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Synthesis of dioxolylethan-1-one-containing isatin-based chalcone derivatives and their antibacterial activity

Saud Nusrat Ali[1], Chandra Shekhar Yadav[2], Mohd Arsh Khan[1], Azhar Kamal[3], Abdul Rahman Khan[1], Iqbal Azad[1], Sabahat Yasmeen Sheikh[1], Firoj Hassan[1*]

[1]Department of Chemistry, Integral University, Lucknow-226026, India
[2]Department of Laboratory Animal Facility, CSIR-CDRI, Lucknow, India
[3]Department of Bioengineering, Integral University, Lucknow-226026, India

***Corresponding author:** Firoj Hassan
*Email: firoz@iul.ac.in

Abstract
Isatin-based chalcone derivatives (**3a-b**) have garnered significant attention due to their versatile biological activities and potential therapeutic applications. Our research goes to the development of new small molecules of isatin-containing druglikeness using the Claisen Schmidt reaction. Actually, analytical techniques like FT-IR, ^{1}H, ^{13}C NMR, and HRMS were deployed for the reporting to successfully characterize. As a preliminary investigation, synthesized compounds were passed through the computed strategy to find out the druglikeness properties. Further, the *in-vitro* analysis was conducted for synthesized compounds against representative bacteria *B. pumilis* (MTCC 160), *B. cerius (*MTCC1305), *E. coli* (ATCC 25923) and *K. pneumoniae* (NCTC418) obtained from NCIM, Pune (INDIA). n this study, the synthesized compound 3a, showed more significant activity against E. coli (ATCC 25923) at 93.5 µg/ml .While other strains like *B. cerius* (MTCC1305), *Klebsiella pneumonia* (NCTC418) and *B. Pumilis* (MTCC 160) at 187.2µg/ml, 156.5µg/ml and 156.25 µg/ml. This study emphasizes the relevance of combining synthetic chemistry and computational approaches to speed up drug development procedures using isatin-based chalcone derivatives.

Keywords: Anti-Bacterial, Claisen-Schmidt, eco-friendly, green chemistry, chalcone, computational.

Introduction

Isatin chalcone derivatives have gained attention in cancer research due to their potential as anticancer agents [1]. These compounds combine the structural features of both isatin and chalcone, offering a unique molecular framework with diverse pharmacological properties [2]. Here's an overview of isatin chalcone derivatives and their role in cancer therapy. Isatin chalcone derivatives have demonstrated the ability to induce apoptosis, or programmed cell death, in cancer cells [3, 48, 49]. They can activate intrinsic apoptotic pathways by modulating Bcl-2 family proteins, mitochondrial function, and caspase activation. This leads to the selective elimination of cancer cells while sparing normal cells [4, 50, 51]. Isatin chalcone derivatives exert inhibitory effects on cancer cell proliferation by interfering with cell cycle progression (**Fig. 1**).

Isatin-based chalcone derivatives have emerged as an intriguing class of compounds in the field of medicinal chemistry due to their broad spectrum of biological activities owing to isatin having >CO-NH- functionality, therefore it showed notably activity against various carcinomas including antimicrobial, anticancer, anti-inflammatory, and antioxidant properties [5]. The isatin scaffold, coupled with the chalcone moiety, offers a versatile framework for structural modifications, enabling the design of molecules with enhanced pharmacological profiles [6]. In recent years, advancements in synthetic methodologies have facilitated the efficient preparation of these derivatives, employing techniques such as green chemistry approaches, microwave-assisted synthesis, and the use of eco-friendly catalysts [7]. These innovations not only enhance the yield and selectivity of the reactions but also align with the principles of sustainable chemistry. Complementing experimental methods, in-silico tools have become indispensable in evaluating the pharmacokinetics, molecular docking, and target interactions of these derivatives [8]. Computational studies provide critical insights into the structural and functional aspects of isatin-based chalcones, enabling the prediction of their biological efficacy and guiding the optimization of lead compounds for drug development [9].

