 (1H, dd, J=7.2, 1.2 Hz, H-4′′), 7.88 (1H, d, J=15.3 Hz, H-3), 8.12 (2H, d, J=7.5 Hz, H-8′, H-12′), 8.38 (1H, dd, J=6.6, 2.1 Hz, H-7′′), 12.20 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 106.79 (C-2′, C-6′), 113.12 (C-4′′), 118.67 (C-1′′), 122.77 (C-6′′, C-7′′), 123.23 (C-5′′), 125.90 (C-2), 126.79 (C-3′′), 129.32 (C-1′), 129.90 (C-9′, C-11′), 130.85 (C-8′, C-12′), 134.96 (C-10′), 135.77 (C-2′′), 137.83 (C-8′′), 140.43 (C-3), 153.02 (C-3′, C-4′, C-5′), 164.48 (CO), 184.42 (C-1); ESI-MS, MeOH (Positive): m/z 428 $[M-H]^+$, 450 $[M+Na]^+$, Negative: 428$[M-H]^-$, $C_{26}H_{21}NO_5$.

***Trans-1-indolyl-3-(4'-hydroxyphenyl)-2-propen-1-one* (2h)**

Dark brown powder,85% yield, obtained and analysed by spectroscopic data as described by an earlier method (Kumar., et al 2010).

***Trans-1-indolyl-3-(2'-methylphenyl)-2-propen-1-one* (2i)**

Obtained as brown solid; 80% yield; mp 140-142°C; IR ν^{max}(KBr): 3422, 1562, 1156, 748 (NH), 1639 (chalcone C=O), 1520, 1442, 1492 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6):δ 2.34(3H, s, CH_3), 7.13-7.18 (5H, m, H-3', H-4', H-5', H-5'', H-6''), 7.40 (1H, dd, J=8.1 Hz, 2.1 Hz, H-4''), 7.63 (1H, d, J=15.6 Hz, H-2), 7.82 (1H, d, J=15.6 Hz, H-3), 7.87 (1H, dd, J=7.5, 2.4 Hz, H-6'), 8.25 (1H, dd, J=6.6, 2.1Hz, H-7''), 8.63 (1H, d, J=3.0 Hz, 1H, H-2''), 12.05 (1H, brs, NH); ^{13}CNMR (75 MHz, DMSO-d_6): δ 20.23 (CH_3), 113.07 (C-4''), 118.54 (C-1''), 122.61 (C-7''), 122.74 (C-6''), 123.99 (C-5''), 126.36 (C-2), 126.80 (C-3''), 127.11 (C-5'), 127.36 (C-6'), 130.41 (C-4'), 131.55 (C-3'), 134.68 (C-1'), 135.63 (C-2''), 137.54 (C-3), 137.76 (C-8''), 138.23 (C-2'), 184.55 (C-1); ESI-MS, MeOH (Positive): m/z 262 [M+H]+, 284 $[M+Na]^+$, Negative: 260 $[M-H]^-$, $C_{18}H_{15}NO$.

***Trans-1-indolyl-3-(thiophenyl)-2-propen-1-one* (2j)**

Creamish white powder;70% yield; mp 181-182°C; IR ν^{max}(KBr): 3448 1578, 1199, 754 (NH), 1632 (chalcone C=O), 1523, 1493, 1438, 1315 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 6.03 (1H, d, J=4.2 Hz, H-2′), 6.10 (2H, m, H-5′′, H-6′′), 6.34 (1H, m, H-4′′), 6.36 (1H, d, J=15.3 Hz, H-2), 6.45 (1H, brs, H-4′), 6.55 (1H, d, J=4.2 Hz, H-3′), 6.66 (1H, d, J=15.3 Hz, H-3), 7.50 (1H, d, J=7.5 Hz, H-7′′), 7.52 (1H, brs, H-2′′), 10.95 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 113.06 (C-4′′), 118.34 (C-1′′), 122.62 (C-7′′), 122.72 (C-6′′), 124.00 (C-5′′), 124.07 (C -2), 126.71 (C-3′′), 129.32 (C-2′), 129.76 (C-3′), 132.06 (C-4′), 133.32 (C-3), 135.40 (C-2′′), 137.73 (C-8′′), 141.06 (C-1′), 184.03 (C-1); ESI-MS, MeOH (Positive): m/z 254 $[M+H]^+$, 276 $[M+Na]^+$, Negative: 252 $[M-H]^-$, $C_{15}H_{11}NOS$.

