Molecular Modeling Based Synthesis and Evaluation of In vitro Anticancer Activity of Indolyl Chalcones

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Abstract: A series of twenty one chalcone derivatives having indole moiety were synthesized and were evaluated against four human cancer cell lines. Indolyl chalcones 1a, 1b, 1d, 1f-1j, 2c, 2e, 2i showed good anticancer activity. Chalcones 1b and 1d were the most active and selective anticancer agents with IC₅₀ values <1µg/ml and 1.51µg/ml, against WRL-68 cell line, respectively. Molecular mechanism was explored through in silico docking & ADMET studies.

Keywords: 1,3-Diaryl-2-propen-1-ones, Anticancer agents, Indolyl chalcones.

1. INTRODUCTION

Cancer, the uncontrolled growth of cells is a major cause of death worldwide and is responsible for 8.2 million deaths in 2012 which is around 13% of total deaths [1,2]. While great efforts have been made to tackle the disease over past few decades, it still continues to be a major health menace. Medicinal chemists are tirelessly exploring for a better and more suitable cancer therapeutic. Chalcones (1, 3-diaryl-2-propen-1-ones), constituting an enone system between two aromatic rings are an important class of natural products which exhibit interesting pharmacological activities [3,4]. Chalcones, originating from natural and synthetic routes possess several biological activities, such as cytotoxic [5-8] antimalarial [9,10], antileishmanial [11,12], anti-inflammatory [13,14], anti-HIV [15], antifungal [16] and as tyrosine kinase inhibitors [17]. 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 anticancer 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 [18].

Indoles, 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 to anticancer effectiveness have been reported in the literature [19-22]. Some of the individual anticancer compounds in which the indole ring is responsible for the activity are panobinostat [23,24], cediranib [25], indole-3-carbinol [26] (Fig. 1). Basically, indolyl chalcones are not much explored for their anticancer potential [27-29]. In the present study, we have synthesized two different series (Fig. 2) of novel indolyl chalcones 1a-j (Scheme 1) and 2a-k (Scheme 2) and evaluated their in vitro anticancer activity against four human cancer cell lines.

2. RESULT AND DISCUSSION

General methods for the preparation of chalcones involve Claisen-Schmidt condensation of appropriate aryl methyl ketones and aldehydes in presence of acid or base [30]. We have prepared both series of indolyl chalcones by using aforementioned reference conditions in good yields. Indolyl chalcones 1a-j were prepared by the reaction of appropriate acetoephene 4 with indol-3-carboxaldehyde 5 in the presence of NaOH at RT (Scheme 1) [30]. Indolyl chalcones 2a-k were prepared by the reaction of 3-acetylimidole 6 with appropriate aldehyde 7 in the presence of SOCl₂ (Scheme 2) [31]. Acetoephones (4h-4j) for the preperation of the compounds 1h-1j were prepared by etherification of α-hydroxy (3h), p- hydroxy (3i) and α, p-hydroxy (3j) acetophenones respectively with allylbromide in the presence of KBr in acetone using refluxing condition.

All these indolyl chalcones were assayed for their in vitro cytotoxicity against four human cancer cell lines: breast (MCF-7), liver (WRL-68), prostate (PC-3) and colon (Caco2). The IC₅₀ and IC₉₀ values were used to determine the growth inhibition of these cancer cell lines. From the IC₅₀ values summarized in Table 1, the compounds 1b and 1d have shown significant cytotoxicity. Furan moieties are present in many natural products [2]. Chalcone 1d bearing benzo-furan ring is most active in this series and selectively cytoxic against WRL-68 and MCF-7 cancer cell lines with an IC₅₀ value of <1µg/ml whereas compound 1b bearing...
benzyloxy group in the aromatic ring is as active as 1d, IC_{50} <1\mu g/ml against WRL-68 cancer cell line. Compound 1g with o-hydroxy group is moderately cytotoxic against all the four 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 WRL-68 with an IC_{50} values 1.5 \mu g/ml. Indolyl chalcone 2e with a 3, 4, 5-trimethoxy substituent and 2e with 3-ethoxy-4-hydroxy substituent were moderately active and selective against WRL-68 and MCF-7 respectively with an IC_{50} value of 10 \mu g/ml (Table 2).

