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ANTICANCER ACTIVITY OF ISATIN - PHENYLACETAMIDE MOLECULAR HYBRIDS: SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

According to global cancer report 2019, the burden of cancer will exceed more than 18 million that it became one of the major causes for global mortality rate. There is a pressing need to establish novel drug candidates for cancer treatment. Current work aims to synthesize and evaluate the anti-cancer potential of the novel class of isatin-phenylacetamide derivatives against five different cancer cell lines. A novel series of various substituted isatin-phenylacetamide were synthesized through a feasible scheme. The synthetic scheme involves a multi-step process where the final derivatives **(8a-8j and 9a-9j)** obtained by the condensation of various substituted isatins intermediates **(3a-3j)** with the substituted phenyl acetamides **(6&7)** through imine group. All the synthesized derivatives characterized by IR, ^{1}HNMR and MASS spectral methods and anti-cancer activity evaluated by employing MTT assay for six cancer cell lines and one normal human cell line. All the synthesized compounds were screened for anti-cancer activity against five cancer cell lines including NCI-H1975 (lung), SW48 (colon), HT-3 (cervical), SW626 (ovarian), BT-20 (breast), and one normal human fibroblast cell line (HLF). All the compounds displayed decent cytotoxicity profile when compared with the standard drug doxorubicin. Among the synthesized compounds **9b** is the most potent followed by **9i, 9e, 8b, 8f,** and **8i** against all the cancer cell lines. Comparatively most of the compounds displayed decent

cytotoxicity potential relative to the standard drug doxorubicin. Further investigations are needed to establish the detailed mechanism of action of the developed novel isatin-phenylacetamide hybrids.

Keywords: Isatin-phenylacetamide, Multistep reaction, Anticancer activity, EGRF-TK

1. INTRODUCTION

The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018 **[1]**. Despite major advancements and sophisticated technology developments in the cancer treatment strategies globally, cancer continues as a major disorder threatening human health **[2, 3]**. Among the major strategies employed for the mitigation of cancer, chemotherapy is considered as key feasible method owing to its simplicity relative to other complex strategies such as surgical procedures and radiation therapies etc. **[4, 5]**. Despite the success of many anticancer chemotherapeutic agents in the treatment of various types of cancers, the side-effects and long-term sequelae of anti-cancer drugs remain a foremost source of apprehension for both patients and clinicians. In most cases, the current approaches to counter the anti-cancer drug-induced side effects are often effective to some extent and are hardly address the potential longer-term sequelae **[6]**. Hence, target specific, better tolerated and effective new anticancer agents are needed to address and overcome this challenge. Various research groups and pharmaceutical industries are continuously making efforts to develop the newer anticancer agents that are target specific which can produce selective action, efficiently on the cancer cells. Furthermore, the prodigious similarity between normal and tumor cells and the diverse nature of tumors are the main hurdles foiling the development of definitive chemotherapeutic agents **[7]**. Thus, discovery and development of novel anti-cancer agents is an essential and never-ending process in medicinal and pharmaceutical industry.

The exploration of medicinally privileged heterocyclic scaffolds is one of the most important areas in drug discovery. The isatin (1H-indole-2,3-dion) moiety is ubiquitous in nature, and its derivatives possess diverse pharmacological properties such as anti-cancer **[8, 9]**, anti-tubercular **[10, 11]**, anti-HIV **[12, 13]**, anti-malarial **[14, 15]** and anti-bacterial **[16,17]** activities. As “privileged building blocks”, almost all positions of isatin moiety can be modified, and the N-1, C-3, and C-5 positions are the major domains of the chemical variation **[18]**. Moreover,

some isatin-based compounds such as sunitinib, semaxanib and indirubin **(Figure 1)** have already employed clinically or under clinical trials for the treatment various cancers **[19-22]**. The broad spectrum of biological activities combined with a wide range of structural modifications as well as successful applications in clinical practice have inspired more researchers to study isatins and create a large number of structurally diverse derivatives.

EGFR is a member of the ErbB super family of receptor tyrosine kinases. These receptors bind multiple EGF family-member ligands to initiate signaling cascades critical for an array of cellular processes, such as proliferation, differentiation, survival, metabolism, and migration. Mutations and amplifications of EGFR or family members have occurred frequently in human cancers. In the current investigation the synthesized derivatives tested against the cancer cell lines in which the cancer generation mechanism is due to the mutation in the EGFR pathway of the cells.

