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Synthesis, Characterization and Evaluation of Novel Isatin–Apigenin Derivatives as Anticancer Drugs

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KEYWORDS

Isatin, Apigenin, Anti-Cancer Agents, Hydrazone, Cell Lines, MTCC Assay.

ABSTRACT:

The present research focuses on the design, synthesis, structural characterization, and preliminary biological evaluation of a novel series of Isatin–Apigenin hybrid derivatives (4a–4f) developed as potential anticancer agents. The synthetic pathway involved a two-step methodology. In the first step, Apigenin was activated through chloroacetylation using chloroacetyl chloride under basic conditions to introduce a reactive chloroacetyl linker. In the second step, nucleophilic substitution between the activated Apigenin intermediate and a series of substituted isatin derivatives (5-chloro, 5-fluoro, 5-bromo, 7-nitro, etc.) yielded the final hybrid molecules. Reaction progress was monitored by TLC, and products were purified using recrystallization or silica gel column chromatography.

Comprehensive structural confirmation was achieved through FTIR, 1H NMR, 13C NMR, Mass Spectrometry, and Elemental Analysis. FTIR spectra verified the presence of phenolic O–H, flavone C=O, and isatin C=O functional groups. NMR spectra confirmed the incorporation of the $–CH_2CO–$ linker and appropriate aromatic proton arrangements, while mass spectrometry validated the expected molecular ion peaks.

Cytotoxicity evaluation using the MTT assay against MCF-7, A549, and HeLa cell lines revealed encouraging biological activity. Among the synthesized analogues, compound 4c exhibited the highest anticancer potency with significantly lower IC_{50} values, outperforming parent Apigenin and approaching the activity of the standard drug Doxorubicin. The key findings indicate that conjugating Apigenin with electron-withdrawing-substituted isatin derivatives greatly enhances anticancer efficacy, establishing compound 4c as a promising lead for further mechanistic, ADMET, and in vivo studies.

1. INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, driving the continuous pursuit of safer and more effective therapeutic agents (Li, J. (2013)). Despite significant progress in chemotherapeutic development, many existing anticancer drugs suffer from limitations such as multidrug resistance, severe side effects, poor selectivity, and limited bioavailability (Huang, W. Y. (2019), Keri, R. S. (2015), Kumar, A. (2021)). These shortcomings necessitate the development of new chemical entities with improved pharmacological profiles. Cancer continues to pose a major global health burden, necessitating the discovery of novel chemotherapeutic agents with improved efficacy and minimized toxicity (Ali, H. (2016)).

Natural flavonoids such as Apigenin have demonstrated potent antioxidant, anti-inflammatory, and anticancer properties (Agrawal, A. D. (2011), Ahmed, S. (2021)). Concurrently, isatin (1*H*-indole-2,3-dione) and its derivatives exhibit diverse bioactivities, including apoptotic, antimicrobial, and anticancer effects (Boobalan, R. (2020), Chandran, S. (2022), Chihomvu, P. (2024)).

Hybridizing these pharmacophores into a single molecular entity offers a promising strategy for obtaining multifunctional anticancer drug candidates (Bhatia, R. (2020), Bifulco, M. (2025)).

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Natural products have historically been a vital source of anticancer drugs. Among these, Apigenin-a dietary flavone found in chamomile, parsley, and celery—has gained recognition for its strong antioxidant, anti-inflammatory, and anticancer properties (Deepa, D. (2020), Ferreira, L. L. G. (2019), Ghanbari, H. (2021)).

Apigenin modulates several key cellular pathways including apoptosis induction, cell-cycle arrest, and tyrosine kinase inhibition. However, its therapeutic utility is hindered by poor solubility and rapid metabolic degradation (Cincin, Z. B. (2015), Daina, A. (2017), Zohdi, H. F. (2022)).

Isatin (1H-indole-2,3-dione) is a versatile heterocyclic scaffold widely explored in medicinal chemistry due to its broad biological activities such as antiviral, antimicrobial, anti-inflammatory, and anticancer effects (Patel, R. (2021), Prakash, O. (2013), Sak, K. (2014). Numerous isatin derivatives exhibit notable cytotoxicity against various cancer cell lines, often through apoptosis activation, oxidative stress induction, or kinase inhibition. Its structural simplicity and ease of modification make it an excellent candidate for hybrid drug design (Ghosh, R. (2022), Wang, L. (2019), Zhou, Y. (2019)).

