

Synthesis, Characterization and In-Vitro Anticancer Evaluation of Novel N-Substituted Indolyl Chalcone Hybrids Incorporating Benzimidazole Moieties

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ABSTRACT

The present study was undertaken to synthesize, characterize, and evaluate a novel series of N-substituted indolyl chalcone hybrids incorporating benzimidazole moieties for their potential anticancer activity. N-1-substituted indole-3-carbaldehyde and N-1-substituted 2-acetylbenzimidazole derivatives were synthesized by alkylation using appropriate alkylating agents in the presence of anhydrous potassium carbonate. Reaction conditions were optimized by varying the molar ratio of potassium carbonate and evaluating the effect of phase-transfer catalysts. PEG-400 provided improved reaction efficiency, with optimized conditions employing a 1:4 molar ratio of substrate to potassium carbonate. Subsequently, thirty indolyl chalcone hybrids (CI-1–CI-30) were synthesized through condensation of the substituted aldehyde and ketone intermediates. The synthesized compounds were characterized by melting point, thin-layer chromatography, FT-IR, and ¹H-NMR spectroscopy. The synthesized chalcones exhibited melting points ranging from 95 to 267 °C. The in-vitro anticancer activity was evaluated using the sulforhodamine B assay against ME-180 cervical cancer, MCF-7 breast cancer, and A-2780 ovarian cancer cell lines, with Adriamycin used as the reference drug. Among the synthesized compounds, CI-27 demonstrated notable activity across the tested cell lines, with GI50 values of 43.2 μM, 28.7 μM, and 30.4 μM against ME-180, MCF-7, and A-2780 cells, respectively. CI-2 also exhibited comparatively promising activity, particularly against MCF-7 and A-2780 cells, with GI50 values of 19.4 μM and 35.5 μM, respectively. Molecular docking results showed favorable Glide scores for several synthesized compounds, although the docking scores did not consistently correlate with the observed cytotoxic activity. Overall, the findings demonstrate that N-substituted indolyl chalcone-benzimidazole hybrids represent a promising structural framework for further investigation as potential anticancer agents.

Keywords: Indole-3-carbaldehyde; Benzimidazole; Indolyl chalcones; N-substitution; Sulforhodamine B assay; Anticancer activity; ME-180; MCF-7; A-2780; Molecular docking.

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INTRODUCTION

Chalcones (1,3-diaryl-2-propen-1-ones) are important flavonoid-related scaffolds characterized by a pharmacologically active α,β -unsaturated carbonyl system. They exhibit diverse biological activities, including anticancer, antimicrobial, anti-inflammatory, antioxidant, antiviral, and antifungal effects, making them valuable structures in medicinal chemistry [1–3].

Indole is a privileged heterocyclic scaffold widely present in bioactive molecules and is associated with diverse pharmacological activities, particularly anticancer activity. Substitution at the N-1 position of indole can significantly influence lipophilicity, molecular interactions, and pharmacological properties [4–6]. Similarly, benzimidazole is an important nitrogen-containing heterocycle found in several biologically active and marketed drugs. Its two nitrogen atoms facilitate hydrogen-bond interactions with biological targets, while N-1 substitution provides opportunities for structural modification and activity optimization [7–9].

Molecular hybridization, which combines two or more biologically active pharmacophores within a single molecular framework, is an established strategy for developing compounds with improved biological properties [10–12]. Accordingly, combining indole, benzimidazole, and chalcone moieties may provide promising hybrid structures with enhanced anticancer potential.

The Claisen–Schmidt condensation is a widely used and efficient method for synthesizing chalcones through the reaction of aromatic aldehydes with aromatic ketones under basic conditions [13–15]. In the present study, thirty N-1-substituted indolyl chalcone hybrids derived from 2-acetylbenzimidazole were synthesized using the Claisen–Schmidt condensation, followed by purification and structural characterization. The synthesized compounds were subsequently evaluated for their anticancer activity.

MATERIAL AND METHODS

All the required chemicals purchased from the Loba Chemicals Pvt. Ltd.