***Trans-1-indolyl-3-(benzodioxanyl)-2-propen-1-one* (2k)**

Light orange,90% yield; mp 154-155°C; IR ν^{max}(KBr): 3449 1580, 1251, 752 (NH), 1638 (chalcone C=O), 1509, 1439, 1291 (aromatics) cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 4.25 (2H, s, H-5′, H-6′), 6.88 (1H, d, J=8.4 Hz, H-3′), 7.21 (2H, m, H-5′′, H-6′′), 7.28 (1H, dd, J=8.4, 1.5 Hz, H-2′), 7.42 (1H, d, J=1.5 Hz, H-8′), 7.48 (1H, dd, J=6.6, 2.7 Hz, H-4′′), 7.52 (1H, d, J=15.3 Hz, H-2), 7.66 (1H, d, J=15.6 Hz, H-3), 8.33 (1H, dd, J=6.3, 2.4 Hz, H-7′′), 8.68 (1H, brs, H-2′′), 12.04 (1H, brs, NH);^{13}C NMR (75 MHz, DMSO-d_6): δ 64.86* (C-4′), 65.21* (C-5′), 122.66 (C-6′′, C-7′′), 123.26 (C-8′), 123.70 (C-2), 123.94 (C-5′′), 126.76 (C-3′′), 129.56 (C-1′), 135.34 (C-2′′), 137.71 (C-8′′), 140.28 (C-3), 144.45 (C-6′), 145.97 (C-3′), 184.69 (C-1) (*=interchangable);ESI-MS, MeOH (Positive): m/z 306 $[M+H]^+$, Negative: 304 $[M-H]^-$, $C_{19}H_{15}NO_3$.

Molecular modelling parameters and energy minimization

To find the possible interactions of indolyl chalcones analogues compounds **1b**, **1f** and **1g** with colon cancer target cyclin-dependent kinase2 (CDK2), we docked compounds at CDK2 binding site. Sybyl X 2.0 interfaced with Surflex–Dock module was used for molecular docking. Program automatically docks ligand into binding pocket of a target protein using protomol based algorithm and empirically produced scoring function. The X-ray crystallographic structures of CDK2 complex with ligand (PDB ID: 2R3J) (Yoon et al., 2013) was taken from the protein data bank and water molecules were removed, H atoms were added and side chains were fixed. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by Gasteiger-Huckel method. In reasonable binding pocket, all the compounds were docked into the binding pocket and 20 possible active docking conformations with different scores were obtained for each compound. During the docking process, all of the other parameters were assigned their default values (Yadav et al., 2014).

Screening through pharmacokinetic properties

During the process of drug discovery, most of drugs fail to cross the clinical trials because of poor pharmacokinetic properties (absorption, Distribution, metabolism, excre-tion, and toxicity) (Yadav et al., 2013). Some properties correlate well e.g., primary determinant of fractional absorption referred to as polar surface area (PSA) (cut-off ≤ 140Å^2) and low molecular weight for absorption). The compound distribution in the body depends on factors such as blood–brain barrier, permeability, the volume of distribution and plasma protein binding. The descriptors' values of 90% orally active compounds follows Lipinski's rule. The bioavailability of com-pounds was evaluated by topological polar surface area value. This descriptor

correlates well with passive molecular transport through membranes. The number of rotatable bonds is a topological parameter as a measure of molecular flexibility (cut-off ≤10) and oral bioavailability (Bhagat et al., 2013).

MTT anti-proliferative activity assay

In vitro anti-cancer activity of phytomolecules is done by using MTT assay. Cytotoxicity testing *in vitro* was done by the method of Woerdenbag et al. 1-2 x 10^4 cells/well were incubated in the 5% CO_2 incubator for 24 hours to enable them to adhere properly to the 96-well polystyrene microplate (Grenier, Germany). Test compounds dissolved in 100% DMSO (Merck, Germany) in at least five doses was added and left for 6 hours after which the compounds plus media was replaced with fresh media and the cells were incubated for another 48 hours in the CO_2 incubator at 37ºC. The concentration of DMSO used in our experiments never exceeded 1.25%, which was found to be non-toxic to cells. Then, 10 µL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma M 2128] was added, and plates were incubated at 37ºC for 4 hours. One hundred microlitres of dimethyl sulfoxide (DMSO, Merck, Germany) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few min at room temperature to ensure that all crystals were dissolved, the plates were read on a spectrofluorometer FLUO star Omega (BMG Labtech) at 570 nm. Plates were normally read within 1 hour of adding the DMSO. The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated as follows:

% inhibition = (1-OD at 570 nm of sample well/OD at 570 nm of control well) x 100

IC_{50} is the concentration mg/mL required for 50% inhibition of cell growth as compared to that of untreated control.