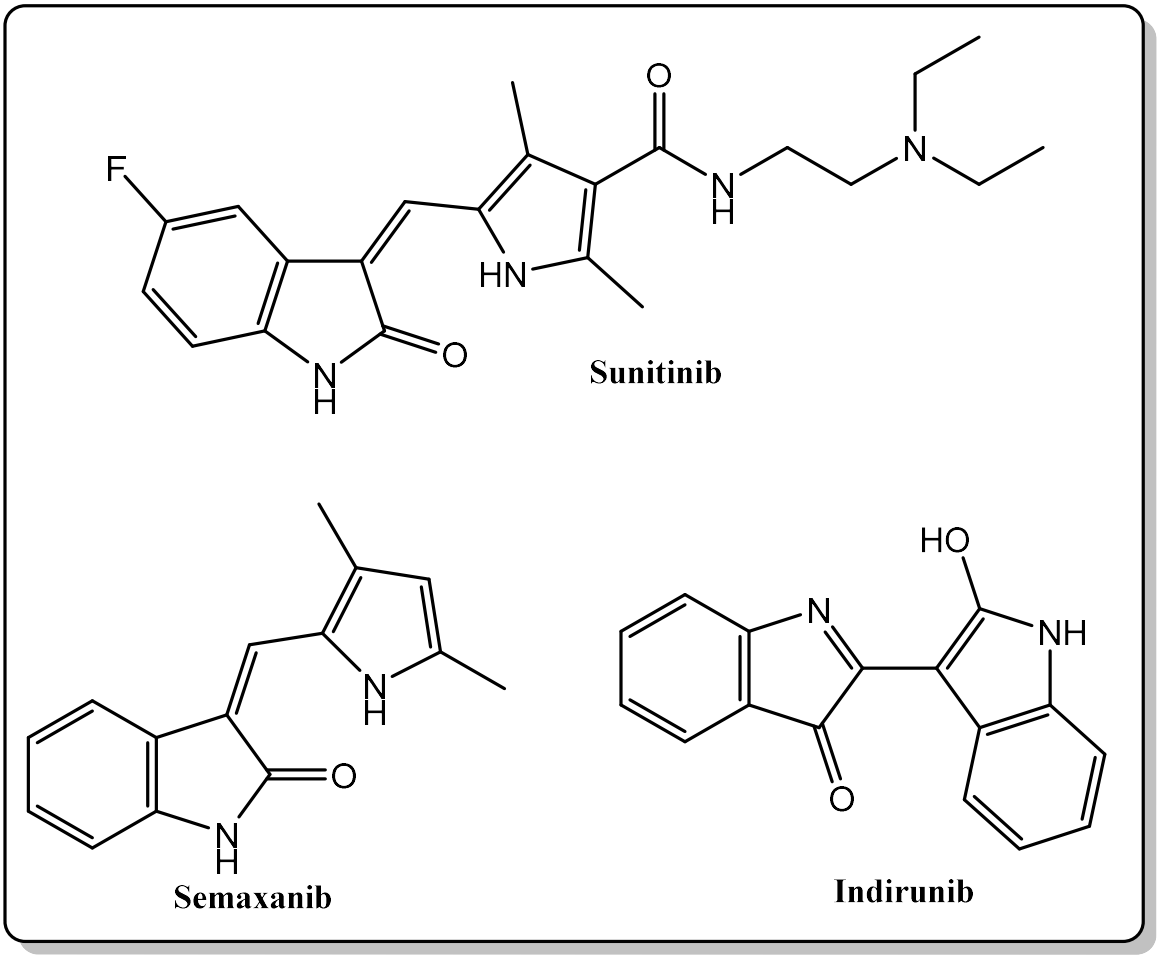

Figure 1: Structures of clinical drugs containing isatin scaffold

2. MATERIALS AND METHODS

2.1. Synthesis

All chemicals and solvents used in this work were of synthetic grade purchased from Sigma-Aldrich, local vendors and used without purification. Merck-precoated aluminum TLC plates of silica gel 60 F254 were employed for the reaction monitoring and the spots visualized with iodine vapors and in UV chamber. Melting points were determined by Remi electronic melting point apparatus. IR spectra were recorded on Agilent FTIR by KBr pellet method. ^{1}H NMR recorded on BRUKER DRX – 500 MHz. Chemical shift values (δ) articulated in ppm with reference to internal standard tetra methyl silane (TMS). The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. MASS recorded on BRUKER ESI-IT MS.

Synthesis of isatin-phenyl acetamide hybrids involves a multistep process. The complete scheme of synthesis depicted in **Figure 2**.

2.1.1. Synthesis of substituted isatins [23]

2.1.1.1. Procedure for the synthesis of isonitrosoacetanilides (2a-2j).

Sodium sulfate (6 equiv) and chloral hydrate (1 equiv) were dissolved in water (100 mL) in a round-bottomed flask. Substituted aniline (**1a-1j,** 1 equiv) dissolved in concentrated HCl (40 mL) was added to the mixture, and the mixture was stirred vigorously for 30 min at room temperature. Hydroxylamine HCl (3 equiv) solution in water (10 mL) was added to the reaction mixture. The resultant mixture was heated to 70 °C for 6−8 h. Once the reaction was completed (monitored by TLC), ice-cold water was added to the reaction mixture. The precipitates of α-isonitrosoacetanilide **(2a-2j)** so obtained were filtered, washed with water, dried, and used in the next step without further purification.