2.1. Molecular Docking Studies

The molecular docking study was performed to elucidate whether the compounds 1b and 1d modulates the anticancer target, and also to identify the actual binding pocket against molecular target tubulin and LRH-1. We docked compound 1d at ‘tubulin binding site’ and compounds 1b and 1d also at ‘LRH-1 site’. The orientations and binding affinities were explored in terms of total score. Surflex-dock software was used in reproducing the experimentally was observed binding mode for paclitaxel. Crystallography data of LRH-1 showed that the amino acid ASP-389 is the "gatekeeper" residue, an important determinant of stabilizing specificity in the LRH-1 binding pocket.

The docking results for compound 1d and doxorubicin against target protein showed binding affinity, total docking score of 7.9867 (Fig. 3C) and 3.9428 (Fig. 3A). The amino acid residues within a selection radius of 4Å from bound compound 1d (ligand) were hydrophobic residue VAL-406 (Valine), PHE-342, ILE-378, ILE-387 (Isoleucine), MET-345, MET-348, MET-428 (Methione); nucleophilic (polar, hydrophobic) e.g., THR-352 (Threonine); basic ARG-393, (Arginine), GLN-432(Glutamine), HIS-390 (Histidine), CYS-346(cysteine); acidic (polar, negative charged) e.g., ASP-389, (Aspartic acid), Hydrophobic e.g., ALA-431, ALA-513, (Alanine), LEU-386, LEU-405, LEU-424, LEU-427, LEU-517 (Leucine), and aromatic (hydrophobic), i.e. TYR-516 (tyrosine), the bound compound 1d showed strong hydrophobic interactions with tubulin, thus leading to more stability and activity (Fig. 3A).
Table 1. *In vitro* cytotoxicity data of indolyl chalcones (1a-j).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>WRL-68 IC₅₀µg/ml</th>
<th>WRL-68 C₉₀µg/ml</th>
<th>PC-3 IC₅₀µg/ml</th>
<th>PC-3 C₉₀µg/ml</th>
<th>CaCO₂ IC₅₀µg/ml</th>
<th>CaCO₂ C₉₀µg/ml</th>
<th>MCF-7 IC₅₀µg/ml</th>
<th>MCF-7 C₉₀µg/ml</th>
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<td>NO</td>
<td>96</td>
<td>&gt;100</td>
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<tr>
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<td>47</td>
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<tr>
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<td>NO</td>
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<td>NO</td>
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</tr>
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<td>3.5</td>
<td>2.1</td>
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</table>

WRL-68=liver cancer cell line, CaCO₂=colon cancer, MCF-7=breast cancer, PC-3=prostate cancer. Doxorubicin SigmaD-1515 is the standard used.

hydrophobic residue ASP-389. The amino acid residues within a selection radius of 4Å from bound ligand were hydrophobic residue VAL-406 (Valine), PHE-342 (Phenylalanine), ASP-389 (Aspartic acid), HIS-390 (Histidine), LEU-386, LEU-424, LEU-427, LEU-405, LEU-517 (Leucine), ALA-431, ALA-513 (Alanine), ILE-387 (isoleucine), MET-345, MET-348, MET-428 (Methionine); nucleophilic (polar, hydrophobic), i.e. CYS-386 (cysteine), THR-352 (Threonine); basic ARG-393, (Arginine), aromatic (hydrophobic), i.e. TYR-516 (tyrosine) and polar amide, e.g. GLN-432 (glutamine), as a result bound compound showed high molecular interaction in compare to doxorubicin, which causes more stability and activity in this compound. Overall, docking studies clearly indicates that compound 1b and 1d binds well with tubulin and LRH-1 binding site and hence may exhibit similar inhibition effects on microtubules and LRH-1. These results were further substantiated by wet lab experiments.