Results and Discussion

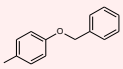
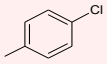
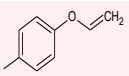
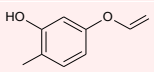
Chalcones are a major class of natural products and are considered as the precursors of flavonoids and isoflavonoids. Chemically, chalcones are 1,3-diaryl-2-propen-1-ones in which two aromatic rings are joined by a three carbon bridge having a carbonyl moiety and α,β unsaturation (Aggarwal et al., 2005). In this study both series of indolyl chalcones found in good yields, which are in line with aforementioned references. All these indolyl chalcones were assayed for their *in vitro* cytotoxicity against human colon (Caco-2) cancer cell lines. The IC_{50} values were used to determine the growth inhibition of these cancer cell lines. From the IC_{50} values summarized in Table I, the compounds **1b**, **1f** and **1g** have shown significant cytotoxicity. Furan moieties are common sub-structures in numerous natural products. Chalcone **1b** bearing benzofuran ring is most active in this series and selectively cytotoxic against colon cancer cell lines (Caco-2) with an IC_{50} value of 7.4 µg/mL whereas compound **1f** bearing benzyloxy group in the aromatic ring is as active as **1g**, IC_{50} 7.8 µg/mL against Caco-2 cancer cell line. Compound **1g** with o-hydroxy group is moderately cytotoxic against all the caco2 cell lines without any selectivity. Introduction of m-allylic group in the aryl ring i.e. compound **1h** is beneficial for the activity as compared to compound **1g**.

In other series, Compound **2i** has displayed significant cytotoxicity against Caco-2 with an IC_{50} value of 7.4 µg/mL. Indolyl chalcone **2c** with a 3,4,5-trimethoxy substituent and **2i** with 3-ethoxy-4-hydroxy substituent were moderately active and selective against caco-2 with an IC_{50} value of 7.4 µg/mL (Table II).

In the study, we explored the orientations and binding affinities (in terms of total score). The docking reliability was validated by using the known crystallized X-ray

Table I

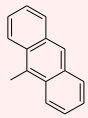
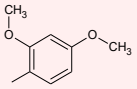
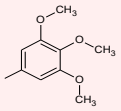
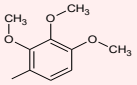
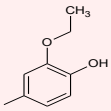
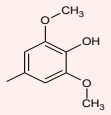
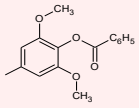
***In vitro* cytotoxicity data of indolyl chalcones (1a–j)**

Compounds	R	Caco-2 IC_{50} (µg/mL)
1a		96
1b		7.4
1c		100
1d		52
1e		NO
1f		7.8
1g		7.8
1h		8.7
1i		NO
1j		NO
Doxorubicin		3.5

Caco-2 = colon cancer; Doxorubicin (SigmaD-1515) is the standard used

Table II

***In vitro* cytotoxicity data of indolyl chalcones (2a–k)**

Compounds	R	Caco-2 IC_{50} (µg/mL)
2a		NO
2b		NO
2c		74
2d		NO
2e		88
2f		NO
2g		32
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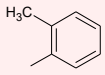
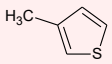
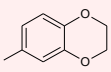
2i		7.4
2j		NO
2k		NO
Doxorubicin		3.5

Caco-2 = colon cancer; Doxorubicin (SigmaD-1515) is the standard used

structure of target protein LRH-1 complex with 3-bromo-5-phenyl-N-(pyridin-3-ylmethyl) pyrazolo [1,5-a]pyrimidin-7-amine. The co-crystallized structure was re-docked into the binding site and the docked conformation with the highest total score of 6.54 was selected as the most probable binding conformation. The low root mean-square deviation (RMSD) of 0.56 Å between the docked and the crystal conformations indicates the high reliability of Surflex-dock software in reproducing the experimentally observed binding mode for doxorubicin. Redocked molecules were almost in the same position with co-crystallized at the active site of paclitaxel. Crystallography data CDK2 showed that the amino acid Asp-86 is the "gatekeeper" residue, an important determinant of inhibiting in the CDK2 binding pocket.