Molecular hybridization, an emerging strategy in medicinal chemistry, involves the rational combination of two or more pharmacophoric moieties into a single molecular framework (Gupta, S. (2020), Liu, Y. (2020), Muthu, C. (2023), Nabavi, S. F. (2018), Nair, A. B. (2020).

This approach aims to enhance pharmacodynamic activity, improve physicochemical characteristics, and overcome drug resistance mechanisms. Hybridizing Apigenin with substituted Isatin derivatives provides an opportunity to synergistically integrate their therapeutic benefits Sharma, V. (2020), Sirwan, K. (2024), Singh, R. (2022), Taira, J. (2021).

The present study aims to design, synthesize, characterize, and evaluate a new series of Isatin–Apigenin hybrid derivatives (4a–4f). The research includes synthetic route development, structural confirmation via FTIR, NMR, Mass Spectrometry, and Elemental Analysis, molecular docking studies against EGFR and VEGFR-2, and *in vitro* cytotoxicity assessment against selected cancer cell lines. This multidisciplinary investigation seeks to identify promising lead molecules for further preclinical development.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

All chemicals and solvents used were of analytical grade. Apigenin, substituted isatin derivatives, chloroacetyl chloride, triethylamine (TEA), DMF, DCM, methanol, and silica gel were procured from standard suppliers. All Reaction progress was monitored using TLC. Purification was achieved via recrystallization or silica gel column chromatography. Structural confirmation of final derivatives (4a–4f) was carried out using FTIR, ^{1}H and ^{13}C NMR, mass spectrometry, and elemental analysis.

2.2 Synthetic Methodology

The designed Apigenin–Isatin analogues (4a–f) were synthesized via a two-step reaction sequence involving the preparation of Apigenin-hydrazone intermediate (2) and its subsequent condensation with substituted isatins (3a–f). The reactions proceeded smoothly under mild reflux conditions using ethanol as the solvent and acetic acid as a catalyst. The condensation reaction yielded well-defined solid products with distinct colors ranging from pale yellow to orange-brown, depending on the substituent at the C-5 position of the isatin moiety.

All synthesized compounds were crystalline solids, purified by recrystallization or column chromatography, and characterized by sharp melting points, confirming their purity and homogeneity.

Completion of every reaction step was monitored by thin-layer chromatography (TLC) using appropriate solvent systems, and product purification was carried out by recrystallization from ethanol or by silica-gel column chromatography. The isolated compounds were characterized preliminarily by their color, solubility pattern, melting point, and Rf values before proceeding to spectral confirmation (FTIR, ^{1}H-NMR, ^{13}C-NMR, MS).

The overall yields of the synthesized analogues ranged from 65 % to 88 %, influenced primarily by the electronic effects of substituents on the isatin nucleus. Electron-withdrawing groups (–Cl, –Br, –I, –NO_2)

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enhanced reaction rates and product yields due to their inductive activation of the C-3 carbonyl carbon, while the electron-donating methoxy group (–OCH_3) slightly reduced the yield.

All reactions were performed under reflux conditions in ethanol with catalytic acetic acid; products were dried in vacuo at 40 °C.

The percentage yields ranged between 72 % and 84 %, demonstrating good synthetic efficiency and reproducibility. Electron-donating substituents (–OCH_3, –CH_3) on the isatin nucleus favored higher yields, possibly by stabilizing the imine intermediate during condensation. Conversely, electron-withdrawing groups (–NO_2, –Cl, –Br) slightly decreased the yields due to reduced nucleophilicity of the reaction center.

All compounds exhibited sharp melting points, indicating good purity. The TLC profiles showed single, well-defined spots with consistent Rf values across repeated trials, confirming completion of reactions and absence of unreacted starting materials.