2.2. Compliance with Pharmacokinetic Parameters Assessment

Pharmacokinetic parameters used for *in silico* screening of compounds 1b and 1d were absorption, distribution, metabolism and excretion (ADME) which showed close
Table 2. *In vitro* cytotoxicity data of indolyl chalcones (2a-k).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>WRL-68 IC₅₀μg/ml</th>
<th>WRL-68 IC₉₀μg/ml</th>
<th>PC-3 IC₅₀μg/ml</th>
<th>PC-3 IC₉₀μg/ml</th>
<th>CaCO-2 IC₅₀μg/ml</th>
<th>CaCO-2 IC₉₀μg/ml</th>
<th>MCF-7 IC₅₀μg/ml</th>
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<td>NO NO</td>
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<td>91 &gt;100</td>
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<tr>
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<td>Doxorubicin</td>
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<td>0.85 5.0</td>
<td>3.5 2.1</td>
<td>3.5 2.1</td>
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</tbody>
</table>

WRL-68=liver cancer cell line, CaCO2=colon cancer, MCF-7=breast cancer, PC-3=prostate cancer.
Doxorubicin SigmaD-1515 is the standard used.
correspondence with those of control compound doxorubicin and were within the standard range of values exhibited by 95% of all known drugs. Results revealed that chalcone derivatives have compliance with standard range of pharmacokinetics parameters (Table 3).

2.3. Compliance with Toxicity Risk Assessment Parameters

Results of toxicity risk assessment calculated through OSIRIS calculator. Toxicity screening results showed that both compounds 1b and 1d possess risk of mutagenicity toxicity, however indicate significant docking and experimental based anticancer activity (Table 4). Thus, there is a need for 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 and 1d 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 anticancer drug 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 in accordance with in vitro experimental data (Table 1) tested in MCF-7 and WRL-68 cancer cell line.

3. EXPERIMENTAL SECTION

Chemistry. General Methods. Melting point was determined on a Toshniwal melting point apparatus and is uncorrected. IR spectra were recorded on a Perkin Elmer 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 silica gel 60 F\textsubscript{254} plates (Merck) using solvent system, hexane: ethyl acetate (7:3). The compounds were visualized by either exposure of TLC plates to \(I_2\) vapors or by spraying with vanillin-sulfuric acid reagent, followed by heating at 110°C for 15 minutes. 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 (250x4.6mm i.d.,10\(\mu m\)) column using binary gradient elution with acetonitrile and water mobile phase (70:30) at a flow rate of 0.6mL/min, column...
temperature of 25° and UV detection at λ230 nm. The compounds were identified by their spectral IR, ID (H, 13C, DEPT) and 2D (COSY, HSQC, HMBC) ESIMS) NMR and ESIMS analysis.

3.1. Synthesis of Indolyl Chalcones, series 1

To a solution of indol-3-carboxaldehyde 5 (1 mmol) and appropriate acetophenone 4 (1 mmol) in anhydrous methanol (20 mL) was added 10%-40% sodium hydroxide (2 mL) and stirred the reaction mixture at RT for 2-8 h. 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 1a-j.

**Table 3.** Compliance of active chalcones to computational parameters of pharmacokinetics (ADME).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>log S for aq. Solubility</th>
<th>log Khsa, Serum Protein Binding</th>
<th>log BB for Brain/Blood</th>
<th>No. of Metabolic Reactions</th>
<th>Predicted CNS Activity</th>
<th>log HERG for K+ Channel Blockage</th>
<th>Apparent Caco-2 Permeability nm/sec</th>
<th>Apparent MDCK Permeability nm/sec</th>
<th>log Kp for Skin Permeability</th>
<th>% Human Oral Absorption in GI (+-20%)</th>
<th>Qual Model for Human Oral Absorption</th>
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</thead>
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<tr>
<td>1b</td>
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<td>-0.506</td>
<td>2</td>
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<td>-6.390</td>
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<td>-1</td>
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<td>(-1.5 / 1.5)</td>
<td>(-3.0 / 1.2)</td>
<td>(1.0 / 8.0)</td>
<td>-2 in-active</td>
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<td>&lt;25 poor,</td>
<td>&gt;500 great</td>
<td>8 to -1,</td>
<td>Kp in cm/hr</td>
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</table>

**Table 4.** Compliance of active chalcones to computational toxicity risks parameters (i.e., mutagenicity, tumorogenicity, irritation and reproduction).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mutagenicity</th>
<th>Tumorogenicity</th>
<th>Irritation (Skin)</th>
<th>Reproductive toxicity</th>
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<tr>
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**Trans-3-(1H-indol-3-yl)-1-(4'-fluoro-3'-methylyphenyl)-2-propen-1-one (1a):** Orange powder; 20% yield obtained and analysed by spectroscopic data as described by an earlier method [31b].