The docking results as shown for compounds **1b**, **1f** and **1g** was docked at CDK2 binding pocket as shown docking score in the form of total score was i.e. 7.1740, 5.9001 and 6.3035 respectively. While, the docking scores of doxorubicin were 4.6772 only. The docked view of compounds **1b** and **1g** shows the formation of a hydrogen bond of length 2.0, 1.8 and 1.8Å to the polar hydrophobic residue Asp-86, Asp-145 and Asn-132. In docking pose, the conserved binding site pocket of amino acid residues within a selection radius of 3Å from bound ligand were hydrophobic residue Val-18 (valine), Phe-80, Phe-82 (phenylalanine), Asp-86, Asp-145 (aspartic acid), HIS-390 (histidine), Leu-83, Leu-134, (leucine), Ala-31, Ala-144 (alanine), Ile-10 (isoleucine), nucleophilic (polar, hydrophobic), i.e. Thr-14 (threonine) and polar amide, e.g. Gln-85, Gln-131 (glutamine) as a result as shown in Table III, bind compound showed a high interaction compare to with doxorubicin show more stability and activity in this compound. Overall, docking studies clearly indicates that compound **1b** and **1g** binds well (Figure 3) with CDK2 binding site and hence may exhibit similar inhibition effects on colon cancer receptor CDK2. These results were further substantiated by wet lab experiments.

In our study, the ADME (absorption, distribution, metabolism and excretion) parameters were calculated for the active chalcone derivatives namely, compounds **1b**, **1f** and **1g**. The values of these parameters also showed close correspondence with those of control compound doxorubicin and were within the standard range of values exhibited by 95% of all known drugs. Typically, low solubility is associated with bad absorption, so the general aim is to avoid poorly soluble compounds. The aqueous solubility (logS) of a compound significantly affects its absorption and distribution characteristics. The calculated logS values of the studied compounds were within the acceptable interval. Other calculations related to solubility, serum protein binding, the blood–brain barrier (log BB and apparent MDCK cell permeability), gut–blood barrier (Caco-2 cell permeability), predicted central nervous system activity, number of likely metabolic reactions, log IC_{50} for hERG K+ channel blockage, skin permeability (Kp), and human oral absorption in the gastrointestinal tract showed that these values for the active chalocone derivatives fell within the standard ranges generally observed for drugs (Table IV).

Toxicity screening results showed that compounds **1b**, **1f** and **1g** possess risk of mutagenicity toxicity, however indicate significant docking and experimental based anti-cancer activity (Table V). Thus, there is a need for

Table III

Comparison of binding affinity of standard drug (control) anti-cancer drugs and active chalcone derivative against colon cancer receptor (PDB ID:2R3J)

Compound name	Total score	Amino acid involved in active pocket in 3Å	Involved group of amino acid	Length of H-bond Å	Number of Hydrogen Bond
1b	7.2	Ile-10, Gly-11, Glu-12, Gly-13, Val-18, Ala-31, Phe-80, Phe-82, Leu-83, His-84, Gln-85, Asp-86, Lys-89, Lys-129, Gln-131, Asn-132, Leu-134, Ala-144	Asp-86	2.0	1
1f	5.9	Ile-10, Val-18, Ala-31, Lys-33, Val-64, Phe-80, Glu-81, Phe-82, Leu-83, His-84, Leu-134, Ala-144, Asp-145	-	-	-
1g	6.3	Ile-10, Glu-12, Gly-13, Thr-14, Val-18, Ala-31, Lys-33, Val-64, Phe-80, Glu-81, Phe-82, Leu-83, Lys-129, Asn-132, Leu-134, Ala-144, Asp-145	Asp-145 Asn-132	1.8 1.8	2
Doxorubicin	4.7	Ile-10, Val-18, Ala-31, Lys-33, Val-64, Phe-80, Phe-82, Leu-83, His-84, Gln-85, Asp-86, Lys-88, Lys-89, Gln-131, Leu-134, Ala-144	Lys-89 Asp-86 Leu-83	2.0 1.9 2.1	3

Surflex-Dock scores (total scores) were expressed in –log10(Kd) units to represent binding affinities