Optimized Methodology

Indole-3-carbaldehyde (1.45g, 0.01mol) was put to a 50 ml round bottom flask along with anhydrous K₂CO₃ (3.528g, 0.04 mol) and dimethyl formamide (20 ml) as a solvent. The appropriate alkylating chemical 3.1 (0.01 mol) was dropped onto this at room temperature. Using a magnetic stirrer, the resulting mixture was stirred for three hours at 10-15 °C. When the reaction was complete, as indicated by TLC, the reaction material was poured into ice cold water. The separated material was filtered, extra water washed off, and dried to get the crude product. The latter were recrystallized from ethanol to get a pure chemical.

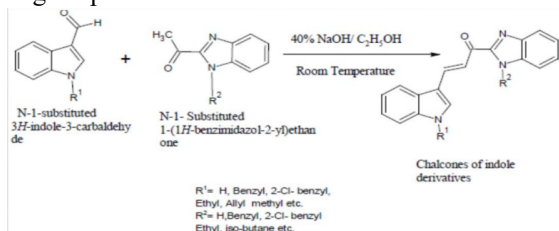


Figure 1. Scheme-IA. : The synthesis of N-1-Derivatives of indole-3-carbaldehyde.

Synthesis of N-1-substituted derivatives of 2-Acetyl benzimidazole (Scheme-IB)

In a 50 ml round bottom flask, the following solvents were used: anhydrous K₂CO₃ (3.528g, 0.04 mol), dimethylformamide (20 ml), and 2-acetylbenzimidazole (1.6g, 0.01 mol). Substitute as mentioned 3.2 (0.01mol), the appropriate alkylating agent was then added to this dropwise while still at room temperature. At a temperature of 10-15 °C, it was then stirred for three hours. TLC was employed to track the reaction. The reaction mass was introduced to a glass of ice-cold water. The separated material was filtered, extra water washed off, and dried to get the crude product. The latter were recrystallized from ethanol to get a pure chemical.

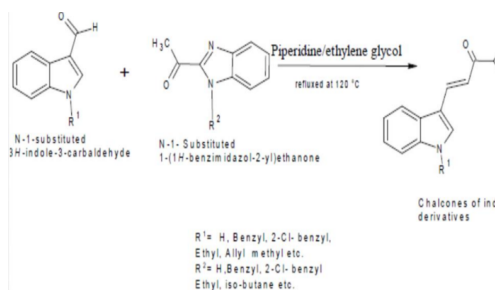


Figure 2. Scheme-IB: The synthesis of N-1-Derivatives of 2- acetyl benzimidazole.

RESULT AND DISCUSSION

Analysis of the compound

Using an EXPO-Hi Tech melting point apparatus with open glass capillaries, the melting points of all compounds were calculated without correction. R_f values recorded on TLC enabled us to confirm the purity of the chemical. The structures of every synthesised molecule were determined from their IR spectra and confirmed by their ¹H-NMR spectra.

The IR spectra were recorded on a Shimadzu FT-IR spectrometer in the 4000-650 cm⁻¹ range using a KBr pellet method. The ¹H-NMR spectra were recorded on a Varian 400 MHz spectrometer using CDCl₃ as the solvent and TMS as an internal standard. By using mass spectrometric analysis, the structures of a few indolyl chalcones have been elucidated.

IR and ¹H-NMR spectra of N-1 substituted Indole-3-carboxaldehyde 1 IR and ¹H-NMR spectra of 1-ethyl-1H-indole-3-carbaldehyde (IA-2)

Table 1. IR & ¹H-NMR Spectrum interpretation of IA-2

Compound	Wave Number (cm ⁻¹)	Chemical shift (δ ppm)
IA-2	3103(C-H str, Ar), 1664(C=O), 1532 (C=C str, Ar).	δ 1.51 (t, 3H, CH ₃), 3.38 (s, 3H, CH ₃), 4.22 (q, 2H, N-CH ₂), 7.25-7.39 (m, 5H, Ar-H), 8.31(m, 1H, H-4), 9.97 (s, 1H, CHO) ppm;

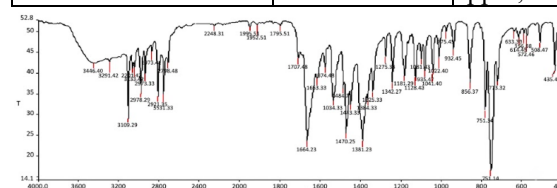


Figure 3. IR spectrum of 1-ethyl-1H-indole-3-carbaldehyde (IA-2)