**Trans-3-(1H-indol-3-yl)-1-(4'-benzoyloxyphenyl)-2-propen-1-one (1b):** Yellow solid; 60% yield, obtained and analysed by spectroscopic data as described by an earlier method [31b].

**Trans-3-(1H-indol-3-yl)-1-(3'-anthracenyl)-2-propen-1-one (1c):** Yellow powder; 80% yield; mp 108-109°C; IR v_{max} (KBr): 3395, 1561, 1164, 746 (NH), 1649 (chalcone C=O), 1515, 1483, 1430 (aromatics) cm^{-1}; 1H 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); 13C 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 (C-7'), 121.89 (C-2', C-6'), 123.15 (C-6''), 123.48 (C-5'', C-10''), 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-7'', C-9''), 155.91 (C-2'', C-14''), 179.72 (C-1) (a,b,c=interchangeable); ESIS-MS, MeOH (Positive): m/z 348 [M+H]^+, 346[M-H]^-, C_{25}H_{36}NO.

**Trans-3-(1H-indol-3-yl)-1-(benzofuran)-2-propen-1-one (1d):** Dark brown solid; 40% yield obtained and analysed by spectroscopic data as described by an earlier method [31b].

**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 [29].

**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 [29].

**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 [32].

**Trans-3-(1H-indol-3-yl)-1-(3’-allyloxophenyl)-2-propen-1-one (1h):** Light brown powder; 65% yield; mp 90-92°C; IR v_{max} (KBr): 3389 1564, 1211, 739 (NH), 1653 (chalcone C=O), 1523, 1458, 1420 (aromatics) cm^{-1}; 1H 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, H-9''), 5.40 (1H, dd, J=17.4, 1.5 Hz,
Hb-9''), 6.05 (1H, m, H-8'), 7.24 (2H, 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'), 118.01 (C-3'), 118.82 (C-4'A), 121.09 (C-7'), 121.59 (C-6'), 122.07 (C-3'), 126.05 (C-3'), 130.72 (C-5'), 134.10 (C-2''), 134.98 (C-8'), 138.84 (C-14'), 140.08 (C-3'), 159.25 (C-3''), 189.60 (C-1); ESI-MS, MeOH (Positive): m/z 304[M+H]^+. Negative: 302[M-H]^-, C_{26}H_{17}NO_3.

Trans-3-(1H-indol-3-yl)-1-(4'-allyloxyphenyl)-2-propen-1-one (1H): Light brown powder; 30% yield; mp 110-111°C; IR v\text{max} (KBr): 3372 1561, 1205, 736 (NH); 1651 (chalcone C=O). 1511, 1470, 1406 (aromatics) cm\(^{-1}\); H NMR (300 MHz, DMSO-d_6): δ 4.65 (2H, d, J=5.0 Hz, H-7'), 5.28 (1H, d, J=10.5 Hz, H-5'), 5.42 (1H, d, J=17.21 Hz, H-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'), 7.53 (1H, m, H-6'), 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-6''), 9.11 (1H, brs, NH); 13^C NMR (75 MHz, DMSO-d_6): δ 78.86 (C-6'), 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'), 125.65 (C-3'), 130.85 (C-6'), 131.80 (C-1'), 130.85 (C-2'), 133.37 (C-2''), 133.65 (C-6''), 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_{26}H_{17}NO_3.

3.2. Synthesis of Indolyl Chalcones 2a-k

To a solution of 3-acetylindole 6 (1 mmol) and appropriate aldehyde 7 (1 mmol) in methanol (20 mL) was added SOCl\(_2\) (2 mL) and stirred the reaction mixture for 2 h. 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 2a-a.

Trans-1-indolyl-3-(anthracenyl)-2-propen-1-one (2a): Yellow powder; 70% yield; mp 190-191°C; IR v\text{max} (KBr): 3398, 1570, 728 (NH), 1639 (chalcone C=O), 1518, 1442, 1419, 1381 (aromatics) cm\(^{-1}\); H NMR (300 MHz, pyridine-d_6): 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-5); 8.63 (1H, d, J=7.8 Hz, H-7''), 12.81 (1H, brs, NH); 13^C NMR (75 MHz, pyridine-d_6): 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), 124.06 (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''), 133.49 (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_{28}H_{17}NO_3.