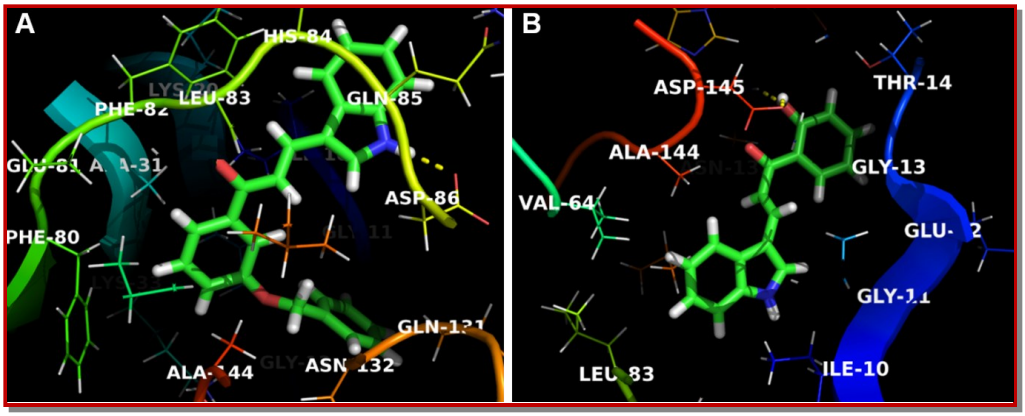


Figure 3: *In silico* molecular docking studies elucidating the possible mechanisms of compound 1b and 1g induced modulation of colon cancer protein (CDK2) receptor (PDB: 2R3J). The docking studies were carried out using SYBYL-X 2.0, Tripos International. Compound 1b and 1g docked on CDK2 form a H-bond of length 2.0 and 1.8Å to the binding pocket residue Asp-86, Asp-145 and Asn-132 and total score 7.1740 (A) and 6.3035 (B) was observed

more qualitative safety evaluation of chalcone. This is particularly important because of the fact that chalcone derivatives are used very frequently in clinical and non-clinical settings. The compliance of active chalcone derivatives namely, compounds **1b**, **1f** and **1g** with computational toxicity risks parameters indicate that these compounds are active and safe except mutagenicity toxicity risk at high doses or long term use similar to standard anti-cancer drugs namely doxorubicin. Therefore lead optimization of these active chalcone derivatives is a subject of further research work. Results of ADMET revealed that the overall drug scores of predicted active compounds are comparable to that of standard drugs and also established through *in vitro* experimental data (Table I) tested in colon (Caco-2) cancer cell line.

Conclusion

The indolyl chalcone analogues synthesized by various methods are potential candidates for further investigation towards the management of colon cancer.

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Conflict of Interest

Authors declare no conflict of interest

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Table IV

Compliance of active indolyl chalcones to computational parameters of pharmacokinetics (ADME)

Compounds	log S for aq. solubility	log Khsa, serum protein binding	log BB for brain/ blood	Number of metabolic reactions	Predicted CNS activity	log HERG for K+ Channel Blockage	Apparent Caco-2 permeability nm/sec	Apparent MDCK Permeability nm/sec	log Kp for skin permeability	% Human oral absorption in GI (+-20%)	Qual model for human oral absorption
1b	-5.293	0.825	-0.506	2	-1	-6.390	2157.263	1135.692	-0.365	100	High
1f	-4.432	-4.432	-0.410	1	-1	-6.179	1783.35	924.50	-1.001	100	High
1g	-3.688	0.303	-0.607	1	-1	-5.424	1136	568	-1.521	100	High
Doxorubicin	-2.162	-0.628	-2.633	9	-2	-5.916	3.982	1.394	-7.423	0	Low
Stand. Range*	(-6.5 / 0.5)	(-1.5 / 1.5)	(-3.0 / 1.2)	(1.0 / 8.0)	-2 inactive+2 active	concern below -5	<25 poor, >500 great	<25 poor, >500 great	-8 to -1, Kp in cm/hr	<25% is poor	>80% is high

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Table V

Compliance of active indolyl chalcones to computational toxicity risks parameters (i.e., mutagenicity, tumorogenicity, irritation and reproduction)

Compounds	Toxicity risk parameters			
Compounds	MUT	TUMO	IRRI	REP
1b	High risk	No risk	No risk	No risk
1f	High risk	No risk	No risk	No risk
1g	High risk	No risk	No risk	No risk
Doxorubicin	No risk	No risk	No risk	No risk

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