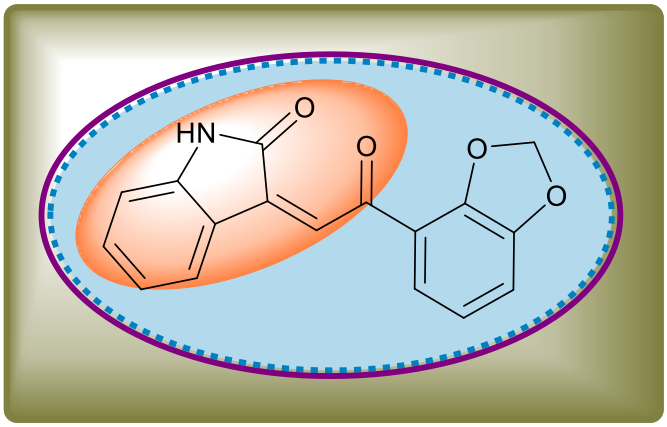


Figure 1. A general structure of Isatin chalcone derivative

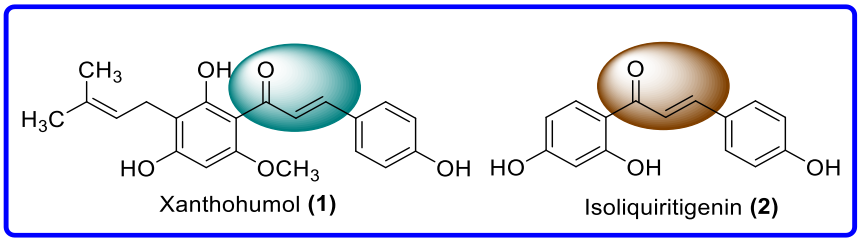


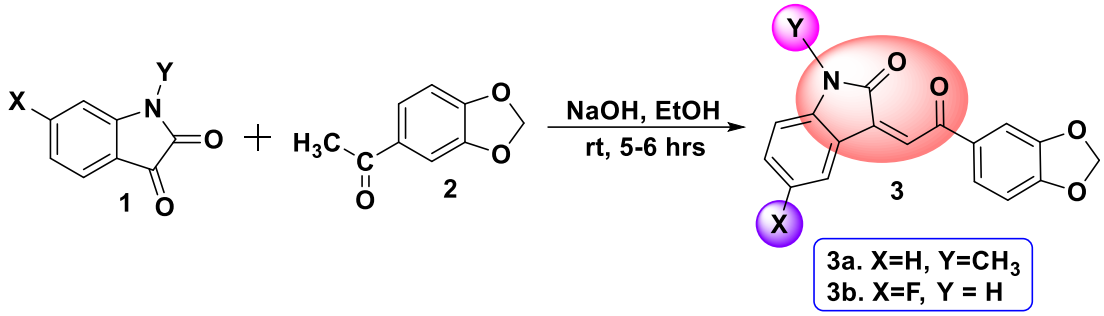
Figure 2. Molecular Structure of Xanthohumol and Isoliquiritigenin

Isatin chalcone derivatives have exhibited selective cytotoxicity towards cancer cells while showing low toxicity towards normal cells. This selectivity may be attributed to differences in the expression of drug targets or metabolic pathways between cancer cells and normal cells, making them promising candidates for cancer therapy with reduced side effects [10]. Isatin chalcone derivatives can synergize with conventional chemotherapeutic agents to enhance their anticancer efficacy. Combinatorial treatments involving isatin chalcone derivatives and standard chemotherapeutic drugs have been explored to overcome drug resistance and improve treatment outcomes in various cancer types [11]. Isatin chalcone derivatives have demonstrated promising anticancer properties by inhibiting cancer cell proliferation, inducing apoptosis, and suppressing tumor growth [12]. They exert their effects through various mechanisms, including modulation of cell cycle progression, inhibition of oncogenic signalling pathways, and induction of oxidative stress-mediated cell death [13] (**Figure 2**). The World Health Organization (WHO) does not specifically endorse individual compounds or derivatives but supports the development of new and effective cancer treatments [14]. Research on isatin-chalcone derivatives aligns with the WHO's goals of advancing cancer research and improving treatment options for cancer patients worldwide [15].

Experimental

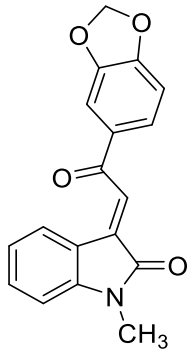
Material and methods

All solvents and chemical reagents were purchased from companies and weren't further purified while being used. Actually, analytical techniques like FT-IR, ^{1}H, ^{13}C NMR, and HRMS were deployed for the reporting to successfully characterize [16]. The sample was dissolved in $CDCl_3$ and DMSO-d6 at a concentration of about 15 mg. The coupling constant, J, and the chemical shift values were calculated in parts per million (ppm) and δ-scale, respectively, and their multiplicities were expressed as m = multiplet, q = quartet, t = triplet, dd = double doublet, d = doublet, and s = singlet. Mass spectrometer was used to record the ESI-MS spectra. The melting points of the synthesized compounds were ascertained using the Stuart SMP10 melting point apparatus. Utilizing a PerkinElmer Nicolet 6700 FTIR spectrometer with attenuated total reflection in the frequency range of 150-750 cm^{-1}, Fourier transform infrared spectroscopy (FTIR) analysis was performed (**Scheme 1**).



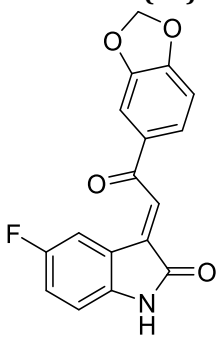
Scheme 1. Synthesis of Isatin chalcone derivatives **(3a-b)**

Spectral characterization of Isatin chalcone derivatives (3a)



Light white solid, yield 76.%, mp149°C, FTIR (KBr) νmax: 2920(C-H aromatic), 1397(C-H), 1613(-C=C- aromatic), 1441(C=C), 1708(C=O), 1091(C-O) 3360(N-H), 1250(C-N) cm^{-1}; 1*H*-NMR (300 MHz, $CDCl_3$) *δH*: 2.17(1H,s), 3.42(1H,s), 3.67(1H,s), 6.82(1H, d, *J* = 8.1Hz), 6.87(1H, d, *J* = 7.8Hz), 7.01-7.06(2H, m), 7.29-7.35(2H, m), 7.37(1H,d, *J*= 1.6Hz), 7.39(1H,d, *J*= 0.6Hz), 7.41(1H,d, *J*= 0.75Hz), 7.49(1H,dd, *J*= 1.7, 8.1Hz); 13*C*-NMR (300 MHz, $CDCl_3$) *δc*: 26.5, 44.0, 74.8, 102.2, 107.9, 108.1, 108.7, 123.2, 124.2, 125.1, 130.1, 130.3, 131.3, 143.7, 148.7, 152.6, 176.3, 196.8; ES-MS (*m/z*): 308 [M+1]+, calculated for $C_{18}H_{13}NO_4$.