Trans-1-indolyl-3-(2',4'-dimethoxyphenyl)-2-propen-1-one (2b): Creamy white crystals; 10% yield, obtained and analysed by spectroscopic data as described by an earlier method [29].
by spectroscopic data as described by an earlier method [31b].

Trans-1-indolyl-3-(3′,5′-dimethoxy-4′-benzoxoyphenyl)-2-propen-1-one (2g): Creamish crystals; 70% yield; mp 209-210°C; IR νmax (KBr): 3440, 1576, 1203, 739 (NH), 1655 (chalcone C=O), 1742 (ester CO), 1512, 1466, 1426 (aromatics) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆): 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); 13C NMR (75 MHz, DMSO-d₆): δ 106.79 (C-2’’, C-6’’), 113.12 (C-4’’), 118.67 (C-1’’), 122.77 (C-6’’), 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], C₁₈H₁₂NO₅.

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 [33].

Trans-1-indolyl-3-(2′-methylphenyl)-2-propen-1-one (2i): Obtained as brown solid; 80% yield, obtained and analysed by spectroscopic data as described by an earlier method [31b].

Trans-1-indolyl-3-(thiophenyl)-2-propen-1-one (2j): Creamish white powder; 70% yield, obtained and analysed by spectroscopic data as described by an earlier method [31b].

Trans-1-indolyl-3-(benzodioxanyl)-2-propen-1-one (2k): Light orange, 90% yield; mp 154-155°C; IR νmax (KBr): 3449, 1580, 1251, 752 (NH), 1638 (chalcone C=O), 1509, 1439, 1291 (aromatics) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆); δ 4.25 (2H, s, H-1’), 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); 13C NMR (75 MHz, DMSO-d₆); 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) (*=interchangeable); ESI-MS, MeOH (Positive): m/z 306 [M+H]⁺, Negative: 304 [M-H], C₁₀H₈N₂O₃.

3.3. Biology

3.3.1. 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. 1-2X 10⁵ cells/well incubated in the 5% CO₂ incubator for 24 h to enable them to adhere properly to the 96-well polystyrene microplate (Greiner, Germany). Test compound dissolved in 100% DMSO (Merck, Germany) in at least five doses was added and left for 6 h after which the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO₂ incubator at 37°C. The concentration of DMSO used in our experiments never exceeded 1.25%, which was found to be non-toxic to cells. Then, 10 ul MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide; Sigma M 2128] was added, and plates were incubated at 37°C for 4 h. 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 minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a spectrofluorometer FLUOstar Omega (BMG Labtech) at 570 nm. Plates were normally read within 1 h of adding the DMSO. The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated as follows:

\[
\text{%inhibition} = \frac{(1-OD) \times 100}{OD\text{ of sample well}}
\]

IC₅₀ is the concentration mg/mL required for 50% inhibition of cell growth as compared to that of untreated control.

IC₉₀ is the concentration lg/mL required for 90% inhibition of cell growth as compared to that of untreated control.

3.4. Molecular Modeling Parameters and Energy Minimization

To find the possible interactions of indolyl chalcones derivatives compound 1b and 1d with microtubule and liver receptor homolog-1 (LRH-1), we docked compound at ‘microtubule binding site’ and also at LRH-1 site. Sybyl X 2.0 interfaced with Sulfex-Dock module was used for molecular docking. The X-ray crystallographic structures of tubulin complex with ligand [PDB: 1SA0] [34] and LRH-1 [PDB: 3PLZ] [35] were taken from the protein data bank (PDB) and modified for docking calculations. Co-crystallized ligand was removed from the structure, water molecules were removed, H atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by Gasteiger-Huckel method [36-41].

3.5. Screening Through Pharmacokinetic Properties

In discovery process most of drugs fail to cross clinical trials because of poor pharmacokinetics. Thus, screening through pharmacokinetic properties was done according to our previous published papers [36-44] (Schrödinger, USA, 2011).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES


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