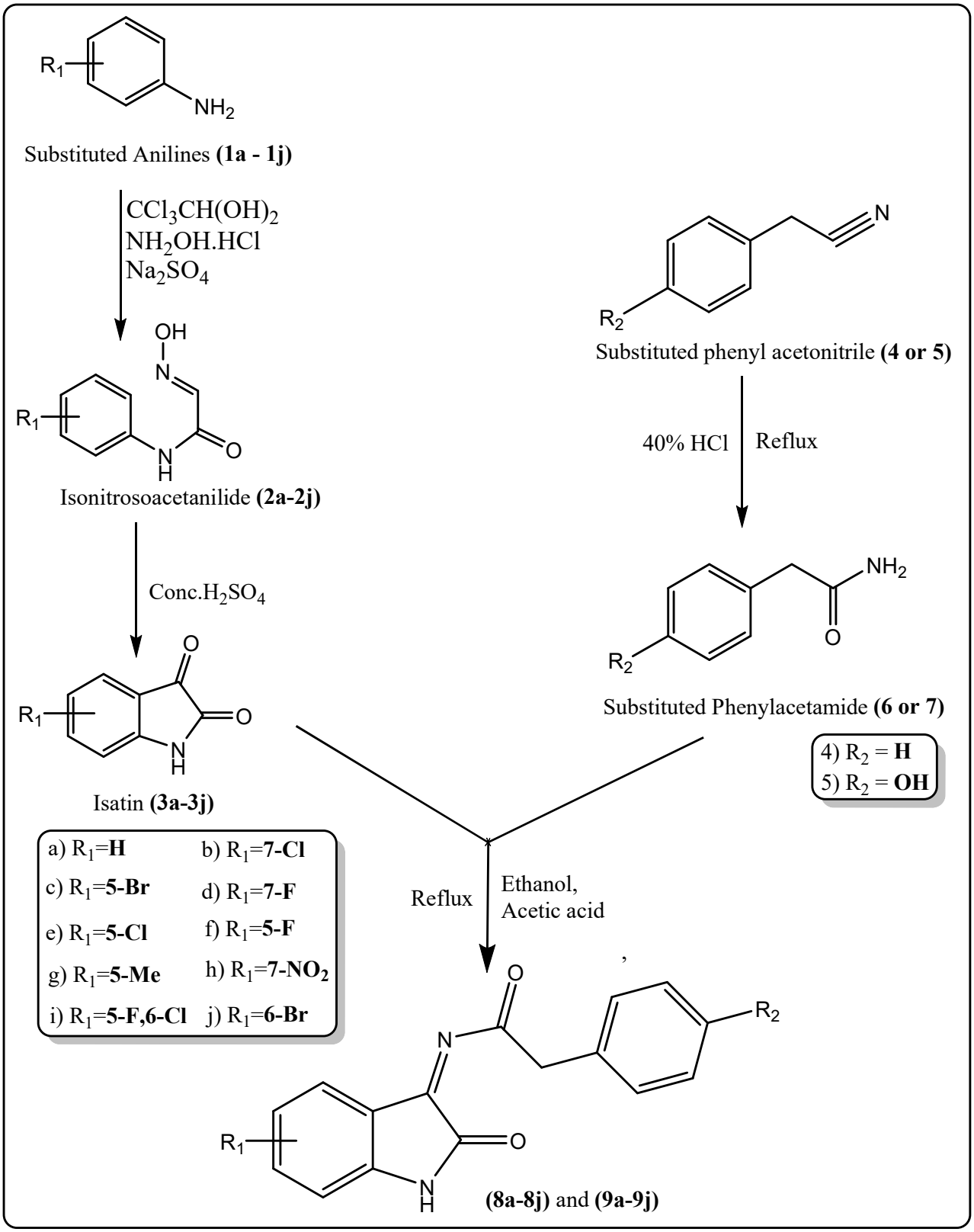

Figure 2: General scheme of synthesis of isatin-phenylacetamide hybrids

2.1.1.2. Procedure for the synthesis of substituted indole 2,3-dione or isatin (3a-3j).

To a round-bottomed flask charged with concentrated sulfuric acid (10 mL) and water (1 mL) was added α-isonitrosoacetanilide (1.0 g) over a period of a few minutes. The resulting deep red solution was heated to 80°C for 4 h and then cooled to room temperature. The reaction mixture was added to a vigorously stirred mixture of ice water (100 mL) and ethyl acetate (50 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (50 mL × 2). The combined red organic phase was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to obtain the substituted isatin compounds **(3a-3j) [23]**.

2.1.2. Procedure for the synthesis of substituted phenyl acetamides (6 & 7)

In a 250ml double round bottomed fitted with addition funnel and thermometer, placed substituted phenyl acetonitrile **(4 or 5)** (mmol) and 40% HCl solution. The reaction mixture kept at 50^0C and stirred vigorously until the clear homogenous solution obtained. The homogenous reaction mixture then added with cold water from the addition funnel drop wise with mild stirring. After complete addition of the cold water the flask kept in an ice bath that results in the formation of substituted phenylacetamide **(6 or 7)**. The crude product of phenyl acetamide filtered and the wet solid was transferred to a conical flask, treated with cold 10% sodium carbonate solution to remove traces of acid. The final product obtained by the suction filtration and dried in vacuum **[24]**.