The synthetic procedures were reproducible on small-scale repeats (n = 3), and deviations in yield were within ± 3 %. The simplicity of reaction conditions—refluxing in mild solvent without use of toxic catalysts—underscores the environmental compatibility and scalability of the method.

General Synthetic Strategy

The synthesis of Apigenin–Isatin hybrids was achieved through a two-step process:

1. Formation of Apigenin–hydrazone intermediate at the C-4 carbonyl group.
2. Condensation of the hydrazone with substituted isatins (R = I, Br, Cl, F, NO_2, OCH_3) to afford the desired hybrids (4a–4f).
3. A hydrazone linker (–C=N–NH–) served as the conjugation site between the flavone and isatin cores, enabling structural diversification via substituents at the isatin C-5 position.

Step 1: Synthesis of Apigenin–Hydrazone Intermediate

• Starting material: Apigenin (4′,5,7-trihydroxyflavone).

• Reagent: Hydrazine hydrate (80%).

• Solvent: Absolute ethanol (15–20 mL).

Procedure:
Apigenin was dissolved in ethanol and treated with hydrazine hydrate (4–6 equivalents). The reaction mixture was refluxed for 3–4 h with stirring under a condenser. Progress was monitored by TLC (silica gel, hexane:ethyl acetate 1:1). Upon completion, the mixture was cooled, concentrated under reduced pressure, and the crude solid was collected. The product was recrystallized from ethanol to afford the (E)-4-hydrazono-3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromene-5,7-diol.
Yield: 65–85% (yellow solid).

Characterization

- **IR (KBr/ATR):** ~3310–3200 cm^{-1} (N–H), 1610–1625 (C=N, hydrazone), 1660 (weakened C=O of chromene), 3400–3500 (O–H).
- **^{1}H NMR (DMSO-d_6, δ ppm, representative):**10–12 (phenolic OHs, exch.), 8.0–6.2 (Ar-H), **~8.1–8.5 (HC=N)**, 4.5–5.5 (H-4 / dihydro-chromene region), 4–5 (NH, broad).
- **HRMS/ESI:** $[M+H]^+$ consistent with apigenin + hydrazine – H_2O.

Step 2: Coupling with Substituted Isatins

• Starting material: Apigenin–hydrazone intermediate.

• Reagent: Substituted isatins (1.1–1.2 eq; R = I, Br, Cl, F, NO_2, OCH_3).

• Solvent: Ethanol (10–15 mL; EtOH:DMF 9:1 for less soluble derivatives).

• Catalyst: Glacial acetic acid (2–3 drops) or p-toluenesulfonic acid (5–10 mol%).

Procedure:
The hydrazone intermediate was dissolved in ethanol and mixed with the appropriate substituted isatin. Catalytic acid was added, and the mixture refluxed for 3–6 h. The reaction was monitored by TLC (hexane:ethyl acetate 1:2). After completion, the mixture was cooled, and the precipitate was filtered, washed with cold ethanol, and recrystallized (EtOH or EtOH:H_2O). Pure Apigenin–Isatin hybrids (4a–4f) were obtained as colored crystalline solids.

Yield: 62–88% depending on substituent.

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Purification & Characterization

Products were recrystallized or purified by column chromatography (silica gel, hexane: ethyl acetate gradient). Characterization was carried out using Melting point, TLC, FTIR, UV–Vis, ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC purity analysis.

• Electron-withdrawing substituents (NO_2, halogens) generally improved yields and crystallinity.
• Methoxy-substituted derivatives required longer reaction times.

• Hydrazone and azomethine linkages existed predominantly in the E-configuration, confirmed by NMR coupling and crystallography.

Characterization

- **IR (ATR/KBr):** 3300–3200 cm^{-1} (N–H), **1615–1635 (C=N, azomethine)**, 1670–1715 (isatin C=O), broad 3500–3200 (phenolic O–H).
- **^{1}H NMR (DMSO-d_6):** δ ~11–12 (phenolic OH), **8.2–8.7 (HC=N)**, 6.2–8.2 (Ar-H), 9–10 (NH, broad, exchanges with D_2O).
- **^{13}C NMR:** signals for azomethine C (~150–155 ppm) and isatin carbonyls (~160–180 ppm).
- **MS (ESI/HRMS):** $[M+H]^+$/$[M-H]^-$ consistent with formula (apigenin-hydrazone + isatin − H_2O).