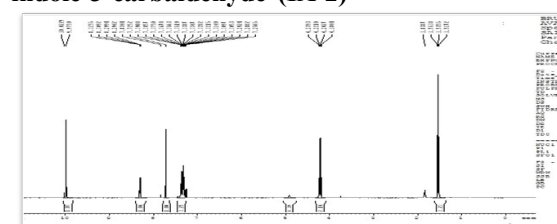
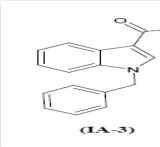


Figure 4. ¹H NMR spectrum of 1-ethyl-1H-indole-3-carbaldehyde (IA-2)

Table 2. IR & ¹H-NMR Spectrum interpretation of IA-4

Compound	Wave Number (cm ⁻¹)	Chemical shift (δ ppm)
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IA-4 	3107,3057(C-H strAr),2881(CH O), 2926 (C-Hstr CH ₂), 1660 (C=O str), 1552, (C=C str, Ar) 838(C-Cl str).	δ , 3.43 (d, 2H, N-CH ₂), 7.14 - 7.30 (m, 7H, Ar-H), 8.34 (m,1H, Ar-H), 9.99 (s, 1H, CHO)ppm;
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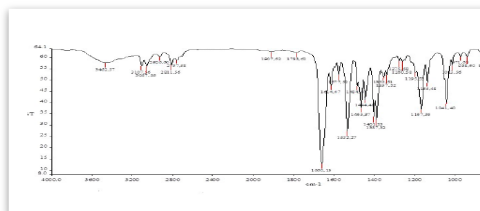


Figure 5. IR spectrum of 1-(2-chlorobenzyl)-1H-indole-3-carbaldehyde (IA-4)

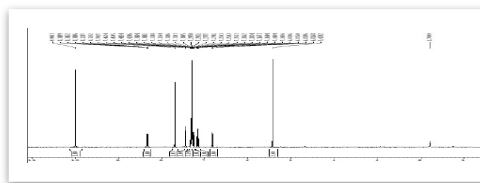
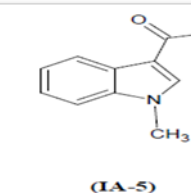


Figure 6. ¹H NMR spectrum of 1-(2-chlorobenzyl)-1H-indole-3-carbaldehyde(IA-4)
Table 3. IR & ¹H-NMR Spectrum interpretation of IA-5

Compound	Wave Number (cm ⁻¹)	Chemical shift (δ ppm)
IA-5 	3106, 3051 (C-H str, Ar), 2806(CH O), 2938(C-H str) 2806(N-CH ₃ str), 1655(C=O), 1536 (C=C str, Ar).	δ 3.7 (s,3 H,N-CH ₃), 7.28- 7.59 (m, 4H, Ar-H),8.27(m, 1H, H-4), 9.97 (s, 1H, CHO) ppm;

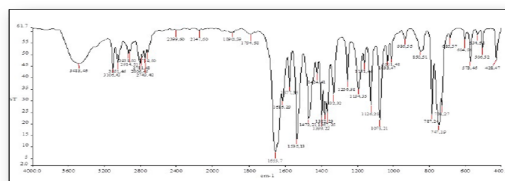


Figure 7. IR spectrum ofN1-Methyl-Indole-3-Carboxaldehyde (IA-5)

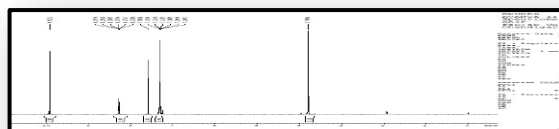
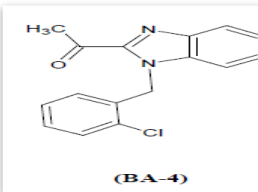


Figure 8. ¹H NMR spectrum of N1-Methyl-Indole-3-Carboxaldehyde (IA-5)

Table 4. IR & ¹H-NMR Spectrum interpretation of BA-4

Compound	Wave Number (cm ⁻¹)	Chemical shift (δ ppm)
BA-4 	3060(C-H str, Ar), 2972 (C-H str, CH ₂), 1686(C=O str), 1581 (C=C strAr), 828(C-Cl str).	δ ,2.84 (s, 3H, CH ₃ -C=O)3.85 (d, 2H, N-CH ₂), 6.33-7.94 (m, 8H, Ar-H), ppm