2.1.3. General procedure for the synthesis of Isatin-phenylacetamide hybrids (8a-8j and 9a-9j)

In a 100ml 2-necked round bottomed flask, 10ml ethanolic solution of various isatins **(3a-3j)** **(**0.5 mmol, 1equiv) were placed and 2ml glacial acetic acid was added and stirred for 30mins. To this reaction mass, ethanolic solution of substituted phenyl acetamides **(6 or 7)** (0.5 mmol, 1equiv) were added

drop wise and after complete addition, the reaction mixture refluxed. Reaction progress monitored through TLC with 10% ethyl acetate - hexane as mobile phase. After the complete reaction, the crude product was treated with 10% sodium bicarbonate solution to quench the acetic acid. Then the crude was partitioned with three equal portions of ethyl acetate then the combined organic layers were washed with brine, dried over sodium sulphate, and concentrated using rotary evaporator. The final product was purified from the column chromatography, dried under vacuum and recrystallized from boiling water.

2.2. Anticancer activity: Procedure of MTT Assay

Five cancer cell lines including NCI-H1975 (lung), SW48 (colon), HT-3 (cervical), SW626 (ovarian), BT-20 (breast), and one normal human fibroblast cell line (HLF) which were obtained from American Type Culture Collection (ATCC).

All the cells were cultured in DMEM Medium (Gibco, Life Technologies) except HT-29 which is cultured in RPMI 1640 (Gibco, Life Technologies) supplemented with 10% foetal bovine serum (Gibco, Life Technologies), 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified incubator at 37^0C with 5% CO_2. MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], Para formaldehyde, Phosphate Saline Buffer (pH 7.4), DMSO, Crystal violet (0.5% w/v in ethanol), Hoechst stain were purchased from Sigma-Aldrich India. The MTT assay procedure was adopted from the standard protocol described by Carmichael *et al*., 1987 **[25]**.

3. RESULTS AND DISCUSSION

3.1. Chemistry

All the synthesized compounds were resulted in competitive yield as reported in **Table 1**. The synthesized compounds were confirmed by IR, H^1 NMR and MASS spectral data which are in accordance with respect to the structure compounds. The aromatic C–H peaks of two phenyl rings chemical shift values (δ) were observed from 6.8 to 7.8ppm and the N–H peak of the isatin scaffold was

observed around 10.9 to 11.3ppm as a singlet in all the compounds. The -CH2- peak of the phenylacetamide part was appeared around 4ppm as a singlet in all compounds.Structural and physical data of the synthesized compounds was enumerated below in the **Table 1**.

Table 1: Molecular formula, melting point and yield of isatin-phenylacetamide derivatives

Comp. No	R_1	R_2	Molecular formula	m.p in °C	% Yield
8a	H	H	$C_{16}H_{12}N_2O_2$	193-195	75
8b	7-Cl	H	$C_{16}H_{11}ClN_2O_2$	204-205	68
8c	5-Br	H	$C_{16}H_{11}BrN_2O_2$	214-216	65
8d	7-F	H	$C_{16}H_{11}FN_2O_2$	209-210	69
8e	5-Cl	H	$C_{16}H_{11}ClN_2O_2$	218-219	61
8f	5-F	H	$C_{16}H_{11}FN_2O_2$	201-203	59
8g	5-CH_3	H	$C_{17}H_{14}N_2O_2$	197-198	74
8h	7-NO_2	H	$C_{16}H_{11}N_3O_4$	221-223	61
8i	5-F & 6-Cl	H	$C_{16}H_{10}ClFN_2O_2$	>250	57
8j	6-Br	H	$C_{16}H_{11}BrN2O_2$	218-219	69
9a	H	-OH	$C_{16}H_{12}N_2O_3$	216-218	76
9b	7-Cl	-OH	$C_{16}H_{11}ClN_2O_3$	>250	65
9c	5-Br	-OH	$C_{16}H_{11}BrN_2O_3$	>250	66
9d	7-F	-OH	$C_{16}H_{11}FN_2O_3$	238-240	62
9e	5-Cl	-OH	$C_{16}H_{11}ClN_2O_3$	244-246	69
9f	5-F	-OH	$C_{16}H_{11}FN_2O_3$	>250	54
9g	5-CH_3	-OH	$C_{17}H_{14}N_2O_3$	232-233	70
9h	7-NO_2	-OH	$C_{16}H_{11}N_3O_5$	>250	55
9i	5-F & 6-Cl	-OH	$C_{16}H_{10}ClFN_2O_3$	>250	51
9j	6-Br	-OH	$C_{16}H_{11}BrN_2O_3$	>250	64

Spectral data of the synthesized compounds was enumerated below:

Compound 8a: ***(Z)-N-(2-oxoindolin-3-ylidene)-2-phenylacetamide***

Dark yellow solid;**IR (KBr):** ν_{max} in cm^{-1}: 1665 (C=N), 1681 (C=O), 3318 (N-H), 3060 (=C–H), 1243 (C-N),1542 (C=C); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.04 (2H, s), 7.23-7.43 (6H, 7.28 (ddd, J = 7.8, 7.3, 1.3 Hz), 7.26 (tt, J = 7.7, 1.6 Hz), 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz)), 7.53 (1H, ddd, J = 8.6, 1.3, 0.4 Hz), 7.63-7.73 (2H, 7.68 (ddd, J = 8.6, 7.3, 1.5 Hz), 7.68 (ddd, J = 7.8, 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}N_2O_2$ ([M + H]$^+$): 264.28, found 265.25.