Spectral characterization of Isatin chalcone derivatives (3b)



Light brown solid, yield 79%, mp211°C, FTIR (KBr) νmax: 2912(C-H aromatic), 1403(C-H), 1614(-C=C-aromatic), 1434(C=C), 1732(C=O), 1088(C-O), 3230(N-H), 1254(C-N), 1041(C-F)cm^{-1}; 1*H*-NMR (300 MHz, $CDCl_3$) *δH*: 1.60(1H,s), 7.47(1H,s), 7.50(1H,s), 7.59(1H,d, *J* = 2.1Hz), 7.61(1H,d, *J* = 2.0Hz), 7.64(1H,d, *J* = 1.9Hz), 7.66(1H,d, *J* = 2.0Hz), 8.02(1H, dd, *J* = 2.0, 3.8Hz), 8.04(1H, s); 13*C*-NMR (300 MHz, $CDCl_3$) *δc*: 26.9, 122.8, 127.2, 129.0, 129.2, 132.3, 138.9, 144.7, 197.8 ;ES-MS (*m/z*): 312 [M+1]+, calculated for $C_{17}H_{10}FNO_4$.

Antibacterial Activity

Determination of Minimum Inhibitory Concentration

The antimicrobial potentiality of the above-mentioned synthesized compounds were determined by standard agar disc diffusion technique and minimal inhibitory concentration (MIC) in accordance with the Clinical and Laboratory Standards Institute (CLSI,2015) against the tested microorganisms. The disk diffusion method [22] was used for the preliminary antibacterial evaluation of compounds **3a** and **3b**. The minimum inhibitory concentrations (MIC) of these derivatives, showing inhibition in the preliminary tests, were determined by the microtitre plate technique using micro dilution method [23]. Briefly, *Bacillus pumilis (MTCC 160)*,

Bacillus cerius (MTCC1305), *Escherichia coli (ATCC 25923)* and *Klebsiella pneumoniae* (NCTC418) which were obtained from NCIM Pune, India. These srains were grown to mid-logarithmic phase and harvested by centrifugation, washed with 10 mM sodium phosphate buffer (SPB) at pH 7.4 and diluted to 2 x 105 colony forming units (CFU) / ml in SPB containing 0.03% nutrient broth (NB). Synthesized compounds were serially diluted in 100 µl of nutrient broth (NB) medium in 96-well microtitre plates to achieve the desired concentrations (62.50-250µg/ml) with bacterial inoculum (5x104 CFU per well). After incubation at 37ºC overnight, the MIC was taken as the lowest synthesized compounds concentration at which growth was inhibited.

MIC=(Lowest concentration inhibit growth+ Highest concentration allow growth) /2

Control

Gentamycin was used as standard antibacterial control and DMSO used as negative control for comparison of results under identical condition.

Determination of Minimum Bactericidal Concentration (MBC µg/ml)

For the determination of MBC (Table-2) the following strain were assayed: ***B. pumilis*** *(MTCC 160) 156.25*µg/ml**,** ***B. cerius*** *(MTCC1305)* 187.5µg/ml, ***E. coli*** *(ATCC 25923)* 93.75µg/ml, *Klebsiella pneumonia* (NCTC418) 93.64µg/ml in case of 3a and 3b ***B. pumilis*** *(MTCC 160) 187.5.*µg/ml**,** ***B. cerius*** *(MTCC1305)* 156.25 µg/ml, ***E. coli*** *(ATCC 25923)* 156.25 µg/ml and *Klebsiella pneumonia* (NCTC418) 156.25µg/ml respectively.

After having determined the MIC of bacteria from the wells of the microtitre plate with no visible growth, samples were removed for serial sub cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubated for 24 and 48 h respectively. The lowest concentration with no visible growth was defined as MBC, indicating 99.97% killing of the original inoculum. The optical density of each well was measured at a wavelength of 595 nm on ELISA Reader (BIORAD United states of America) and compared with a blank. Solvent [di-methyl sulphoxide (DMSO)] was used as a negative control. Two replicates were done for each compound and each experiment was repeated thrice.

Results and discussion

Chemistry

The synthesis of new compounds of isatin-containing molecules (**3a-b**) was reported in favour of valuable finding pure yields. For that, isatin **1** and 1-(benzo[d][1,3]dioxol-5-yl)ethan-1-one **2** reactants were used in ethanol solvent for 5 to 6 hours in the presence of ambient temperature. Such formation as C-C bond development among them successfully conducted under Claisen-Schmidt reaction. Generally, the reactions are quite fast and afford the desired Claisen-Schmidt condensation products with moderate to good yields; thus, it provides a convenient and efficient approach for the synthesis of allylic alcohols under mild conditions [20].

In silico drug likeness and ADMET study

In silico calculations of the synthesized derivatives 3a and 3b were done against the standard drugs Isoliquiritigenin and Xanthohumol by the freely available online web-tools like AdmetLab3.0 [24-47] and AdmetSAR3.0 (https:// lmmd.ecust. edu.cn/ admetsar3/predict.php).

Physicochemical Properties

Physicochemical properties of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Molecular Weight, Volume, Density, nHA, nHD, nRot, nRing, MaxRing, nHet, fChar, nRig, Flexibility, Stereo Centers, TPSA, logS, logP, logD7.4, pka (Acid), pka (Base), Melting point and Boiling point.

Table 1. Physico chemical properties

Parameters	3b	3a	Isoliquiritigenin	Xanthohumol
Molecular Weight (MW)	311.06	307.08	256.07	354.15
Volume	296.859	308.088	264.953	374.882
Density	1.048	0.997	0.966	0.945
nHA	5.0	5.0	4.0	5.0
nHD	1.0	0.0	3.0	3.0
nRot	2.0	2.0	3.0	6.0
nRing	4.0	4.0	2.0	2.0
MaxRing	9.0	9.0	6.0	6.0
nHet	6.0	5.0	4.0	5.0
fChar	0.0	0.0	0.0	0.0
nRig	23.0	23.0	14.0	15.0
Flexibility	0.087	0.087	0.214	0.4
Stereo Centers	0.0	0.0	0.0	0.0