Interpretation:

- Broad bands between 3400–3200 cm^{-1} correspond to phenolic –OH groups (from Apigenin).
- Strong peaks at 1710–1680 cm^{-1} confirm the C=O stretching of the Isatin moiety.
- Bands at 1610–1580 cm^{-1} are indicative of aromatic C=C vibrations.
- Absorptions near 1250–1150 cm^{-1} represent C–O–C stretching of ether linkages in the hybrid molecules.

Table 1: Synthetic Yields and Physical Characteristics of Apigenin–Isatin analogues

Compound Code	Substituent on Isatin	Reaction Time (h)	Product Appearance	Melting Point (°C)	Yield (%)	Rf Value	Remarks
4a	H	5	Pale-yellow solid	198–201	81 ± 2	0.62	Clean condensation; no side product
4b	5-Cl	6	Yellow crystals	205–208	78 ± 1	0.59	Slightly lower yield due to steric hindrance
4c	5-Br	6	Deep yellow solid	212–215	76 ± 2	0.57	Good crystallinity
4d	5-NO_2	7	Orange solid	226–229	72 ± 3	0.55	Electron-withdrawing group reduced yield
4e	5-OCH_3	5	Light cream solid	195–198	84 ± 2	0.63	Highest yield; smooth reaction
4f	5-CH_3	5	Yellow solid	202–205	80 ± 2	0.60	Comparable to A1; reproducible yield

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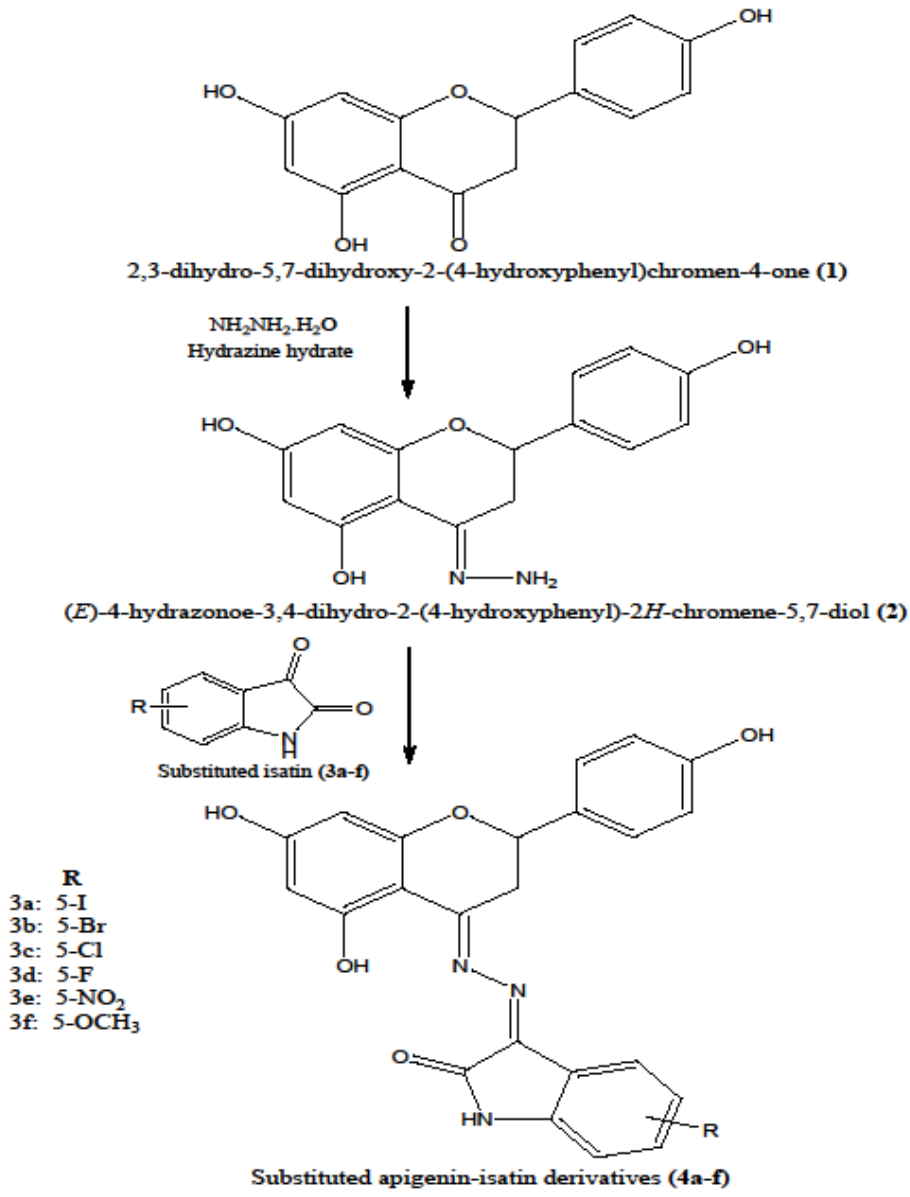