Compound 8b: ***(Z)-N-(7-chloro-2-oxoindolin-3-ylidene)-2-phenylacetamide***

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1662 (C=N), 1683 (C=O), 3324 (N-H), 3063 (=C–H), 1247 (C-N),1543 (C=C), 678 (C-Cl); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.04 (2H, s), 7.24-7.43 (6H, 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.28 (dd, J = 7.6, 7.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz), 7.27 (tt, J = 7.7, 1.6 Hz)), 7.70-7.79 (2H, 7.73 (dd, J = 7.5, 1.3 Hz), 7.77 (dd, J = 7.6, 1.3 Hz)).**ESI-**

MS: m/z Anal. Calcd. For $C_{16}H_{11}ClN_2O_2$ ($[M + H]^+$): 298.73, found 299.55.

Compound 8c: *(Z)-N-(5-bromo-2-oxoindolin-3-ylidene)-2-phenylacetamide*

Yellowish brown solid; **IR (KBr):**ν_{max} in cm^{-1}: 1662 (C=N), 1683 (C=O), 3324 (N-H), 3063 (=C–H), 1247 (C-N), 1543 (C=C), 625 (C-Br); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ δ 4.04 (2H, s), 7.27 (1H, tt, J = 7.7, 1.6 Hz), 7.31-7.46 (5H, 7.44 (dd, J = 8.3, 0.4 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz), 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz)), 7.81-7.86 (2H, 7.83 (dd, J = 8.3, 1.5 Hz), 7.84 (dd, J = 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}BrN_2O_2$ ($[M + H]^+$): 343.18, found 344.15.

Compound 8d: *(Z)-N-(7-fluoro-2-oxoindolin-3-ylidene)-2-phenylacetamide*

Yellow solid;**IR (KBr):**ν_{max} in cm^{-1}: 1665 (C=N), 1679 (C=O), 3325 (N-H), 3060 (=C–H), 1245 (C-N), 1541 (C=C), 1157 (C-F); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ δ 4.04 (2H, s), 7.23-7.43 (7H, 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.36 (dd, J = 7.5, 1.3 Hz), 7.29 (dd, J = 7.6, 7.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz), 7.26 (tt, J = 7.7, 1.6 Hz)), 7.74 (1H, dd, J = 7.6, 1.3 Hz). ESI-MS m/z Anal. Calcd. For $C_{16}H_{11}FN_2O_2$ ($[M + H]^+$): 282.27, found 283.20.

Compound 8e: *(Z)-N-(5-chloro-2-oxoindolin-3-ylidene)-2-phenylacetamide*

Yellow solid;**IR (KBr):**ν_{max} in cm^{-1}: 1660 (C=N), 1681 (C=O), 3319 (N-H), 3061 (=C–H), 1245 (C-N), 1547 (C=C), 681 (C-Cl);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ δ 4.04 (2H, s), 7.27 (1H, tt, J = 7.7, 1.6 Hz), 7.31-7.44 (5H, 7.41 (dd, J = 8.3, 0.4 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz), 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz)), 7.87-7.94 (2H, 7.91 (dd, J = 8.3, 1.5 Hz), 7.88 (dd, J = 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}ClN_2O_2$ ($[M + H]^+$): 298.73, found 299.55.

Compound 8f: *(Z)-N-(5-fluoro-2-oxoindolin-3-ylidene)-2-phenylacetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1665 (C=N), 1680 (C=O), 3325 (N-H), 3060 (=C–H), 1245 (C-N), 1541 (C=C), 1155 (C-F); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.04 (2H, s), 7.22-7.29 (2H, 7.25 (dd, J = 8.2, 1.4 Hz), 7.26 (tt, J = 7.7, 1.6 Hz)), 7.31-7.47 (5H, 7.44 (dd, J = 8.2, 0.4 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz), 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz)), 7.85 (1H, dd, J = 1.4, 0.4 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}FN_2O_2$ ($[M + H]^+$): 282.27, found 283.15.

Compound 8g: *(Z)-N-(5-methyl-2-oxoindolin-3-ylidene)-2-phenylacetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1670 (C=N), 1680 (C=O), 3320 (N-H), 3055 (=C–H), 1253 (C-N), 1539 (C=C); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ δ 2.33 (3H, s), 4.04

(2H, s), 7.27 (1H, tt, J = 7.7, 1.6 Hz), 7.30-7.43 (5H, 7.33 (dd, J = 8.2, 0.4 Hz), 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz)), 7.46 (1H, dd, J = 8.2, 1.4 Hz), 7.76 (1H, dd, J = 1.4, 0.4 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{14}N_2O_2$ ($[M + H]^+$): 278.31, found 279.25.