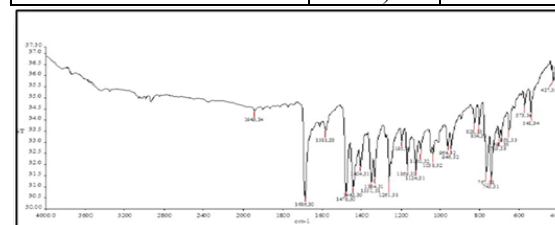


Figure 9. IR spectrum of1-[1-(2-chlorobenzyl)-1H-benzimidazol-2-yl]ethanone (BA-4)

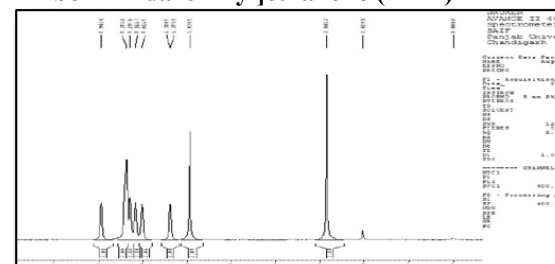


Figure 10. ¹H NMR spectrum of1-[1-(2-chlorobenzyl)-1H-benzimidazol-2-yl] ethanone (BA-4)

Table 5. IR & ¹H-NMR Spectrum interpretation of BA-5

Compound	Wave Number (cm ⁻¹)	Chemical shift (δ ppm)
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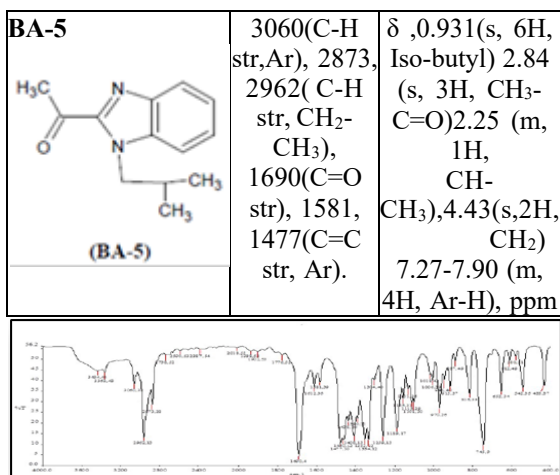


Figure 11. IR spectrum of 1-[1-(2-methylpropyl)-1H-benzimidazol-2-yl] ethanone (BA-5)

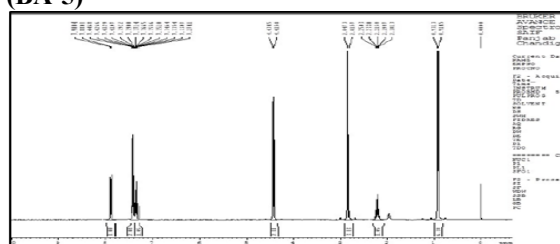


Figure 12. ¹H NMR spectrum of 1-[1-(2-methylpropyl)-1H-benzimidazol-2-yl] ethanone (BA-5)

Synthesis

In this study, 30(CI-1 to CI-30) indolylchalcone hybrids were created by combining N-1 substituted 2-acetyl-benzimidazole and N-1 substituted indole-3-carboxaldehyde. The N-alkylation/arylation and mono-methylation on indole 3-carboxaldehyde were enhanced by using alkylating and arylating chemicals in the presence of environmentally friendly catalysts such as anhydrous potassium carbonate (K₂CO₃) and PEG-400, which serve as phase transfer catalysts. 6.1.1 The creation of derivatives of indole-3-carbaldehyde using N-1 substitutes and 2-acetyl benzimidazole.

All N-1 substituted indole-3-carbaldehyde derivatives (Scheme-IA) and 2-acetyl benzimidazole (Scheme-IB (i-iii)) were synthesised by using alkylating and arylating agents to optimise these reaction to get maximum yield of the compound, various trials by varying mole ratio of K₂CO₃ and by using with PTC and without PTC were conducted.