TPSA	64.63	55.84	77.76	86.99
logS	-4.442	-3.704	-3.457	-4.568
logP	2.497	2.385	2.521	3.829
logD7.4	2.543	2.615	2.502	3.445
pka (Acid)	8.739	7.603	8.102	9.355
pka (Base)	3.15	3.614	5.562	5.756
Melting point	213.935	151.59	221.077	165.143
Boiling point	348.608	323.259	361.376	347.523

Absorption

Absorption properties of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Caco-2 Permeability, MDCK Permeability, PAMPA, Pgp inhibitor, Pgp substrate and HIA were analyed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table 2. Details of druglikeness of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
Caco-2 Permeability	-4.885	-4.855	-4.839	-5.056
MDCK Permeability	-4.581	0.0	-4.798	0.0
PAMPA	---	---	--	--
Pgp inhibitor	+++	+++	--	+
Pgp substrate	---	---	---	---
HIA	---	---	---	---

Distribution

Distribution properties of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like PPB, VDss, BBB, Fu, OATP1B1 inhibitor, OATP1B3 inhibitor, BCRP inhibitor, MRP1 inhibitor and BSEP inhibitor were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table 3. Details of inhibitory effect of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
PPB	95.7%	94.7%	96.1%	95.6%
VDss	0.168	1.431	-0.344	1.7
BBB	+	---	---	---
Fu	4.9%	5.2%	5.0%	4.6%
OATP1B1 inhibitor	+++	++	+++	+++
OATP1B3 inhibitor	+++	+++	+++	+++
BCRP inhibitor	---	---	+	+
MRP1 inhibitor	---	---	---	---
BSEP inhibitor	+++	+++	++	+++

Metabolism

Metabolism properties of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like inhibitor and substrate of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP2B6, CYP2C8 and HLM Stability were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table 4. Details of toxicity of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
CYP1A2 inhibitor	+++	+++	+++	+++
CYP1A2 substrate	--	+	--	---
CYP2C19 inhibitor	+++	+++	+++	+++
CYP2C19 substrate	---	---	---	---
CYP2C9 inhibitor	+++	+++	+++	+++
CYP2C9 substrate	---	---	+++	++
CYP2D6 inhibitor	+++	+++	+++	+++
CYP2D6 substrate	--	---	+++	+++
CYP3A4 inhibitor	+++	+++	+++	+++
CYP3A4 substrate	---	---	---	---
CYP2B6 inhibitor	+++	+++	+++	+++
CYP2B6 substrate	---	---	---	---
CYP2C8 inhibitor	+++	+++	+++	+++
HLM Stability	-	++	--	+++

Medicinal Chemistry

Metabolism properties of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like QED, SAscore, GASA, Fsp^3, MCE-18, NPscore, Lipinski Rule, Pfizer Rule, GSK Rule, GoldenTriangle, PAINS, Alarm_NMR Rule, BMS Rule, Chelating Rule, Colloidal aggregators, FLuc inhibitors, Blue fluorescence and Green fluorescence were analysed.

Table 5. Details of druglikeness effects of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
QED	0.684	0.632	0.583	0.411
SAscore	Easy	Easy	Easy	Easy
GASA	Easy	Easy	Easy	Easy
Fsp^3	0.059	0.111	0.0	0.19
MCE-18	44.333	44.1	12.0	15.0
NPscore	-0.637	-0.423	0.7	1.633
Lipinski Rule	Accepted	Accepted	Accepted	Accepted
Pfizer Rule	Accepted	Accepted	Accepted	Accepted
GSK Rule	Accepted	Accepted	Accepted	Accepted
GoldenTriangle	Accepted	Accepted	Accepted	Accepted
PAINS	0	0	0	0
Alarm_NMR Rule	2	2	3	3
BMS Rule	0	0	0	0
Chelating Rule	0	0	0	0
Colloidal aggregators	0.813	0.36	0.923	0.987
FLuc inhibitors	0.99	0.998	1.0	0.998
Blue fluorescence	0.506	0.572	0.485	0.593
Green fluorescence	0.63	0.426	0.387	0.836
Reactive compounds	0.004	0.045	0.988	0.583
Promiscuous compounds	0.329	0.224	0.583	0.203

Toxicophore Rules

Toxicophore rules of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like Aquatic Toxicity Rule, Genotoxic Carcinogenicity Mutagenicity Rule, NonGenotoxic Carcinogenicity Rule, Skin Sensitization Rule, Acute Toxicity Rule, NonBiodegradable, SureChEMBL Rule and FAF-Drugs4 Rule were analysed.

Table 6. Details of toxicophore effects of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
Aquatic Toxicity Rule	4	3	2	2
Genotoxic Carcinogenicity Mutagenicity Rule	2	2	1	1
NonGenotoxic Carcinogenicity Rule	2	1	1	1
Skin Sensitization Rule	6	3	5	6
Acute Toxicity Rule	0	0	0	0
NonBiodegradable	1	0	1	1
SureChEMBL Rule	0	0	0	1
FAF-Drugs4 Rule	3	3	2	2

Toxicity

Toxicity parameters of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like hERG Blockers, Carcinogenicity, hERG Blockers (10 um), Skin Sensitization, DILI, AMES Toxicity, Rat Oral Acute Toxicity, FDAMDD, Eye Corrosion, Eye Irritation, Drug-induced Nephrotoxicity, Respiratory, Human Hepatotoxicity, Drug-induced Neurotoxicity, Ototoxicity, Hematotoxicity, Genotoxicity, RPMI-8226 Immunitoxicity, RPMI-8226 Immunitoxicity, A549 Cytotoxicity, Hek293 Cytotoxicity, BCF, IGC50, LC50DM and LC50FM were analysed.