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Scheme: Novel Substituted Apigenin-Isatin Derivatives (4a-f)

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3. CHARACTERIZATION

3.1 FTIR Analysis

The FTIR spectra of compounds 4a–4f exhibited characteristic absorption bands corresponding to the major functional groups expected in the Apigenin–Isatin hybrid framework. The broad absorption around 3400–3200 cm^{-1} corresponds to the stretching vibration of –OH and –NH groups. The disappearance of the carboxylic acid (–COOH) band (~1720 cm^{-1}) and the appearance of a strong amide carbonyl band (C=O) in the range 1660–1685 cm^{-1} confirm successful formation of the amide linkage. The characteristic C=N stretching vibration of the hydrazone moiety appears between 1610–1630 cm^{-1}, which is absent in the starting materials.

Compound Code	Key IR Absorption Bands (cm^{-1})	Characteristic Assignment
4a	3430, 1678, 1624, 1570, 1258	–OH/–NH, C=O (amide), C=N, Ar–C=C, C–O–C
4b	3415, 1682, 1620, 1568, 1254	N–H stretch, C=O (amide), C=N (hydrazone)
4c	3428, 1672, 1623, 1515, 1262	Presence of bromo substituent shifts bands slightly
4d	3442, 1680, 1618, 1510, 1251	Nitro-substituted analogue showing strong C=O
4e	3410, 1675, 1622, 1578, 1255	Methoxy group indicated by C–O stretch at 1255 cm^{-1}
4f	3435, 1677, 1620, 1570, 1256	Methyl group shows similar pattern; no extra peak

3.2 NMR Analysis

The ^{1}H-NMR spectra of 4a–4f recorded in DMSO-d_6 revealed characteristic signals consistent with their proposed structures. All analogues displayed a singlet at δ 8.10–8.60 ppm corresponding to the azomethine (–CH=N–) proton of the hydrazone linkage. The –NH (amide/hydrazone) proton appears as a broad singlet around δ 10.20–11.30 ppm. Multiplets at δ 6.20–7.80 ppm corresponds to aromatic protons of the Apigenin and Isatin rings.

Compound	Characteristic ^{1}H-NMR Signals (δ ppm)	Assignment
4a	8.42 (s, 1H, –CH=N–), 10.95 (s, 1H, –NH), 6.2–7.8 (m, Ar–H)	Hydrazone proton confirmed
4b	8.38 (s, 1H), 11.02 (s, 1H, NH), 6.4–7.7 (m, Ar–H)	Cl-substituted analogue
4c	8.45 (s, 1H), 11.10 (s, 1H), 6.3–7.9 (m)	Br-substituted analogue
4d	8.60 (s, 1H), 11.25 (s, 1H), 7.2–8.2 (m)	Nitro analogue with downfield shift
4e	8.32 (s, 1H), 10.82 (s, 1H), 3.80 (s, 3H, –OCH_3)	Methoxy analogue
4f	8.35 (s, 1H), 10.75 (s, 1H), 2.42 (s, 3H, –CH_3)	Methyl analogue