Synthesis of 2-acetylbenzimidazole as shown in scheme IB (i) & (ii)

In this, 2-(hydroxyethyl) benzimidazole was produced as a crude product by condensing o-Phenyldiamine with lactic acid at Philips conditions. K₂Cr₂O₇ was used to oxidise the 2-(hydroxyethyl) benzimidazole, resulting in 2-acetylbenzimidazole. By employing the solvent system of chloroform: ethyl acetate (10:1), the

melting point and R_f values of the chemical were used to verify its purity.

Optimization of % yield of fused heterocyclic ring

The mole ratio of anhydrous K₂CO₃ is quite important. Similar to how the mole ratio of anhydrous K₂CO₃ increases, the percentage yield of N-1 alkyl and aryl derivatives of fused heterocyclic rings also increases. The effects of PEG-400 and TEBAC (as a phase transfer catalyst) on the % yields of N-1 substitutions of indole-3-carboxaldehyde and 2-acetyl-benzimidazole are shown graphically in Figures 13 and 14, respectively. The results show that the product yield increases as the ratio of anhydrous K₂CO₃ increases. The product yield percentage increases when PEG-400 is used as a PTC.

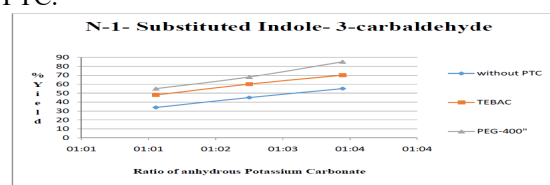


Figure 13. Effect of ratio of K₂CO₃ on % yield of N-1 substituted of Indole-3-carbaldehyde

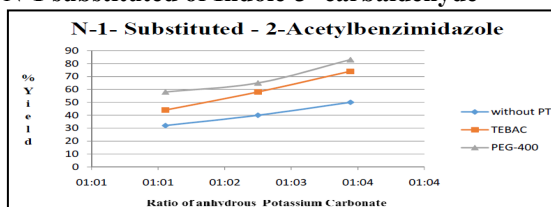


Figure 14. Effect of ratio of K₂CO₃ on % yield of N-1 substituted of 2-acetylbenzimidazole.

Optimization of Time

The reaction time, comprising PEG-400 and TEBAC, with and without PTC is influenced by the anhydrous K₂CO₃ molar ratio. The reaction takes less time to complete as the mole ratio of anhydrous K₂CO₃ increases. The reaction required less time to complete when K₂CO₃ was employed in a mole 1:4 ratio and PEG-400 was used as the PTC. Tables 6, for indole-3-carbaldehyde and 2-acetyl benzimidazole, respectively, provide the findings.

Table 6 Comparisons of the result for the synthesis of N-1-Substituted Indole-3-carbaldehyde derivatives with various PTC and mole ratios of anhydrous K₂CO₃

	N-1-Substituted Indole-3-carbaldehyde derivatives with various PTC and mole ratios of anhydrous K ₂ CO ₃	N-1-Substituted 2-acetylbenzimidazole derivatives with various PTC and mole ratios of anhydrous K ₂ CO ₃

T r i a l	Ra tio of an hy dr ou s K2 C O3	T i m e i n h o u r s	A % Yi el d W it h o u t P T C	B % Yi el d T E B A C	C % Yi el d W it h P E G- 40 0	T i m e i n h o u r s	A % Yi el d W it h o u t P T C	B % Yi el d T E B A C	C % Yi el d W it h P E G- 40 0
a	1:2	10	34			10	32		
b	1:2	9		48		9		44	
c	1:2	5			55	8			58
d	1:3	8	45			8	40		
e	1:3	7		60		7		58	
f	1:3	4			68	5			65
g	1:4	4	55			6	50		
h	1:4	3		70		5		74	
i	1:4	2			85	2			83

Table 7. Melting points, Rf values and percent (%) yield of N-1 substituted 2- acetyl-benzimidazole

Compou nd	Name of the compound	Meltin g point	Rf	% Yiel d
BA-1	2-Acetyl- benzimidaz ole	177- 179°C	0.8 0	80.
BA-2	N-1-benzyl- 2 acetyl benzimidaz ole	97-99 °C	0.6 5	70
BA-3	N-1- ethyl- 2- acetyl benzimidaz ole	103- 105 °C	0.4 9	66
BA-4	N-(2-Cl- benzyl)- 2 acetyl benzimidaz ole	106- 108oC	0.5 9	74
BA-5	N-(iso- butane)-2- acetyl-	96-98 °C	0.6 1	68

	benzimidaz ole			
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Mobile phase – Hexane: Ethyl acetate(9:1)