Table 7. Details of toxic nature of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
hERG Blockers	0.247	0.218	0.187	0.129
hERG Blockers (10um)	0.438	0.461	0.494	0.577
DILI	0.953	0.854	0.238	0.414
AMES Toxicity	0.615	0.625	0.29	0.547
Rat Oral Acute Toxicity	0.58	0.461	0.105	0.427
FDAMDD	0.792	0.583	0.714	0.587
Skin Sensitization	0.103	0.306	0.857	0.944
Carcinogenicity	0.746	0.821	0.196	0.248
Eye Corrosion	0.002	0.012	0.146	0.002
Eye Irritation	0.825	0.777	0.998	0.923
Respiratory	0.499	0.593	0.618	0.923
Human Hepatotoxicity	0.829	0.675	0.491	0.628
Drug-induced Nephrotoxicity	0.899	0.642	0.118	0.485
Drug-induced Neurotoxicity	0.886	0.822	0.506	0.605
Ototoxicity	0.385	0.309	0.109	0.339
Hematotoxicity	0.658	0.595	0.034	0.138
Genotoxicity	0.997	0.979	0.81	0.881
RPMI-8226 Immunitoxicity	0.099	0.116	0.051	0.094
A549 Cytotoxicity	0.157	0.149	0.239	0.404
Hek293 Cytotoxicity	0.512	0.522	0.837	0.783
BCF	1.377	1.279	1.31	1.642
IGC50	3.955	4.35	4.16	4.608
LC50DM	5.599	5.653	4.677	6.001
LC50FM	4.99	5.282	4.469	5.466

TOX21 Pathway

Tox21 Pathway parameters of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetLab3.0. Parameters like NR-AhR, NR-AR, NR-AR-LBD, NR-Aromatase, NR-ER, NR-ER-LBD, NR-PPAR-gamma, SR-ARE, SR-ATAD5, SR-HSE, SR-MMP and SR-p53 were analysed. The following range of values are represented by the symbols i.e. 0-0.1 (---), 0.1-0.3 (--), 0.3-0.5 (-), 0.5-0.7 (+), 0.7-0.9 (++), and 0.9-1.0 (+++).

Table 8. Details of tox21 pathway nature of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
NR-AhR	++	++	++	+++
NR-AR	---	---	---	---
NR-AR-LBD	++	+	+	+
NR-Aromatase	-	-	-	++
NR-ER	---	--	+++	++
NR-ER-LBD	---	---	+++	+
NR-PPAR-gamma	---	--	+	+++
SR-ARE	+++	+++	+++	+++
SR-ATAD5	-	+	+	-
SR-HSE	+++	+++	+++	+++
SR-MMP	+++	++	+++	+++
SR-p53	+++	+++	+++	+++

Radar View

Radar View of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were generated by the freely available online web-tool AdmetLab3.0.