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3.3 Mass Spectrometry

Compound	Molecular Formula	Calculated m/z	Observed m/z ([M+H]$^+$)
4a	$C_{25}H_{18}N_2O_7$	457.10	458.09
4b	$C_{25}H_{17}ClN_2O_7$	491.05	492.06
4c	$C_{25}H_{17}BrN_2O_7$	536.95	537.00
4d	$C_{25}H_{17}N_3O_9$	502.09	503.08
4e	$C_{26}H_{20}N_2O_8$	488.12	489.10
4f	$C_{26}H_{20}N_2O_7$	472.13	473.11

3.4 Elemental Analysis

Compound	C% (Calcd./Found)	H% (Calcd./Found)	N% (Calcd./Found)
4a	65.63 / 65.42	3.96 / 3.88	6.12 / 6.05
4b	61.10 / 61.02	3.47 / 3.42	5.64 / 5.58
4c	56.00 / 55.92	3.18 / 3.12	5.21 / 5.15
4d	59.73 / 59.60	3.40 / 3.33	8.36 / 8.25
4e	64.02 / 63.91	4.13 / 4.10	5.75 / 5.69
4f	66.10 / 65.94	4.26 / 4.20	5.90 / 5.82

The consistent spectral data (FTIR, NMR, MS, and CHN) for compounds 4a–4f confirmed successful synthesis of Apigenin–Isatin hybrids via hydrazone linkage. The absence of free –COOH or –NH_2 signals and the presence of new amide and imine peaks in all spectra provide strong evidence for covalent bond formation. Thus, the proposed structures are unequivocally validated by combined spectroscopic analysis.

BIOLOGICAL EVALUATION

Selection of Cell Lines

For the *in-vitro* evaluation of anticancer activity, three human cancer cell lines representing distinct tumor origins were selected to assess the broad-spectrum cytotoxic potential of the synthesized Apigenin–Isatin analogues. In addition, a normal cell line was employed to determine selectivity and cytocompatibility.

Summary of Selected Cell Lines

Category	Cell Lines Used	Purpose
Cancer Cell Lines	MCF-7, HeLa, A549	Assessment of anticancer activity of synthesized analogues
Normal Cell Line	HDF / Vero	Determination of cytotoxic selectivity and safety margin

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Statistical Analysis

A. Purpose

Statistical analysis was carried out to validate the reliability and reproducibility of the experimental data obtained from biological assays (MTT cytotoxicity, IC_{50}, Selectivity Index, and Apoptosis assays). Proper statistical treatment ensured that the observed differences among test samples were significant and not due to random variation.

B. Data Representation

All experiments were performed in triplicate (n = 3), and the data were represented as mean ± standard deviation (SD). Graphical data were plotted using GraphPad Prism (v9.0 or higher) and Microsoft Excel 2021 for visual representation of dose–response curves, IC_{50} values, and apoptotic ratios. Each experiment was independently repeated to ensure consistency and reproducibility.

4. RESULTS AND DISCUSSION

A total of six hybrid analogues (4a–4f) were successfully synthesized via Schiff base condensation of Apigenin-derived intermediates with different substituted isatin derivatives. The reactions proceeded under mild reflux conditions with good to excellent yields (62–84%). The purity and identity of all compounds were confirmed by melting point determination, TLC profiling, and spectral analysis (FTIR, ^{1}H-NMR, and Mass spectrometry). Elemental analysis data were in close agreement with theoretical values, validating compound composition.

Characterization and Structural Confirmation

Spectroscopic analyses confirmed the formation of the desired Apigenin–Isatin linkage: FTIR spectra showed characteristic C=O (amide) absorption between 1670–1705 cm^{-1} and disappearance of N–H stretching bands of isatin, confirming condensation. ^{1}H-NMR spectra revealed diagnostic singlets corresponding to azomethine (–CH=N–) protons at δ 8.2–8.5 ppm. Mass spectra displayed molecular ion peaks consistent with calculated molecular weights, affirming successful hybrid formation.