Synthesis of indolyl chalcone hybrids of 2-acetylbenzimidazole

Throughout the synthesis of all the indolyl chalcone, the temperature and mole ratio of the basic catalyst were adjusted to optimise the yield %. From various N-1-substituted indole-3-carbaldehydes and N-1-substituted 2-acetyl-benzimidazoles serving as aldehyde and ketone moiety, respectively, this procedure produces 30 indolyl chalcones (CI-1 to CI-30).

The catalyst in one method (Scheme-I) was 40% NaOH at room temperature, whereas the catalyst in the other (Scheme-II) was piperidine at reflux with ethylene glycol as the solvent. The average yield for all indolyl chalcone compounds synthesised using the piperidine catalyst in reflux method ranged from 60 to 84%. When these compounds were produced using 40% NaOH at room temperature, the typical yield ranged from 50 to 70 percent. Therefore, the method used in Scheme I helped to increase compound yield by around 18%. The reaction times for the compounds' synthesis in Scheme-I and Scheme-II, respectively, varied from 12 to 27 hours and 24 to 48 hours, respectively. As a result, in Scheme-II, utilising piperidine as a catalyst under refluxed conditions for synthesis not only helped to shorten the reaction time but also enhanced the quality and yield of the synthesised indolyl chalcone hybrids of 2-acetylbenzimidazole. By 10 hours, the response time had drastically shortened.

Table 8. The comparison of the reaction time and yield of indolyl chalcone by using NaOH 40% and piperidine/ ethylene glycol as a catalyst

Comp ound	Comp ound	Reaction time in houres		% yield	
		NaO H 40% /RT	Piper idine in ethyl ene glyco l /reflu x	NaO H 40% /RT	Piper idine in ethyl ene glyco l /reflu x
CI-1	AI-1	36	18	70	77
CI-2	AI-2	48	12	62	65
CI-3	AI-3	34	17	62	68
CI-4	AI-4	33	16	67	65
CI-5	BH-1	31	20	68	70
CI-6	BH-2	28	16	50	69

CI-7	BH-3	29	17	67	72
CI-8	BH-4	44	20	61	75
CI-9	EB-1	45	16	50	55
CI-10	EB-2	24	27	55	60
CI-11	EB-3	35	16	63	65
CI-12	EB-4	33	15	65	77
CI-13	CL-1	29	14	68	79
CI-14	CL-2	27	15	65	81
CI-15	CL-3	29	18	60	84
CI-16	CL-4	31	19	62	68
CI-17	A-1	38	13	64	65
CI-18	A-2	39	17	66	68
CI-19	A-3	46	20	65	71
CI-20	A-4	32	21	65	60
CI-21	A-5	34	19	64	70
CI-22	MH-1	33	18	58	71
CI-23	MH-2	31	17	53	65
CI-24	MH-3	23	16	60	66
CI-25	MH-4	25	16	65	80
CI-26	MH-5	29	18	67	79
CI-27	IB-1	31	19	58	65
CI-28	IB-2	33	22	54	70
CI-29	IB-3	38	18	65	73
CI-30	IB-4	45	19	60	68

Table 9. Melting points and Rf values of synthesized indolyl chalcone.

Compound	Name of the compound	Melting point °C	Rf
CI-1	(2Z)-1-(1H-benzimidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one	265-267	0.6123*
CI-2	(2Z)-1-(1H-benzimidazol-2-yl)-3-(1-ethyl-1H-indol-3-yl)prop-2-en-1-one	213-214 °C	0.1711*