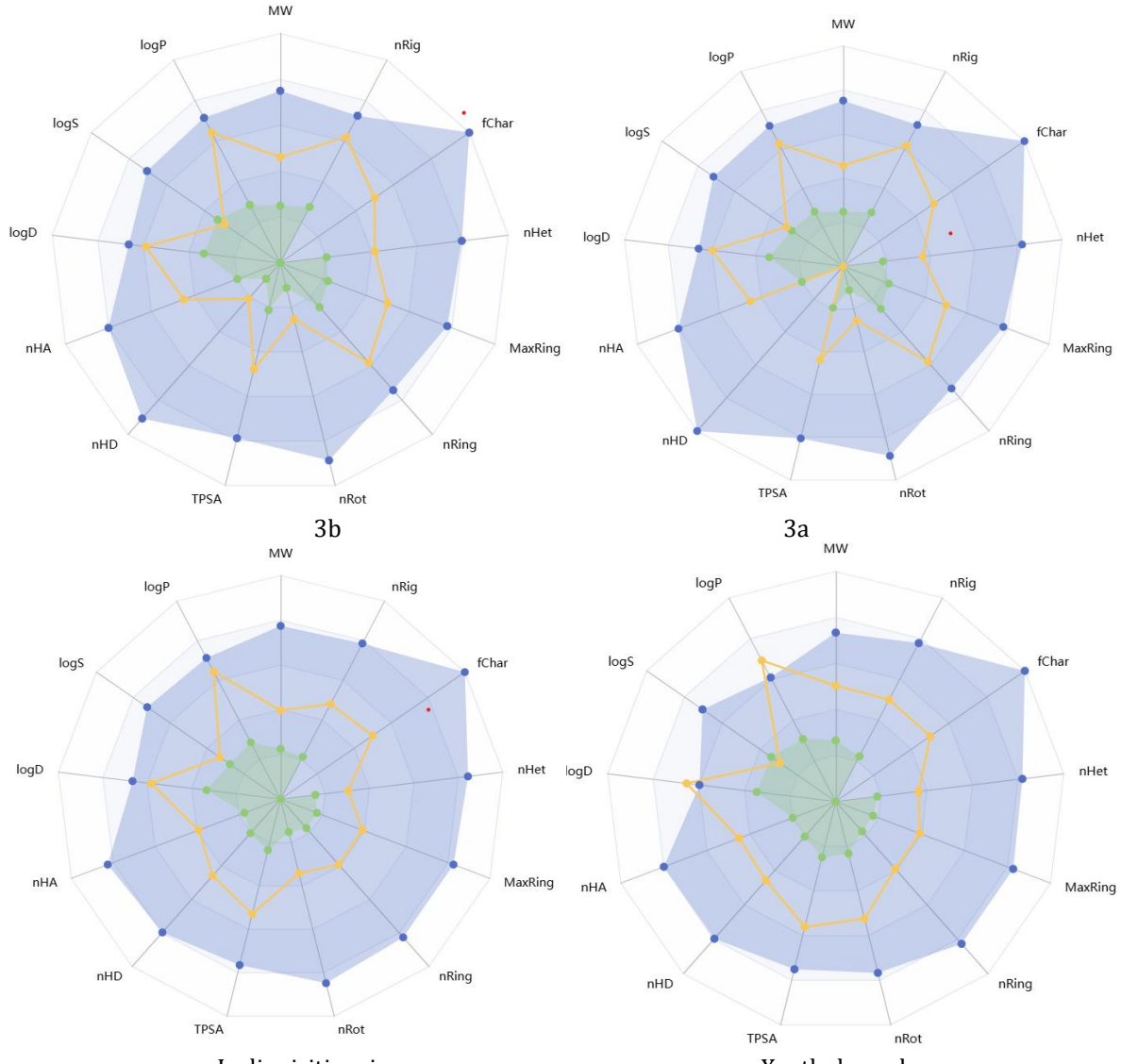


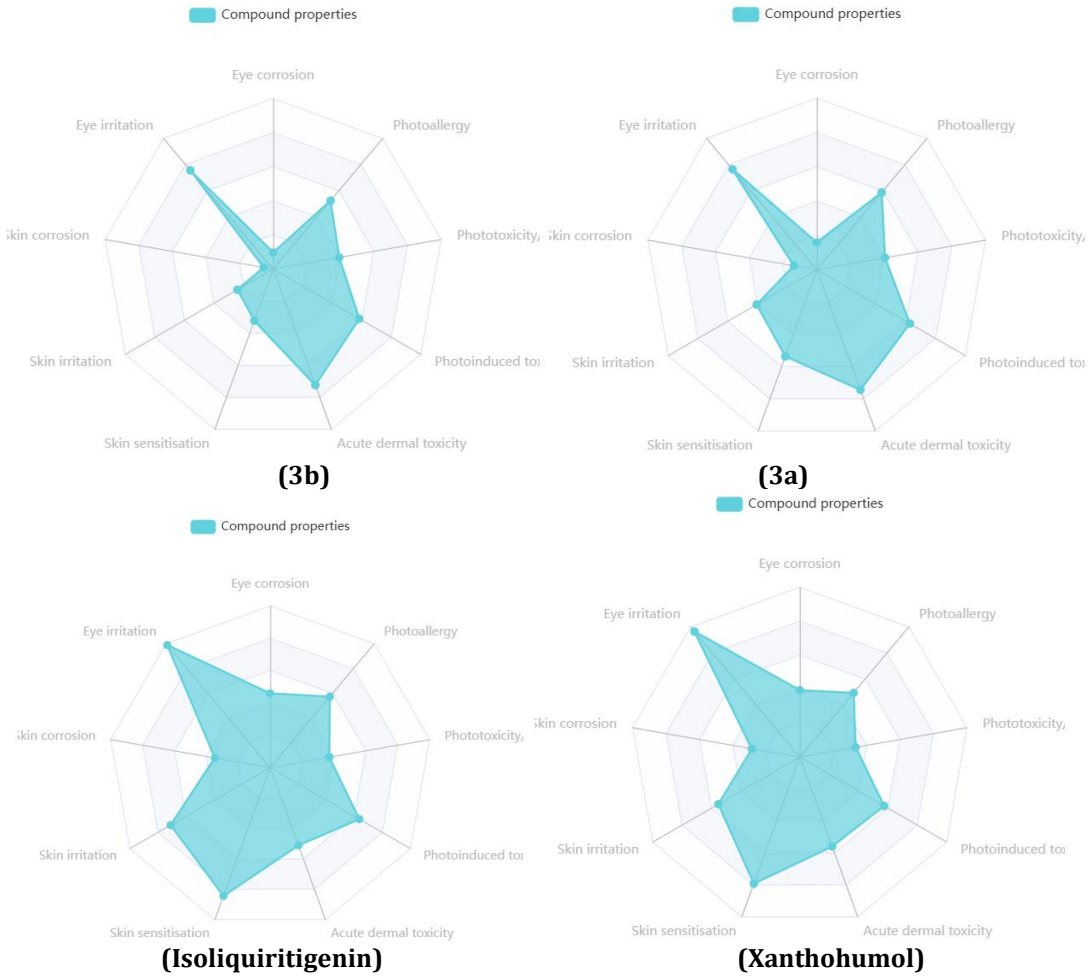
Figure 3. A view of Radar detailing for synthesized compounds and FDA drugs

Cosmetic Risk Assessment

Cosmetic Risk Assessment parameters of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were calculated by the freely available online web-tool AdmetSAR3.0. Parameters like Eye corrosion, Eye irritation, Skin corrosion, Skin irritation, Skin sensitisation, Acute dermal toxicity, Photoinduced toxicity, Phototoxicity and Photoallergy. https://lmmd.ecust. edu.cn/admetsar3/ predict. php(web-link).

Table 9. Details of cosmetic risk of compounds

Parameter	3b	3a	Isoliquiritigenin	Xanthohumol
Eye corrosion	0	0	0	0
Eye irritation	1	1	1	1
Skin corrosion	0	0	0	0
Skin irritation	0	0	1	1
Skin sensitisation	0	1	1	1
Acute dermal toxicity	1	1	1	1
Photoinduced toxicity	1	1	1	1
Phototoxicity	0	0	0	0
Photoallergy	1	1	1	0



(3b) **(3a)**

(Isoliquiritigenin) **(Xanthohumol)**

Figure 4. Detailing of cosmetic risk of synthesized compounds and FDA approved drugs

Boiled Egg

Boiled Egg of the synthesized 3a and 3b along with the standard drugs like Isoliquiritigenin and Xanthohumol were generated by the freely available online web-tool SwissADME.