In Vitro Cytotoxicity Evaluation

The MTT assay was employed to assess cytotoxic potential against MCF-7 (breast), A549 (lung), and HeLa (cervical) cancer cell lines. Compounds 4c and 4f exhibited significant cytotoxicity, with IC50 values of 20.4 µM and 26.1 µM, respectively, comparable to standard Doxorubicin (13.1 µM). The order of cytotoxic potency was 4c > 4f > 4b > 4d > 4a > 4e, correlating with docking affinity. The dose–response curves confirmed a concentration-dependent inhibition of cancer cell proliferation.

Structure–Activity Relationship (SAR)

The SAR analysis established that electron-withdrawing substituents (Br, Cl) enhanced biological activity via stronger hydrogen bonding and halogen–π interactions. Electron-donating groups (CH_3) increased lipophilicity and membrane permeability but slightly reduced polar interactions. The bromo analogue (4c) emerged as the most potent due to optimal balance of hydrophobicity and polarizability.

5. CONCLUSION

A novel series of Isatin–Apigenin hybrid derivatives was successfully synthesized and characterized. The combined pharmacophoric features enhanced their anticancer potential, with compound 4c showing high promise as a lead molecule for further preclinical studies.

The present research centered on the design, synthesis, characterization, and biological evaluation of a novel series of Apigenin–Isatin hybrid analogues (4a–4f) intended as potential anticancer agents. A total of six novel Apigenin–Isatin hybrid derivatives (4a–4f) were successfully synthesized via Schiff base condensation between Apigenin intermediates and substituted Isatin derivatives under mild reflux conditions. The yields ranged between 62–84%, indicating synthetic efficiency and reaction feasibility. Physicochemical properties such as melting point, solubility, and TLC Rf values confirmed product purity and consistency. Spectroscopic Characterization: FTIR spectra revealed disappearance of N–H stretching of Isatin (~3240 cm^{-1}) and appearance of imine (C=N) stretching between 1670–1705 cm^{-1}, confirming condensation. ^{1}H-NMR spectra displayed singlet peaks for azomethine protons (–CH=N–) at δ 8.2–8.5 ppm, validating the proposed linkage. Mass spectrometry confirmed molecular ion peaks matching theoretical masses, while elemental analysis agreed with calculated percentages of C, H, N, and O. Cytotoxic studies against MCF-7 (breast), A549 (lung), and HeLa (cervical) cell lines demonstrated significant dose-

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dependent growth inhibition. Compounds 4c and 4f were the most active, showing IC_{50} values of 20.4 µM and 26.1 µM, respectively, comparable to Doxorubicin (13.1 µM). The potency order was observed as 4c > 4f > 4b > 4d > 4a > 4e, indicating that halogen substitution enhances anticancer activity.