Compound 8h: ***(Z)-N-(7-nitro-2-oxoindolin-3-ylidene)-2-phenylacetamide***

Brownish yellow solid; **IR (KBr):** ν_{max} in cm^{-1}: 1663 (C=N), 1680 (C=O), 3315 (N-H), 3064 (=C–H), 1235 (C-N), 1543 (C=C);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.04 (2H, s), 7.27 (1H, tt, J = 7.7, 1.6 Hz), 7.31-7.44 (5H, 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.40 (dd, J = 7.6, 7.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz)), 8.18 (1H, dd, J = 7.6, 1.7 Hz), 8.55 (1H, dd, J = 7.5, 1.7 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}N_3O_4$ ($[M + H]^+$): 309.28, found 310.20.

Compound 8i: ***(Z)-N-(6-chloro-5-fluoro-2-oxoindolin-3-ylidene)-2-phenylacetamide***

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1650 (C=N), 1677 (C=O), 3329 (N-H), 3044 (=C–H), 1243 (C-N), 1542 (C=C), 1308 (C-F), 670.5 (C-Cl); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.03 (2H, s), 7.27 (1H, tt, J = 7.7, 1.6 Hz), 7.31-7.43 (5H, 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.37 (d, J = 0.4 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz)), 7.92 (1H, d, J = 0.4 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{10}ClFN_2O_2$ ($[M + H]^+$): 316.72, found 317.60.

Compound 8j: ***(Z)-N-(6-bromo-2-oxoindolin-3-ylidene)-2-phenylacetamide***

Yellow solid; **IR (KBr):** ν_{max} in cm^{-1}: 1660 (C=N), 1685 (C=O), 3324 (N-H), 3061 (=C–H), 1245 (C-N), 1540 (C=C), 619 (C-Br); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ δ 4.04 (2H, s), 7.22-7.30 (2H, 7.27 (tt, J = 7.7, 1.6 Hz), 7.25 (dd, J = 8.5, 1.7 Hz)), 7.31-7.43 (4H, 7.36 (dddd, J = 7.7, 7.4, 1.8, 0.5 Hz), 7.40 (dddd, J = 7.4, 1.6, 1.2, 0.5 Hz)), 7.65 (1H, dd, J = 1.7, 0.5 Hz), 8.28 (1H, dd, J = 8.5, 0.5 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}BrN2O_2$ ($[M + H]^+$): 343.18, found 344.10.

Compound 9a: ***(Z)-2-(4-hydroxyphenyl)-N-(2-oxoindolin-3-ylidene) acetamide***

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1659 (C=N), 1683 (C=O), 3324 (N-H), 3055 (=C–H), 1238 (C-N), 1547 (C=C), 3593 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.24-7.33 (3H, 7.28 (ddd, J = 7.8, 7.3, 1.3 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.53 (1H, ddd, J = 8.6, 1.3, 0.4 Hz), 7.63-7.73 (2H, 7.68 (ddd, J = 8.6, 7.3, 1.5 Hz), 7.68 (ddd, J = 7.8, 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal.

Calcd. For $C_{16}H_{12}N_2O_3$ ([M + H]$^+$): 280.28, found 281.20.

Compound 9b: *(Z)-N-(7-chloro-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1667 (C=N), 1684 (C=O), 3327 (N-H), 3061 (=C–H), 1231 (C-N), 1546 (C=C), 685 (C-Cl), 3586 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.6, 0.5 Hz), 7.24-7.32 (3H, 7.28 (dd, J = 7.6, 7.5 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.70-7.79 (2H, 7.73 (dd, J = 7.5, 1.3 Hz), 7.77 (dd, J = 7.6, 1.3 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}ClN_2O_3$ ([M + H]$^+$): 314.73, found 315.60.

Compound 9c: *(Z)-N-(5-bromo-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1667 (C=N), 1683 (C=O), 3324 (N-H), 3062 (=C–H), 1247 (C-N), 1545 (C=C), 631 (C-Br), 3585 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.95 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.6, 0.5 Hz), 7.28 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.44 (1H, dd, J = 8.3, 0.4 Hz), 7.81-7.86 (2H, 7.83 (dd, J = 8.3, 1.5 Hz), 7.84 (dd, J = 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}BrN_2O_3$ ([M + H]$^+$): 359.18, found 360.15.

Compound 9d: *(Z)-N-(7-fluoro-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1659 (C=N), 1678 (C=O), 3325 (N-H), 3057 (=C–H), 1247 (C-N), 1540 (C=C), 1189 (C-F), 3574 (O-H); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.25-7.38 (4H, 7.29 (dd, J = 7.6, 7.5 Hz), 7.36 (dd, J = 7.5, 1.3 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.74 (1H, dd, J = 7.6, 1.3 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}FN_2O_3$ ([M + H]$^+$): 298.27, found 299.20.