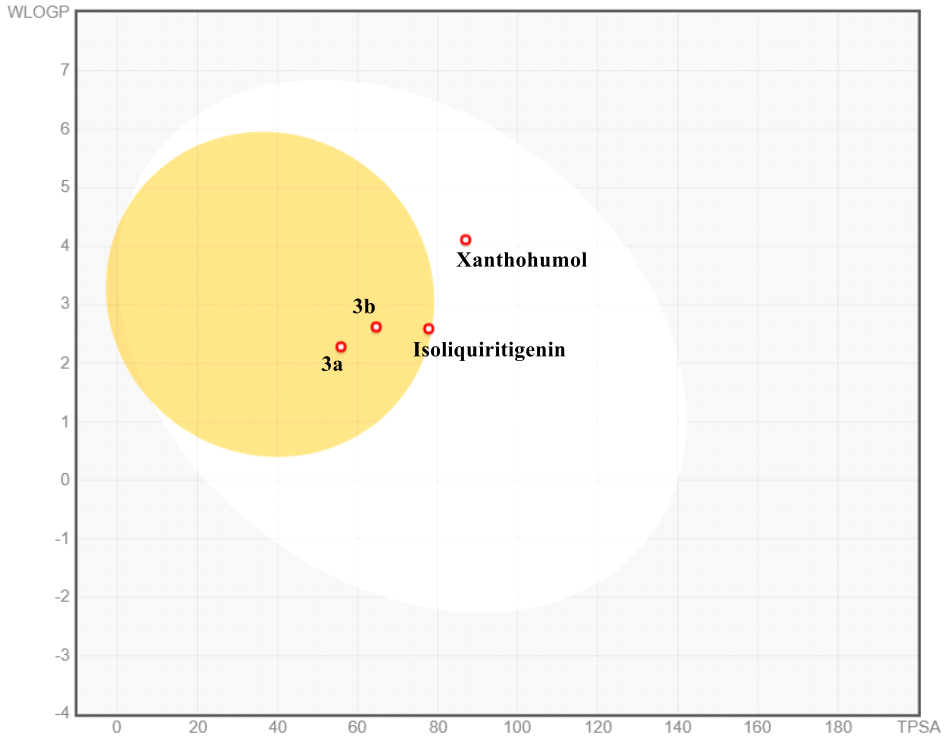


Figure 5. Boiled egg of the synthesized derivative 3a and 3b against Isoliquiritigenin and Xanthohumol.

Anti-Bacterial Activity:
It can be seen in Figures, compounds **3a** and **3b** were active against ***B. pumilis***, ***B. cerius, E. coli*** *and* ***K. Pneumonia*** *respectively.*

Table 1. Antibacterial (MIC µg/ml) of Compound **3a**

Microbial Strains	MIC Value of 3a	MIC of Gentamycin	SD	ER. BAR
B. cerius	187.5	80	±0.5769	0.43852
K. pneumoniae	156.25	80	±0.47585	0.39827
E. coli	93.5	80	±0.32856	0.33094
B. pumilis	156.5	80	±0.31939	0.32629

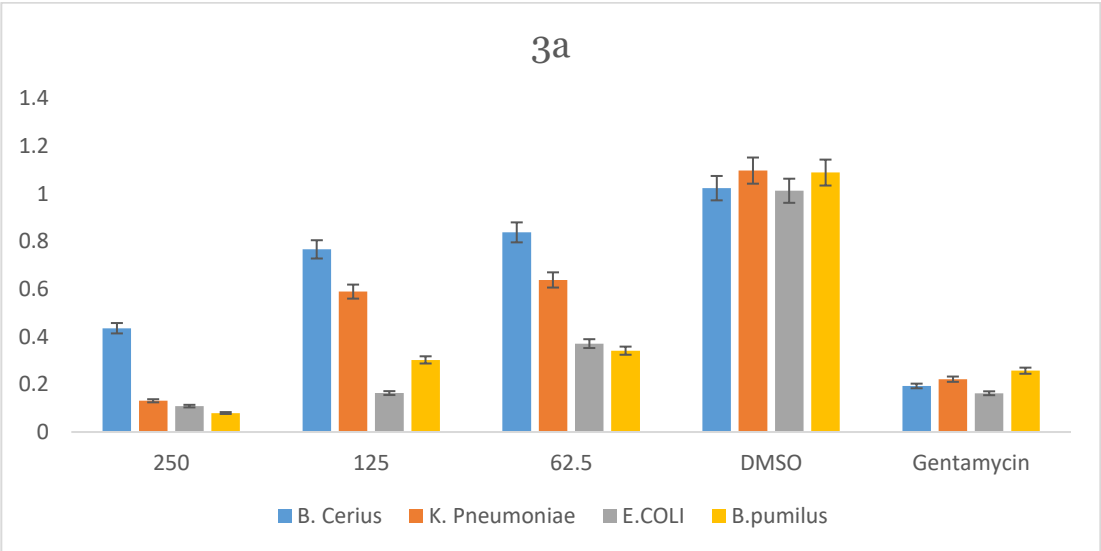


Figure 6. Growth inhibition of bacterial strains at different concentration of 3a was recorded after 18-20 hrs.