CI-3	(2Z)-1-(1H-benzimidazol-2-yl)-3-(1-benzyl-1H-indol-3-yl)prop-2-en-1-one	249-251 °C	0.45234*
CI-4	1-(1H-benzimidazol-2-yl)-3-[1-(2-chlorobenzyl)-1H-indol-3-yl]prop-2-en-1-one	223-226 °C	0.2105*
CI-5	(Z)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one	139-142 °C	0.2630*
CI-6	(Z)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)-3-(1-ethyl-1H-indol-3-yl)prop-2-en-1-one	155-158 °C	0.756#
CI-7	(Z)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)-3-(1-benzyl-1H-indol-3-yl)prop-2-en-1-one	183-186 °C	0.842#
CI-8	(Z)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)-3-(1-(2-chlorobenzyl)-1H-indol-3-yl)prop-2-en-1-one	171-174 °C	0.754#
CI-9	(2Z)-1-(1-ethyl-1H-benzimidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one	205-207 °C	0.641*
CI-10	(2Z)-1-(1-ethyl-1H-benzimidazol-2-yl)-3-(1-ethyl-1H-indol-3-yl)prop-2-en-1-one	175-177 °C	0.6728*

CI-11	(Z)-3-(1-benzyl-1H-indol-3-yl)-1-(1-ethyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	214-216 °C	0.5553 *
CI-12	(Z)-3-(1-(2-chlorobenzyl)-1H-indol-3-yl)-1-(1-ethyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	147-149 °C	0.453*
CI-13	(Z)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one	217-219 °C	0.2156 *
CI-14	(Z)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)-3-(1-ethyl-1H-indol-3-yl)prop-2-en-1-one	181-184 °C	0.7800 #
CI-15	(Z)-3-(1-benzyl-1H-indol-3-yl)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	232 - 235 °C	0.8600 #
CI-16	(Z)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)-3-(1-(2-chlorobenzyl)-1H-indol-3-yl)prop-2-en-1-one	226-228 °C	0.8039 *
CI-17	(2Z)-1-(1H-benzimidazol-2-yl)-3-[1-(prop-2-en-1-yl)-1H-indol-3-yl]prop-2-en-1-one	122-126 °C	0.1428 *
CI-18	(2Z)-1-(1-ethyl-1H-benzimidazol-	101-105 °C	0.3564 *

	2-yl)-3-[1-(prop-2-en-1-yl)-1H-indol-3-yl]prop-2-en-1-one		
CI-19	(Z)-3-(1-allyl-1H-indol-3-yl)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	132-135 °C	0.5870 *
CI-20	(Z)-3-(1-allyl-1H-indol-3-yl)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	137-139 °C	0.5612 *
CI-21	(Z)-3-(1-allyl-1H-indol-3-yl)-1-(1-isobutyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	95-99 °C	0.4012 #
CI-22	(2Z)-1-(1H-benzimidazol-2-yl)-3-(1-methyl-1H-indol-3-yl)prop-2-en-1-one	142-144 °C	0.1451 #
CI-23	(2Z)-1-(1-ethyl-1H-benzimidazol-2-yl)-3-(1-methyl-1H-indol-3-yl)prop-2-en-1-one	224-227 °C	0.4166 *
CI-24	(Z)-1-(1-benzyl-1H-benzo[d]imidazol-2-yl)-3-(1-methyl-1H-indol-3-yl)prop-2-en-1-one	231-234 °C	0.4833 *
CI-25	(Z)-1-(1-(2-chlorobenzyl)-1H-benzo[d]imidazol-2-yl)-3-(1-methyl-1H-indol-3-yl)prop-2-en-1-one	220-222 °C	0.6353 *

CI-26	(2Z)-3-(1-methyl-1H-indol-3-yl)-1-[1-(2-methylpropyl)-1H-benzimidazol-2-yl]prop-2-en-1-one	139-141 °C	0.5833 *
CI-27	(2Z)-3-(1H-indol-3-yl)-1-[1-(2-methylpropyl)-1H-benzimidazol-2-yl]prop-2-en-1-one	129-131 °C	0.6857 *
CI-28	(Z)-3-(1-ethyl-1H-indol-3-yl)-1-(1-isobutyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	116-118 °C	0.5421 *
CI-29	(Z)-3-(1-benzyl-1H-indol-3-yl)-1-(1-isobutyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	127-129 °C	0.5483 *
CI-30	(Z)-3-(1-(2-chlorobenzyl)-1H-indol-3-yl)-1-(1-isobutyl-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one	123-125 °C	0.6140 *

*Mobile Phase = Hexane: Ethyl acetate (8:2);

#Mobile Phase= Chloroform: Hexane:Ethyl acetate (4:4:1)

Anticancer Activity

In-vitro anticancer activity: Sulforhodamine B assay (SRB) on ME-180 cell line

The ME-180 cervical cancer cell line was utilised in this study to evaluate the in vitro anticancer activity of all indolyl chalcone compounds using the sulforhodamine B assay. The results of the test were compared to the G score, lethal concentration (LC50), total growth inhibition (TGI), and 50% growth inhibition (GI 50) of indolylchalcone..