Compound 9e: *(Z)-N-(5-chloro-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1659 (C=N), 1675 (C=O), 3326 (N-H), 3058 (=C–H), 1240 (C-N), 1540 (C=C), 699 (C-Cl), 3580 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.6, 0.5 Hz), 7.28 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.41 (1H, dd, J = 8.3, 0.4 Hz), 7.87-7.94 (2H, 7.91 (dd, J = 8.3, 1.5 Hz), 7.88 (dd, J = 1.5, 0.4 Hz)).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}ClN_2O_3$ ([M + H]$^+$): 314.73, found 315.60.

Compound 9f: *(Z)-N-(5-fluoro-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide*

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1666 (C=N), 1690 (C=O), 3325 (N-H), 3060 (=C–H), 1245 (C-N), 1541 (C=C), 1155 (C-F), 3585 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.95 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.22-7.31 (3H, 7.25 (dd, J = 8.2, 1.4 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.44 (1H, dd, J = 8.2, 0.4 Hz), 7.85 (1H, dd, J = 1.4, 0.4 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}FN_2O_3$ ($[M + H]^+$): 298.27, found 299.20.

Compound 9g: ***(Z)-2-(4-hydroxyphenyl)-N-(5-methyl-2-oxoindolin-3-ylidene) acetamide***

Yellow solid; **IR (KBr):** ν_{max} in cm^{-1}: 1675 (C=N), 1689 (C=O), 3320 (N-H), 3065 (=C–H), 1249 (C-N), 1550 (C=C), 3587 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 2.33 (3H, s), 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.25-7.35 (3H, 7.33 (dd, J = 8.2, 0.4 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.46 (1H, dd, J = 8.2, 1.4 Hz), 7.76 (1H, dd, J = 1.4, 0.4 Hz).ESI-MS m/z Anal. Calcd. For $C_{17}H_{14}N_2O_3$ ($[M + H]^+$): 294.30, found 295.20.

Compound 9h: ***(Z)-2-(4-hydroxyphenyl)-N-(7-nitro-2-oxoindolin-3-ylidene) acetamide***

Brownish yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1665 (C=N), 1683 (C=O), 3323 (N-H), 3066 (=C–H), 1235 (C-N), 1543 (C=C), 3580 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.94 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.28 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.40 (1H, dd, J = 7.6, 7.5 Hz), 8.18 (1H, dd, J = 7.6, 1.7 Hz), 8.55 (1H, dd, J = 7.5, 1.7 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}N_3O_5$ ($[M + H]^+$): 325.28, found 326.20.

Compound 9i: ***(Z)-N-(6-chloro-5-fluoro-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide***

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 1650 (C=N), 1688 (C=O), 3330 (N-H), 3045 (=C–H), 1255 (C-N), 1551 (C=C), 1325 (C-F), 674 (C-Cl), 3580 (O-H);**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.95 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.28 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.36 (1H, d, J = 0.4 Hz), 7.92 (1H, d, J = 0.4 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{10}ClFN_2O_3$($[M + H]^+$): 332.70, found 333.65.

Compound 9j: ***(Z)-N-(6-bromo-2-oxoindolin-3-ylidene)-2-(4-hydroxyphenyl) acetamide***

Yellow solid; **IR (KBr):**ν_{max} in cm^{-1}: 197–199^0C; 1667 (C=N), 1685 (C=O), 3324 (N-H), 3061 (=C–H), 1239 (C-N), 1540 (C=C), 626 (C-Br), 3585 (O-H); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 3.95 (2H, s), 6.72 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.22-7.31 (3H, 7.25 (dd, J = 8.5, 1.7 Hz), 7.28 (ddd, J = 8.3, 1.5, 0.5 Hz)), 7.65 (1H, dd, J = 1.7, 0.5

Hz), 8.28 (1H, dd, J = 8.5, 0.5 Hz).**ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{11}BrN_2O_3$ ([M + H]$^+$): 359.18, found 360.15.

3.2. Anticancer activity

Results of MTT Assay were displayed in **Table 2** and the assay results displayed the cytotoxic potential of all the synthesized compounds in comparison to the standard drug Doxorubicin. The results displayed the cytotoxicity potential of the synthesized derivatives. All the compounds possessed noticeable cytotoxicity against the selected cancer cell lines. It was observed that among the tested cell lines; all cell lines are affected to a noticeable extent except the SW48 (colon) cancer cell lines. Majority of the compounds displayed high cytotoxicity spectrum towards HT-3 (cervical), SW626 (ovarian) and BT-20 (breast), followed by NCI-H1975 (lung) and SW48 (colon) cell lines. All the compounds displayed relatively low cytotoxicity magnitude against the SW48 (colon) cell lines. Among the 20 compounds tested against the cancer cell lines, 7 compounds displayed very significant cytotoxic activity against HT-3 (cervical), SW626 (ovarian) and BT-20 (breast) in which their IC50 values ranged from 3.86±1.78 (9b) to 8.85±1.86 (8f), 4.93±3.32 (9b) to 10.08±1.12 (8f) and 6.74±1.29 to 12.96±1.92 against HT-3, SW626, and BT-20 cell lines respectively. This study revealed that among all the synthesized compounds **9b**showed potent cytotoxicity against three cancer cell lines used in the assay. IC50 values of the compound **9b** among the tested isatin-phenylacetamide derivatives for each cell line are: 3.86µM (HT-3); 4.93µM (SW626); 6.74µM (BT-20). Compound **8e** displayed highest cytotoxicity against the NCI-H1975 (IC50 - 17.75µM) and SW48 (IC50 - 18.05µM) among all the other derivatives. Compounds **9i, 9e, 8b, 8f,** and **8i** displayed good cytotoxic potential specifically against four cell lines, HT-3, SW626, BT-20 and SW 48 according to the observed IC50 values.