Table 9. Antibacterial (MIC µg/ml) of 3b

Microbial Strains	MIC Value of 3b	MIC of Gentamycin	SD	ER. BAR
B. cerius	156.25	80	±0. 325135	0.190069
K. pneumoniae	156.25	80	±0.3 15744	0.187304
E. coli	156.25	80	±0. 293468	0.180576
B. pumilis	187.50	80	±0. 310288	0.185678

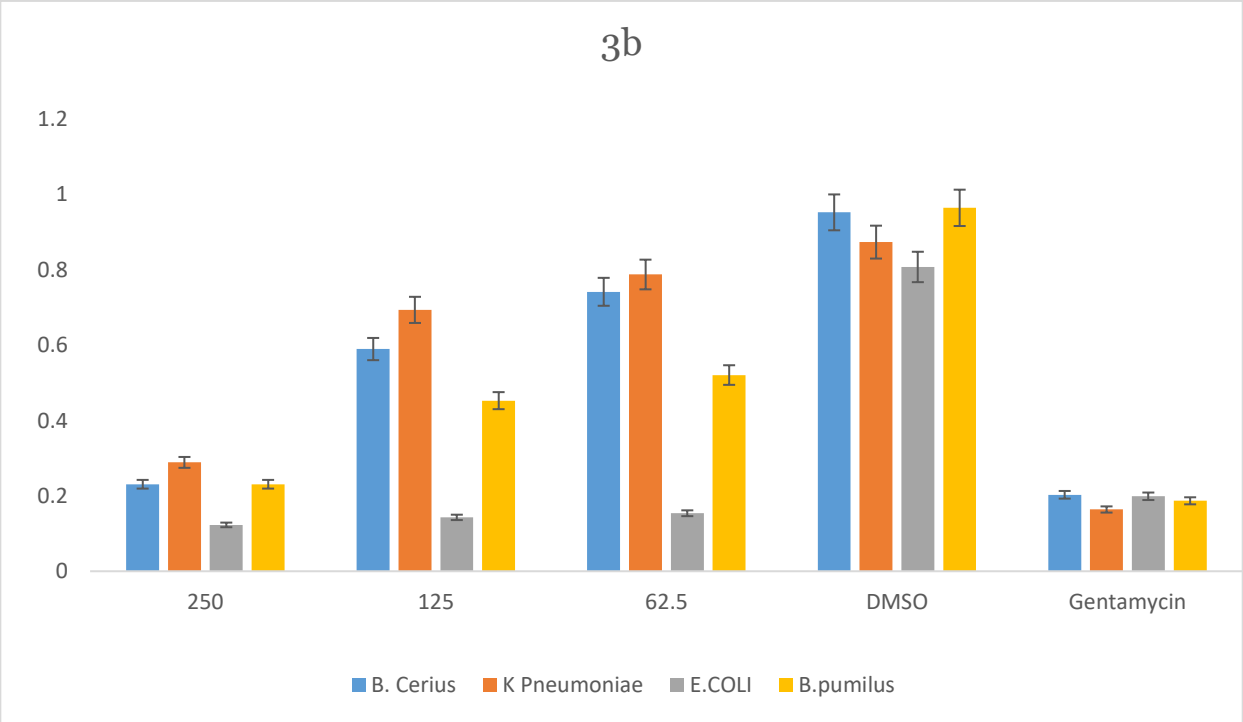


Figure 7. Growth inhibition of bacterial strains at different concentration of synthesized compound was recorded after 18-20 hrs and showed the more significants against *E. coli.*

The antibacterial growth inhibitions of synthesized compounds (3a and 3b) were presented in Fig. 6 and 7. These Compounds were evaluated for antibacterial activity against representative bacteria ***B. pumilis*** *(MTCC 160)*, ***B. cerius*** *(MTCC1305)*, ***E. coli*** *(ATCC 25923)* and ***K. pneumoniae*** (NCTC418) obtained from NCIM, Pune (INDIA). In this study, the synthesized compound 3a, showed more significant activity against ***E. coli*** *(ATCC 25923) at 93.5 µg/ml* .While other strains like ***B. cerius*** *(MTCC1305),* ***Klebsiella pneumonia*** (NCTC418) and ***B. Pumilis*** *(MTCC 160) at 187.2*µg/ml**, 156.5**µg/ml and **156.25** µg/ml**.**

In case of **3b** showed the highest MIC activity against ***Klebsiella pneumonia*** (NCTC418) ***E. coli*** *(ATCC 25923)* and ***B. pumilis*** *(MTCC 160) at 156.25*µg/ml and 187.5 µg/ml.

Conclusion

The investigation into isatin-based chalcone derivatives **(3a-b)** highlights their significant potential in medicinal chemistry, owing to their diverse biological activities and structural versatility. (Z)-3-(2-(benzo[d][1,3]dioxol-5-yl)-2-oxoethylidene)indolin-2-onederivatives were synthesized via the Claisen-Schmidt condensation reaction, employing isatin and substituted aromatic ketones under optimized reaction conditions. Additionally, the in-silico evaluation of these compounds has been explored to predict their pharmacokinetics, binding affinities, and potential as drug candidates. These Compounds were evaluated for antibacterial activity against representative bacteria *B. pumilis* (MTCC 160), *B. cerius (*MTCC1305), *E. coli* (ATCC 25923) and *K. pneumoniae* (NCTC418) obtained from NCIM, Pune (INDIA). n this study, the synthesized compound 3a, showed more significant activity against E. coli (ATCC 25923) at 93.5 µg/ml. While other strains like *B. cerius* (MTCC1305), *Klebsiella pneumonia* (NCTC418) and *B. Pumilis* (MTCC 160) at 187.2µg/ml, 156.5µg/ml and 156.25 µg/ml. The integration of computational methods with synthetic chemistry has proven to be a powerful strategy for accelerating drug discovery. Future research should focus on optimizing these derivatives for specific biological targets and expanding their application across diverse pharmacological domains.

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Conflict of interest

The authors declare no competing interests.

Author contributions

Saud Nusrat Ali (Synthesis and writing the original manuscript), Chandra Shekhar Yadav (Making Schemes and Literature Survey), Mohd Arsh Khan (Technical Support and ADMET calculations), Azhar Kamal (Anti-Bacterial Evaluations), Abdul Rahman Khan (Supervision), Iqbal Azad (Technical Support and proof reading), Sabahat Yasmeen Sheikh (Literature Survey and making schemes), Firoj Hassan (Supervision and writing manuscript).

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