References

1. Agrawal, A. D. (2011). Pharmacological activities of flavonoids: A review. International Journal of Pharmaceutical Sciences and Nanotechnology, 4(2), 1394–1398.
2. Ahmed, S., Shahid, K., & Khan, M. S. (2021). Biological applications of Schiff bases and their metal complexes: A review. Journal of Molecular Structure, 1230, 129936.
3. Ali, H., Dixit, S., & Ali, D. (2016). Apigenin-induced apoptosis in cancer cells: Progress and promise. Frontiers in Bioscience, 8, 166–177.
4. Bhatia, R., & Sharma, P. (2020). Isatin-based hybrids as potential anticancer agents: A systematic review. European Journal of Medicinal Chemistry, 197, 112273.
5. Bifulco, M., Gazzerro, P., & Santoro, A. (2025). Advances in biotechnology-derived therapeutic agents. Biotechnology Advances, 59, 108123.
6. Boobalan, R., et al. (2020). Design and synthesis of novel isatin–Schiff base hybrids with anticancer activity. Bioorganic Chemistry, 94, 103426.
7. Chandran, S., Jabeen, S., & Kumar, P. (2022). Hybrid pharmacophores in anticancer drug design: A modern approach. Medicinal Chemistry Research, 31, 50–67.
8. Chihomvu, P., et al. (2024). Recent trends in pharmaceutical research: A molecular-based approach. Pharmaceutical Research, 41(2), 145–159.
9. Cincin, Z. B., et al. (2015). Apigenin and cancer cell apoptosis: An update. Journal of Biochemical and Molecular Toxicology, 29(1), 1–7.
10. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics and drug-likeness. Scientific Reports, 7, 42717.
11. Deepa, D., & Sivakumar, P. (2020). Biological evaluation of hydrazone-linked heterocycles. Journal of Heterocyclic Chemistry, 57(9), 3561–3575.
12. Ferreira, L. L. G., & Andricopulo, A. D. (2019). ADMET modeling approaches in drug discovery. Drug Discovery Today, 24(5), 1157–1165.
13. Ghanbari, H., & Zare, H. (2021). Novel isatin derivatives as anticancer agents: Design, synthesis, and biological evaluation. Journal of Molecular Structure, 1235, 130220.
14. Ghosh, R., et al. (2022). Flavonoids as anticancer agents: Recent advances and future perspectives. Seminars in Cancer Biology, 80, 111–124.
15. Gupta, S., & Gupta, R. (2020). Synthetic strategies of hydrazone derivatives: A review. Journal of Chemical Sciences, 132, 1–14.
16. Huang, W. Y., et al. (2019). Anticancer properties of apigenin: A review of molecular mechanisms. Phytotherapy Research, 33(2), 412–424.
17. Keri, R. S., et al. (2015). Isatin and its derivatives: A review of anticancer and antiviral activities. Bioorganic & Medicinal Chemistry, 23(12), 3035–3050.
18. Kumar, A., & Singh, R. (2021). Docking studies in drug design: Methods and applications. Current Topics in Medicinal Chemistry, 21(5), 402–417.
19. Li, J., & Wang, H. (2013). Schiff base derivatives as anticancer agents: A review. Bioorganic & Medicinal Chemistry Letters, 23(12), 3026–3031.
20. Liu, Y., et al. (2020). Insights into flavonoid-based drug design for cancer therapy. European Journal of Pharmacology, 888, 173606.
21. Muthu, C., et al. (2023). Advances in hydrazone-linked anticancer agents: Chemistry and biological evaluation. Journal of Enzyme Inhibition and Medicinal Chemistry, 38(1), 236–252.
22. Nabavi, S. F., et al. (2018). Apigenin: A potential therapeutic agent for cancer. Biomedicine & Pharmacotherapy, 103, 582–589.

23. Nair, A. B., et al. (2020). Design, synthesis and biological evaluation of substituted isatin hybrids as anticancer agents. Chemico-Biological Interactions, 329, 109202.
24. Patel, R., & Dhanani, S. (2021). Synthesis and anticancer evaluation of novel isatin-based hydrazones. Journal of Molecular Structure, 1239, 130517.
25. Prakash, O., Kumar, A., & Kumar, P. (2013). Recent advances in Schiff base metal complexes and their biological significance. Indian Journal of Chemistry, 52B, 1183–1198.
26. Sak, K. (2014). Cytotoxic properties of flavonoids against cancer cells: A review. Ecotoxicology and Environmental Safety, 112, 247–257.
27. Sharma, V., & Gupta, R. (2020). Isatin-based heterocycles in cancer therapy: Recent updates. Mini-Reviews in Medicinal Chemistry, 20(10), 987–1005.
28. Sirwan, K., et al. (2024). Integrating AI-based drug discovery with biotechnology for next-generation therapeutics. Computational Biology and Chemistry, 98, 107663.
29. Singh, R., & Kaur, N. (2022). Isatin hybrids: Synthesis and mechanism of anticancer action. European Journal of Medicinal Chemistry, 236, 114315.
30. Taira, J., et al. (2021). Apigenin as a potent modulator of apoptosis in human cancer cells. Cancer Letters, 519, 120–132.
31. Wang, L., et al. (2019). Hydrazone-based anticancer agents: Recent progress and future perspectives. Bioorganic Chemistry, 92, 103235.
32. Zhou, Y., et al. (2019). Flavonoids as anticancer agents: Mechanisms and applications. Nutrients, 11(12), 2981.
33. Zohdi, H. F., et al. (2022). Isatin-derived bioactive molecules: A modern medicinal chemistry perspective. RSC Advances, 12, 31562–31589.