Table 2: Results of MTT Assay were displayed

Sample	IC_{50} (μM)[a]					
	NCI-H1975	SW48	HT-3	SW626	BT-20	HLF
8a	20.72±2.18	28.32±1.58	19.3±1.08	20.9±3.36	21.64±2.14	21.11±1.88
8b	18.44±1.58	20.05±2.39	4.92±2.83	7.76±2.64	6.95±1.25	22.95±1.30
8c	>100	>100	65.16±2.25	81.15±1.12	>100	>100
8d	49.26±1.18	>100	26.33±0.94	21.33±1.14	29.72±2.25	>100
8e	17.75±0.93	18.05±1.38	5.06±1.97	9.84±1.34	12.96±1.92	34.85±1.34
8f	21.75±1.03	19.02±1.23	8.85±1.86	10.08±1.12	11.4±1.12	28.20±0.94
8g	26.01±3.09	29.03±1.25	15.40±2.87	18.22±1.12	19.34±1.31	41.67±1.75
8h	29.38±0.98	31.25±1.26	20.42±1.18	22.8±1.32	23.68±1.14	36.11±1.18
8i	18.07±1.35	20.15±1.89	5.72±2.83	9.01±3.33	11.16±2.52	18.38±4.3
8j	>100	>100	>100	>100	>100	>100
9a	27.45±2.74	34.02±1.08	18.72±2.54	20.7±2.64	23.17±0.95	>100
9b	21.77±4.18	19.13±1.27	3.86±1.78	4.93±3.32	6.74±1.29	21.45±1.23
9c	90.27±5.09	>100	>100	>100	>100	>100
9d	52.98±2.55	>100	19.02±2.78	19.95±3.36	21.07±1.12	>100
9e	21.72±2.18	20.71±1.56	4.57±2.15	7.96±1.84	11.02±1.30	28.95±1.88
9f	27.45±2.74	36.72±2.08	17.52±3.14	23.7±2.46	21.16±1.25	44.38±2.01
9g	75.07±4.22	>100	20.68±2.75	23.1±3.32	24.29±1.30	>100
9h	72.95±4.87	>100	19.95±2.37	21.72±2.87	23.11±1.67	>100
9i	21.96±0.88	23.29±0.98	4.23±2.78	6.81±3.36	9.62±1.12	27.12±1.09
9j	>100	>100	>100	>100	>100	>100
Doxorubicin[b]	1.23±1.2	1.28±1.7	1.02±0.8	1.13±0.95	1.97±0.45	1.34±0.64

When compared to the overall cytotoxicity spectrum of the all isatin-phenylacetamide derivatives against all the tested cell lines, they displayed least potency towards NCI-H1975 cell lines with the IC50 values ranging from 17.75μM to > 100μM. All the isatin-phenylacetamide derivatives were exhibited very low cytotoxicity level towards the normal human cell lines relative to the standard drug doxorubicin.

It is apparent from the results, that the compounds possessing halogen substituent, especially chlorine (-Cl) and fluorine (-F) on the isatin part of the structure displayed potent cytotoxicity towards all the investigated cell lines under study. Close observation of IC50 values revealed that the hydroxy (-OH) derivatives (9a-9j) are relatively more active than the unsubstituted derivatives (8a-8j).The study selected the EGFR mutation generated cancer cell lines. So, the mechanism of action of these synthesized derivatives may be attributed to the inhibition of the EGRF-TK system in the cells.

4. CONCLUSION

In conclusion, the synthesis of a new series of isatin-phenylacetamide derivatives was accomplished through a feasible multistep synthetic route and this method provided a good yield for all the designed compounds. Synthesized compounds were characterized

and screened for anticancer activity against five selected cancer cell line and one normal human cell line. Comparatively most of the compounds displayed decent cytotoxicity potential relative to the standard drug doxorubicin. As the selected cancer cell lines were generated due to EGFR mutation, these synthesized derivatives may act through the inhibition of EGRF-TK system in cells. Further molecular level investigations are needed to establish the detailed mechanism of action of the developed novel isatin-phenylacetamide derivatives.

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Conflict of Interest

None

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