Table 10. Result of LC50, TGI and GI50 along with G-score on ME-180 cell line

Compound	Concentration of indolyl chalcone(μM)			Glide score
	LC50	TGI	GI50	

CI-1	>100	>100	>100	-7.42218
CI-2	>100	>100	63.5	-7.09569
CI-3	>100	>100	>100	-7.95768
CI-4	>100	>100	>100	-7.38003
CI-5	>100	>100	53.9	-4.92451
CI-6	>100	>100	>100	-5.54851
CI-7	>100	>100	>100	5.48183
CI-8	>100	>100	>100	-6.03964
CI-9	>100	>100	51.2	-7.32011
CI-10	>100	>100	>100	-6.12143
CI-11	>100	>100	>100	-5.36871
CI-12	>100	>100	>100	-6.73868
CI-13	>100	>100	55.9	-3.61662
CI-14	>100	>100	>100	-5.8018
CI-15	>100	>100	>100	-5.12298
CI-16	>100	>100	>100	-7.421
CI-17	>100	>100	>100	-5.5432
CI-18	>100	>100	>100	-5.321
CI-19	>100	>100	>100	-4.451
CI-20	>100	>100	>100	-5.712
CI-21	>100	>100	>100	-7.69258
CI-22	>100	>100	>100	-5.75487
CI-23	>100	>100	>100	-6.49431
CI-24	>100	>100	>100	-5.59073
CI-25	>100	>100	>100	-6.00896
CI-26	>100	>100	>100	-7.00579
CI-27	>100	>100	43.2	-6.5031
CI-28	>100	>100	>100	-5.74418
CI-29	>100	>100	>100	-5.84687
CI-30	>100	>100	>100	-6.75183
Std . Adriamycin	>100	<0.1	<0.1	-

All indolyl chalcone compounds are inactive at the first three concentrations, however the 10-4 M concentration range displayed significant cytotoxicity activity. The compounds with the chemical codes CI-2, CI-5, CI-10, CI-13, and CI-27 effectively decrease the growth of ME-180 cells by

50% at doses of 63.5 M, 53.9 M, 51.2 M, 55.9 M, and 43.2 M, respectively.

CONCLUSION

The present investigation successfully established a synthetic approach for the preparation of N-substituted indole-3-carbaldehyde and 2-acetylbenzimidazole derivatives and their subsequent conversion into thirty indolyl chalcone hybrids (CI-1–CI-30). Optimization studies demonstrated that increasing the potassium carbonate ratio improved the reaction efficiency, while the use of PEG-400 as a phase-transfer catalyst resulted in higher yields and shorter reaction times. The synthesized compounds were characterized using melting point, TLC, FT-IR, and ¹H-NMR spectroscopy, supporting the formation of the proposed structures.

The anticancer potential of the synthesized indolyl chalcones was investigated against ME-180, MCF-7, and A-2780 cancer cell lines using the SRB assay. Most compounds showed moderate or limited cytotoxic activity; however, selected derivatives demonstrated comparatively better growth-inhibitory effects. CI-27 emerged as one of the most promising compounds, exhibiting GI₅₀ values of 43.2 μM, 28.7 μM, and 30.4 μM against ME-180, MCF-7, and A-2780 cells, respectively. CI-2 also showed notable activity, particularly against MCF-7 cells, with a GI₅₀ of 19.4 μM, and against A-2780 cells with a GI₅₀ of 35.5 μM. Although none of the synthesized compounds exhibited activity comparable to Adriamycin, the results indicate that structural modification of the indole and benzimidazole nitrogen atoms can influence anticancer activity. Molecular docking further supported favorable interactions for several compounds, although docking scores were not directly proportional to the experimental cytotoxicity. Overall, the synthesized indolyl chalcone-benzimidazole hybrids, particularly CI-2 and CI-27, provide useful lead structures for further structural optimization and detailed biological investigation.

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